COVID-19 mRNA "vaccine" harms research collection

Compiled by Dr. Martin Wucher, MSC Dent Sc (eq DDS), Dr Byram Bridle, PhD, Dr. Steven Hatfill, Erik Sass, et al.

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Version 2, last updated July 1, 2025. Corresponding author: eriksass@gmail.com.

This compilation originated with the authors' contributions to TOXIC SHOT: Facing the Dangers of the COVID "Vaccines" (Foreword by Sen. Ron Johnson)

I. Spike protein pathogenicity research library (n=375)
Originally part of the outer coat of the SARS-CoV2 virus, where it functions as a "key" to "unlock" (infect) cells, spike proteins are also produced in large amounts by the mRNA "vaccines," triggering a short-lived immune response in the form of antibodies. However, a growing body of evidence has shown that the spike protein is harmful by itself, including over 370 peer-reviewed scientific papers collected in section I.

II. Spike protein and "vaccine" mRNA biodistribution studies (n=61)
In addition to the pathogenic characteristics of the spike protein antigen, over 60 peer-reviewed studies have demonstrated that both the "vaccine" mRNA encoding for the spike protein antigen and the spike protein itself can penetrate distant tissues, causing systemic harms.

III. Spike protein and "vaccine" mRNA persistence studies (n=41)
Over 40 peer-reviewed studies confirm that "vaccine" mRNA and the resulting spike protein antigen persist in the tissues of human vaccine recipients and animal test subjects far longer than claimed by public health officials; viral spike proteins, resulting from natural infection, have been shown to persist even longer, bolstering concerns that the identical "vaccine" spike may also last longer than anticipated.

IV. Lipid nanoparticle toxicity and allergenicity studies (n=80)
80 peer-reviewed papers show that ionizable lipid nanoparticles (LNPs) used in the experimental mRNA injections are highly inflammatory on their own, including their polyethylene glycol (PEG) component, an established cause of anaphylaxis (an extreme allergic reaction).

V. COVID-19 "vaccine" immune imprinting library (n=140)
Immune imprinting, dubbed "original antigenic sin" by Thomas Francis Jr., occurs when memory B lymphocytes produced in response to an initial viral infection dominate subsequent responses to related viruses. 140 peer-reviewed

papers suggest that COVID "vaccines" imprinted the immune systems of recipients through exposure to the "wild type" spike protein from the original Wuhan strain, shaping their response to subsequent variants in potentially harmful ways.

VI. SARS-CoV2 vaccine and viral variant research library (n=70)

In addition to the pathogenicity, distribution, and long persistence of the "vaccine" spike protein, this collection of 70 peer-reviewed papers suggests the "vaccines" applied strong selective pressure to the fast-mutating SARS-CoV2 virus, quickly giving rise to "vaccine"-resistant variants.

I. COVID-19 spike protein pathogenicity research library

Compiled by Dr. Martin Wucher, MSC Dent Sc (eq DDS), Erik Sass, et al.

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Version 3, last updated July 1, 2025. Corresponding author: eriksass@gmail.com

Originally part of the outer coat of the SARS-CoV2 virus, where it functions as a "key" to "unlock" (infect) cells, spike proteins are also produced in large amounts by the mRNA "vaccines," triggering a short-lived immune response in the form of antibodies. However, a growing body of evidence has shown that the spike protein is harmful by itself, independent of the rest of the virus.

The following (**I**. Alphabetical List) collects over 370 (**n=375**) peer-reviewed scientific studies confirming that the spike protein is highly pathogenic on its own; most *in vitro* studies cited here used recombinant spike proteins or spike proteins in pseudoviral vectors, and produced pathological effects not reliant on the SARS-CoV2 viral machinery.

The second section (**II**. Categories) organizes the research into broad categories including affected tissues and organ systems, mechanisms, and evidence from clinical pathology. Because these areas overlap, many articles appear more than once in the second section.

This compilation originated with Dr. Wucher's contribution to *TOXIC SHOT: Facing the Dangers of the COVID "Vaccines,"* (Chapter 4: The Spike Protein **I**s Harmful By **I**tself).

I. ALPHABETICAL LIST (n=375)

1. Abdi A et al., "Biomed Interaction of SARS-CoV-2 with cardiomyocytes: **I**nsight into the underlying molecular mechanisms of cardiac injury and pharmacotherapy," *Pharmacother.* 2022, 146: 112518. doi: 10.1016/j.biopha.2021.112518

2. Aboudounya MM and RJ Heads, "COV**I**D-19 and Toll-Like Receptor 4 (TLR4): SARS-CoV-2 May Bind and Activate TLR4 to Increase ACE2 Expression, Facilitating Entry and Causing Hyperinflammation," *Mediators Inflamm.* 2021, 8874339. doi: https://doi.org/10.1155/2021/8874339

3. Acevedo-Whitehouse K and R Bruno, "Potential health risks of mRNA-based vaccine therapy: A hypothesis," *Med. Hypotheses* 2023, 171: 111015. doi: https://doi.org/10.1016/j.mehy.2023.111015

4. Ahn WM et al., “SARS-CoV-2 Spike Protein Stimulates Macropinocytosis in Murine and Human Macrophages via PKC-NADPH Oxidase Signaling,” *Antioxidants* 2024, 13, 2: 175. doi: https://doi.org/10.3390/antiox13020175

5. Ait-Belkacem I et al., “SARS-CoV-2 spike protein induces a differential monocyte activation that may contribute to age bias in COVID-19 severity,” *Sci. Rep.* 2022, 12: 20824. doi: https://doi.org/10.1038/s41598-022-25259-2

6. Aksenova AY et al., “The increased amyloidogenicity of Spike RBD and pH-dependent binding to ACE2 may contribute to the transmissibility and pathogenic properties of SARS-CoV-2 omicron as suggested by in silico study,” *Int. J. Mol. Sci.* 2022, 23, 21: 13502. doi: https://doi.org/10.3390/ijms232113502

7. Al-Kuraishy HM et al., “Changes in the Blood Viscosity in Patients With SARS-CoV-2 Infection,” *Front. Med.* 2022, 9: 876017. doi: 10.3389/fmed.2022.876017

8. Al-Kuraishy HM et al., “Hemolytic anemia in COVID-19,” *Ann. Hematol.* 2022, 101: 1887–1895. doi: 10.1007/s00277-022-04907-7

9. Albornoz EA et al., “SARS-CoV-2 drives NLRP3 inflammasome activation in human microglia through spike protein,” *Mol. Psychiatr.* 2023, 28: 2878–2893. doi: https://doi.org/10.1038/s41380-022-01831-0

10. Almehdi AM et al., “SARS-CoV-2 Spike Protein: Pathogenesis, Vaccines, and Potential Therapies,” *Infection* 2021, 49, 5: 855–876. doi: https://doi.org/10.1007/s15010-021-01677-8

11. Alghmadi A et al., “Altered Circulating Cytokine Profile Among mRNA-Vaccinated Young Adults: A Year-Long Follow-Up Study,” *Immun. Inflamm. Dis.* 2025, 13, 4: e70194. doi: https://doi.org/10.1002/iid3.70194

12. Alves V et al., “SARS-CoV-2 Spike protein alters microglial purinergic signaling *Front. Immunol.* 2023, 14: 1158460. doi: 10.3389/fimmu.2023.1158460

13. Anft M et al., “Effect of immunoadsorption on clinical presentation and immune alterations in COVID-19–induced and/or aggravated ME/CFS,” *Mol. Ther.* 2025, 33, 6: 2886-2899. doi: 10.1016/j.ymthe.2025.01.007

14. Angeli F et al., “COVID-19, vaccines and deficiency of ACE2 and other angiotensinases. Closing the loop on the ‘Spike effect’,” *Eur J. Intern. Med.* 2022, 103: 23–28. doi: 10.1016/j.ejim.2022.06.015

15. Angeli F et al., “The spike effect of acute respiratory syndrome coronavirus 2 and coronavirus disease 2019 vaccines on blood pressure,” *Eur. J. Intern. Med.* 2022, 109: 12-21. doi: 10.1016/j.ejim.2022.12.004

16. Ao Z et al., “SARS-CoV-2 Delta spike protein enhances the viral fusogenicity and inflammatory cytokine production,” *iScience* 2022, 25, 8: 104759. doi: 10.1016/j.isci.2022.104759

17. Appelbaum K et al., “SARS-CoV-2 spike-dependent platelet activation in COVID-19 vaccine-induced thrombocytopenia,” *Blood Adv.* 2022, 6: 2250–2253. doi: 10.1182/bloodadvances.2021005050

18. Arjsri P et al., “Hesperetin from root extract of *Clerodendrum petasites* S. Moore inhibits SARS-CoV-2 spike protein S1 subunit-induced Nlrp3 inflammasome in A549 lung cells via modulation of the Akt/Mapk/Ap-1 pathway,” *Int. J. Mol. Sci.* 2022, 23, 18: 10346. doi: https://doi.org/10.3390/ijms231810346

19. Asandei A et al., “Non-Receptor-Mediated Lipid Membrane Permeabilization by the SARS-CoV-2 Spike Protein S1 Subunit,” *ACS Appl. Mater. Interfaces* 2020, 12, 50: 55649–55658. doi: https://doi.org/10.1021/acsami.0c17044

20. Avolio E et al., “The SARS-CoV-2 Spike Protein Disrupts Human Cardiac Pericytes Function through CD147 Receptor-Mediated Signalling: A Potential Non-infective Mechanism of COVID-19 Microvascular Disease,” *Clin. Sci.* 2021, 135, 24: 2667–2689. doi: https://doi.org/10.1042/CS20210735

21. Azzarone B et al., “Soluble SARS-CoV-2 Spike glycoprotein: considering some potential pathogenic effects,” *Front. Immunol.* 2025, 16 (Sec. Cytokines and Soluble Mediators in Immunity). doi: 10.3389/fimmu.2025.1616106

22. Baldari CT et al., “Emerging Roles of SARS-CoV-2 Spike-ACE2 in Immune Evasion and Pathogenesis,” *Trends Immunol.* 2023, 44, 6. doi: 10.1016/j.it.2023.04.001

23. Balzanelli MG et al., “The Role of SARS-CoV-2 Spike Protein in Long-term Damage of Tissues and Organs, the Underestimated Role of Retrotransposons and Stem Cells, a Working Hypothesis,” *Endocr Metab Immune Disord Drug Targets* 2025, 25, 2: 85-98. doi: 10.2174/0118715303283480240227113401

24. Bansal S et al., “Cutting Edge: Circulating Exosomes with COVID Spike Protein Are Induced by BNT162b2 (Pfizer-BioNTech) Vaccination prior to Development of Antibodies: A Novel Mechanism for Immune Activation by mRNA Vaccines,” *J. Immunol.* 2021, 207, 10: 2405–2410. doi: 10.4049/jimmunol.2100637

25. Barhoumi T et al., “SARS-CoV-2 coronavirus Spike protein-induced apoptosis, inflammatory, and oxidative stress responses in THP-1-like-macrophages: potential role of angiotensin-converting enzyme inhibitor (perindopril),” *Front Immunol.* 2021, 12: 728896. doi: https://doi.org/10.3389/fimmu.2021.728896

26. Barreda D et al., “SARS-CoV-2 Spike Protein and Its Receptor Binding Domain Promote a Proinflammatory Activation Profile on Human Dendritic Cells,” *Cells* 2021, 10, 12: 3279. doi: https://doi.org/10.3390/cells10123279

27. Baumeier C et al., “Intramyocardial Inflammation after COVID-19 Vaccination: An Endomyocardial Biopsy-Proven Case Series,” *Int. J. Mol. Sci*. 2022, 23: 6940. doi: https://doi.org/10.3390/ijms23136940

28. Becker RC et al., “The COVID-19 thrombus: distinguishing pathological, mechanistic, and phenotypic features and management,” *J. Thromb. Thrombolysis* 2025, 58: 15-49. doi: https://doi.org/10.1007/s11239-024-03028-4

29. Bellavite P et al., “Immune response and molecular mechanisms of cardiovascular adverse effects of spike proteins from SARS-coV-2 and mRNA vaccines,” *Biomedicines* 2023, 11, 2: 451. doi: 10.3390/biomedicines11020451

30. Bellucci M et al., “Post-SARS-CoV-2 infection and post-vaccine-related neurological complications share clinical features and the same positivity to anti-ACE2 antibodies,” *Front. Immunol.* 2024, 15 (Sec. Multiple Sclerosis and Neuroimmunology). doi: https://doi.org/10.3389/fimmu.2024.1398028

31. Bhargavan B and GD Kanmogne, “SARS-CoV-2 spike proteins and cell–cell communication inhibits TFPI and induces thrombogenic factors in human lung microvascular endothelial cells and neutrophils: implications for COVID-19 coagulopathy pathogenesis,” *Int. J. Mol. Sci.* 2022, 23, 18: 10436. doi: https://doi.org/10.3390/ijms231810436

32. Bhattacharyya S and JK Tobacman, “SARS-CoV-2 spike protein-ACE2 interaction increases carbohydrate sulfotransferases and reduces N-acetylgalactosamine-4-sulfatase by p38 MAPK,” *Signal Transduct Target Ther* 2024, 9, 39. doi: https://doi.org/10.1038/s41392-024-01741-3

33. Biancatelli RMLC et al., “The SARS-CoV-2 spike protein subunit S1 induces COVID-19-like acute lung injury in Kappa18-hACE2 transgenic mice and barrier dysfunction in human endothelial cells,” *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2021, 321, L477–L484. doi: https://doi.org/10.1152/ajplung.00223.2021

34. Biering SB et al., “SARS-CoV-2 Spike Triggers Barrier Dysfunction and Vascular Leak via Integrins and TGF-β Signaling,” *Nat. Commun.* 2022, 13: 7630. doi: https://doi.org/10.1038/s41467-022-34910-5

35. Bocquet-Garcon A, “Impact of the SARS-CoV-2 Spike Protein on the Innate Immune System: A Review,” *Cureus* 2024, 16, 3: e57008. doi: 10.7759/cureus.57008

36. Boretti A, “PQQ Supplementation and SARS-CoV-2 Spike Protein-Induced Heart Inflammation,” *Nat. Prod. Commun.* 2022, 17, 1934578x221080929. doi: https://doi.org/10.1177/1934578X221080929

37. Boros LG et al., “Long-lasting, biochemically modified mRNA, and its frameshifted recombinant spike proteins in human tissues and circulation after COVID-19 vaccination,” *Pharmacol Res Perspect* 2024, 12, 3: e1218. doi: https://doi.org/10.1002/prp2.1218

38. Bortolotti D et al., “SARS-CoV-2 Spike 1 Protein Controls Natural Killer Cell Activation via the HLA-E/NKG2A Pathway,” *Cells* 2020, 9, 9: 1975. doi: https://doi.org/10.3390/cells9091975

39. Boschi C et al., “SARS-CoV-2 Spike Protein Induces Hemagglutination: Implications for COVID-19 Morbidities and Therapeutics and for Vaccine Adverse Effects,” *Int. J. Biol. Macromol.* 2022, 23, 24: 15480. doi: 10.3390/ijms232415480

40. Brady M et al., “Spike protein multiorgan tropism suppressed by antibodies targeting SARS-CoV-2,” *Comm. Biol.* 2021, 4, 1318. doi: 10.1038/s42003-021-02856-x

41. Braga L et al., “Drugs that inhibit TMEM16 proteins block SARS-CoV-2 spike-induced syncytia,” *Nature* 2021, 594: 88–93. doi: 10.1038/s41586-021-03491-6

42. Buoninfante A et al., “Myocarditis associated with COVID-19 vaccination,” *npj Vaccines* 2024, 122. doi: https://doi.org/10.1038/s41541-024-00893-1

43. Burkhardt A, “Pathology Conference: Vaccine-Induced Spike Protein Production in the Brain, Organs etc., now Proven,” Report24.news. 2022, https://report24.news/pathologie-konferenz-impfinduzierte-spike-produktion-in-gehirn-u-a-organen-nun-erwiesen/

44. Burnett FN et al., “SARS-CoV-2 Spike Protein Intensifies Cerebrovascular Complications in Diabetic hACE2 Mice through RAAS and TLR Signaling Activation,” *Int. J. Mol. Sci.* 2023, 24, 22: 16394. doi: 10.3390/ijms242216394

45. Buzhdygan TP et al., “The SARS-CoV-2 Spike Protein Alters Barrier Function in 2D Static and 3D Microfluidic in-Vitro Models of the Human Blood-Brain Barrier,” *Neurobiol. Dis.* 2020, 146, 105131. doi: 10.1016/j.nbd.2020.105131

46. Bye AP et al., “Aberrant glycosylation of anti-SARS-CoV-2 spike IgG is a prothrombotic stimulus for platelets,” *Blood* 2021, 138, 6: 1481–9. doi: https://doi.org/10.1182/blood.2021011871

47. Cao JB et al., “Mast cell degranulation-triggered by SARS-CoV-2 induces tracheal-bronchial epithelial inflammation and injury,” *Virol. Sin.* 2024, 39, 2: 309-318. doi: https://doi.org/10.1016/j.virs.2024.03.001

48. Cao S et al., “Spike Protein Fragments Promote Alzheimer’s Amyloidogenesis,” *ACS Appl. Mater. Interfaces* 2023, 15, 34: 40317-40329. doi: 10.1021/acsami.3c09815

49. Cao X et al., “Spike protein of SARS-CoV-2 activates macrophages and contributes to induction of acute lung inflammation in male mice,” *FASEB J.* 2021, 35, e21801. doi: https://doi.org/10.1096/fj.202002742RR

50. Cao X et al., “The SARS-CoV-2 spike protein induces long-term transcriptional perturbations of mitochondrial metabolic genes, causes cardiac fibrosis, and reduces myocardial contractile in obese mice,” *Mol. Metab.* 2023, 74, 101756. doi: https://doi.org/10.1016/j.molmet.2023.101756

51. Caohuy H et al., “Inflammation in the COVID-19 airway is due to inhibition of CFTR signaling by the SARS-CoV-2 spike protein,” *Sci. Rep.* 2024, 14: 16895. doi: https://doi.org/10.1038/s41598-024-66473-4

52. Cappalletti G et al., “iPSC-derived human cortical organoids display profound alterations of cellular homeostasis following SARS-CoV-2 infection and Spike protein exposure,” *FASEB J* 2025 39, 4: e70396. doi: 10.1096/fj.202401604RRR

53. Cari L et al., “Differences in the expression levels of SARS-CoV-2 spike protein in cells treated with mRNA-based COVID-19 vaccines: a study on vaccines from the real world,” *Vaccines* 2023, 11, 4: 879. doi: 10.3390/vaccines11040879

54. Carnevale R et al., “Toll-Like Receptor 4-Dependent Platelet-Related Thrombosis in SARS-CoV-2 Infection,” *Circ. Res.* 2023, 132, 3: 290– 305, doi: https://doi.org/10.1161/CIRCRESAHA.122.321541

55. Castro-Robles B et al., “Distinct response patterns of endothelial markers to the BNT162b2 mRNA COVID-19 booster vaccine are associated with the spike-

specific IgG antibody production," *Front. Immunol.* 2025, 15 (Sec. Vaccines and Molecular Therapeutics). doi: https://doi.org/10.3389/fimmu.2024.1471401

56. Cattin-Ortolá J et al., "Sequences in the cytoplasmic tail of SARS-CoV-2 Spike facilitate expression at the cell surface and syncytia formation," *Nat Commun* 2021, 12, 1: 5333. doi: https://doi.org/10.1038/s41467-021-25589-1

57. Chakrabarti SS et al., "Rapidly Progressive Dementia with Asymmetric Rigidity Following ChAdOx1 nCoV-19 Vaccination," *Aging Dis.* 2022, 13, 3: 633-636. doi: 10.14336/AD.2021.1102

58. Chang A et al., "Recovery from antibody-mediated biliary ductopenia and multiorgan inflammation after COVID-19 vaccination," *NPJ Vaccines* 2024, 9, 75. doi: https://doi.org/10.1038/s41541-024-00861-9

59. Chang MH et al., "SARS-CoV-2 Spike Protein 1 Causes Aggregation of α-Synuclein via Microglia-Induced Inflammation and Production of Mitochondrial ROS: Potential Therapeutic Applications of Metformin," *Biomedicines* 2024, 12, 6: 1223. doi: https://doi.org/10.3390/biomedicines12061223

60. Chaves JCS et al., "Differential Cytokine Responses of *APOE3* and *APOE4* Blood–brain Barrier Cell Types to SARS-CoV-2 Spike Proteins," *J. Neuroimmune Pharmacol.* 2024, 19, 22. doi: https://doi.org/10.1007/s11481-024-10127-9

61. Chen Y et al., "New-onset autoimmune phenomena post-COVID-19 vaccination," *Immunology* 2022, 165, 4: 386-401. doi: https://doi.org/10.1111/imm.13443

62. Cheng MH et al., "Superantigenic character of an insert unique to SARS-CoV-2 spike supported by skewed TCR repertoire in patients with hyperinflammation," *Proc Natl Acad Sci* 2020, 117: 25254–25262. doi: 10.1073/pnas.201072211

63. Cheng MY et al., "Clinical Research into Central Nervous System Inflammatory Demyelinating Diseases Related to COVID-19 Vaccines," *Diseases* 2024, 12, 3: 60. doi: https://doi.org/10.3390/diseases12030060

64. Cheung CCL et al., "Residual SARS-CoV-2 viral antigens detected in GI and hepatic tissues from five recovered patients with COVID-19," *Gut* 2022, 71, 1: 226–9. doi: https://doi.org/10.1136/gutjnl-2021-324280

65. Chiok K et al., "Proinflammatory Responses in SARS-CoV-2 and Soluble Spike Glycoprotein S1 Subunit Activated Human Macrophages," *Viruses* 2023, 15, 3: 754. doi: https://doi.org/10.3390/v15030754

66. Chrestia JF et al., “A Functional Interaction Between Y674-R685 Region of the SARS-CoV-2 Spike Protein and the Human α7 Nicotinic Receptor,” *Mol. Neurobiol.* 2022, 59: 676-690. doi: https://doi.org/10.1007/s12035-022-02947-8

67. Chittasupho C et al., “Inhibition of SARS-CoV-2-Induced NLRP3 Inflammasome-Mediated Lung Cell Inflammation by Triphala-Loaded Nanoparticle Targeting Spike Glycoprotein S1,” *Pharmaceutics* 2024, 16, 6: 751. doi: https://doi.org/10.3390/pharmaceutics16060751

68. Chittasupho C et al., “Targeting spike glycoprotein S1 mediated by NLRP3 inflammasome machinery and the cytokine releases in A549 lung epithelial cells by nanocurcumin,” *Pharmaceuticals* (Basel) 2023, 16, 6: 862. doi: https://doi.org/10.3390/ph16060862

69. Choi JY et al., “SARS-CoV-2 spike S1 subunit protein-mediated increase of beta-secretase 1 (BACE1) impairs human brain vessel cells,” *Biochem. Biophys. Res. Commun.* 2022, 625, 20: 66-71. doi: https://doi.org/10.1016/j.bbrc.2022.07.113

70. Clemens DJ et al., “SARS-CoV-2 spike protein-mediated cardiomyocyte fusion may contribute to increased arrhythmic risk in COVID-19,” *PLoS One* 2023, 18, 3: e0282151. doi: https://doi.org/10.1371/journal.pone.0282151

71. Clough E et al., “Mitochondrial Dynamics in SARS-COV2 Spike Protein Treated Human Microglia: Implications for Neuro-COVID,” *Journal of Neuroimmune Pharmacology* 2021, 16, 4: 770–784. doi: 10.1007/s11481-021-10015-6

72. Codoni G et al., “Histological and serological features of acute liver injury after SARS-CoV-2 vaccination,” *JHP Rep.* 2023, 5, 1: 100605. doi: https://doi.org/10.1016/j.jhepr.2022.100605

73. Colmenero I et al., “SARS-CoV-2 endothelial infection causes COVID-19 chilblains: histopathological, immunohistochemical and ultrastructural study of seven paediatric cases,” *Br J Dermatol.* 2020, 183: 729-737. doi: 10.1111/bjd.19327/

74. Coly M, et al., “Subacute monomelic radiculoplexus neuropathy following Comirnaty(c) (Pfizer-BioNTech COVID-19) vaccination: A case report,” *Revue Neurologique* 2023, 179, 6: 636-639. doi: 10.1016/j.neurol.2023.02.063

75. Corpetti C et al., “Cannabidiol inhibits SARS-Cov-2 spike (S) protein-induced cytotoxicity and inflammation through a PPARγ-dependent TLR4/NLRP3/Caspase-1 signaling suppression in Caco-2 cell line,” *Phytother. Res.* 2021, 35, 12: 6893–6903. doi: https://doi.org/10.1002/ptr.7302

76. Correa Y et al., “SARS-CoV-2 spike protein removes lipids from model membranes and interferes with the capacity of high-density lipoprotein to exchange lipids,” *J. Colloid Interface Sci.* 2021, 602: 732-739. doi: https://doi.org/10.1016/j.jcis.2021.06.056

77. Cory TJ et al., “Metformin Suppresses Monocyte Immunometabolic Activation by SARS-CoV-2 Spike Protein Subunit 1,” *Front. Immunol.* 2021, 12 (Sec. Cytokines and Soluble Mediators in Immunity): 733921. doi: 10.3389/fimmu.2021.733921

78. Cosentino M and Franca Marino, “Understanding the Pharmacology of COVID- 19 mRNA Vaccines: Playing Dice with the Spike?” *Int. J. Mol. Sci.* 2022, 23, 18: 10881. doi: https://doi.org/10.3390/ijms231810881

79. Cosenza LC et al., “Inhibitory effects of SARS-CoV-2 spike protein and BNT162b2 vaccine on erythropoietin-induced globin gene expression in erythroid precursor cells from patients with β-thalassemia,” *Exp. Hematol.* 2024, 129, 104128. doi: https://doi.org/10.1016/j.exphem.2023.11.002

80. Craddock V et al., “Persistent circulation of soluble and extracellular vesicle-linked Spike protein in individuals with postacute sequelae of COVID-19,” *J Med. Virol.* 2023, 95, 2: e28568. doi: https://doi.org/10.1002/jmv.28568

81. Dadonite B et al., “SARS-CoV-2 neutralizing antibody specificities differ dramatically between recently infected infants and immune-imprinted individuals,” *J. Virol.* 2025, 99, 4. doi: https://doi.org/10.1128/jvi.00109-25

82. Das T et al., “N-glycosylation of the SARS-CoV-2 spike protein at Asn331 and Asn343 is involved in spike-ACE2 binding, virus entry, and regulation of IL-6,” *Microbiol. Immunol.* 2024, 68, 5: 165-178. doi: 10.1111/1348-0421.13121

83. De Melo BP et al., “SARS-CoV-2 Spike Protein and Long COVID—Part 1: Impact of Spike Protein in Pathophysiological Mechanisms of Long COVID Syndrome,” *Viruses* 2025, 17, 5: 617. doi: https://doi.org/10.3390/v17050617

84. De Michele M et al., “Evidence of SARS-CoV-2 Spike Protein on Retrieved Thrombi from COVID-19 Patients,” *Journal of Hematology Oncology* 2022, 15, 108. doi: https://doi.org/10.1186/s13045-022-01329-w

85. De Michele M et al., “Vaccine-induced immune thrombotic thrombocytopenia: a possible pathogenic role of ChAdOx1 nCoV-19 vaccine-encoded soluble SARS-CoV-2 spike protein,” *Haematologica* 2022, 107, 7: 1687–92. doi: https://doi.org/10.3324/haematol.2021.280180

86. De Sousa PMB et al., “Fatal Myocarditis following COVID-19 mRNA Immunization: A Case Report and Differential Diagnosis Review,” *Vaccines* 2024, 12, 2: 194. doi: https://doi.org/10.3390/vaccines12020194

87. Del Re A et al., “Intranasal delivery of PEA-producing *Lactobacillus paracasei F19* alleviates SARS-CoV-2 spike protein-induced lung injury in mice,” *Transl. Med. Commun.* 2024, 9, 9. doi: https://doi.org/10.1186/s41231-024-00167-x

88. Del Re A et al., “Ultramicronized Palmitoylethanolamide Inhibits NLRP3 Inflammasome Expression and Pro-Inflammatory Response Activated by SARS-CoV-2 Spike Protein in Cultured Murine Alveolar Macrophages,” *Metabolites* 2021, 11, 9: 592. doi: 10.3390/metabo11090592

89. Delgado JF et al., “SARS-CoV-2 spike protein vaccine-induced immune imprinting reduces nucleocapsid protein antibody response in SARS-CoV-2 infection,” *J. Immunol. Res.* 2022: 8287087. doi: https://doi.org/10.1155/2022/8287087

90. DeOre BJ et al., “SARS-CoV-2 Spike Protein Disrupts Blood-Brain Barrier Integrity via RhoA Activation,” *J Neuroimmune Pharmacol.* 2021, 16, 4: 722-728. doi: https://doi.org/10.1007/s11481-021-10029-0

91. Devaux CA and L. Camoin-Jau, “Molecular mimicry of the viral spike in the SARS-CoV-2 vaccine possibly triggers transient dysregulation of ACE2, leading to vascular and coagulation dysfunction similar to SARS-CoV-2 infection,” *Viruses* 2023, 15, 5: 1045. doi: https://doi.org/10.3390/v15051045

92. DeVries A et al., “SARS-CoV-2 Spike Protein is Sufficient to Induce Enhanced Pro-inflammatory Transcriptional Responses in Nasal Epithelial Cells from Atopic Asthmatics,” *J Allergy Clin Immunol* 2025, 155, 2: AB85. doi: 10.1016/j.jaci.2024.12.271

93. Dhandapani S et al., “Lipid-encapsulated gold nanoparticles: an advanced strategy for attenuating the inflammatory response in SARS-CoV-2 infection,” *J. Nanobiotechnology* 2025, 23, 15. doi: 10.1186/s12951-024-03064-5

94. Diaz M et al., “SARS-CoV-2 spike peptide analysis reveals a highly conserved region that elicits potentially pathogenic autoantibodies: implications to pan-coronavirus vaccine development,” *Front. Immunol.* 2025, 16 (Sec. B Cell Biology). doi: https://doi.org/10.3389/fimmu.2025.1488388

95. Dissook S et al., “Luteolin-rich fraction from Perilla frutescens seed meal inhibits spike glycoprotein S1 of SARS-CoV-2-induced NLRP3 inflammasome lung cell inflammation via regulation of JAK1/STAT3 pathway: A potential anti-inflammatory

compound against inflammation-induced long-COVID," *Front. Med.* 2023, 9: 1072056. doi: https://doi.org/10.3389/fmed.2022.1072056

96. Du Preez HN et al., "COVID-19 vaccine adverse events: Evaluating the pathophysiology with an emphasis on sulfur metabolism and endotheliopathy," *Eur J Clin Invest.* 2024, 54, 10: e14296. doi: https://doi.org/10.1111/eci.14296

97. Duarte C, "Age-dependent effects of the recombinant spike protein/SARS-CoV-2 on the M-CSF- and IL-34-differentiated macrophages in vitro," *Biochem. Biophys. Res. Commun.* 2021, 546: 97–102. doi: 10.1016/j.bbrc.2021.01.104

98. Elrashdy F et al., "Autoimmunity roots of the thrombotic events after COVID-19 vaccination," *Autoimmun. Rev.* 2021, 20, 11: 102941. doi: 10.1016/j.autrev.2021.102941

99. Erdogan MA, "Prenatal SARS-CoV-2 Spike Protein Exposure Induces Autism-Like Neurobehavioral Changes in Male Neonatal Rats," *J Neuroimmune Pharmacol.* 2023, 18, 4 :573-591. doi: 10.1007/s11481-023-10089-4

100. Erickson MA et al., "Blood-brain barrier penetration of non-replicating SARS-CoV-2 and S1 variants of concern induce neuroinflammation which is accentuated in a mouse model of Alzheimer's disease," *Brain Behav Immun* 2023, 109: 251-268. doi: https://doi.org/10.1016/j.bbi.2023.01.010

101. Fajloun Z et al., "COVID-19 and Ehlers-Danlos Syndrome: The Dangers of the Spike Protein of SARS-CoV-2," *Infect. Disord. Drug Targets* 2023, 23, 3: 26-28. doi: https://doi.org/10.2174/1871526523666230104145108

102. Fajloun Z et al., "SARS-CoV-2 or Vaccinal Spike Protein can Induce Mast Cell Activation Syndrome (MCAS)," *Infect Disord Drug Targets,* 2025, 25, 1: e300424229561. doi: 10.2174/0118715265319896240427045026

103. Fajloun Z et al., "Unveiling the Role of SARS-CoV-2 or mRNA Vaccine Spike Protein in Macrophage Activation Syndrome (MAS)," *Infect. Disord. Drug Targets* 2025, 25, 2: E220724232138. doi: 10.2174/0118715265341206240722050403

104. Ferrer MD et al., "Nitrite Attenuates the In Vitro Inflammatory Response of Immune Cells to the SARS-CoV-2 S Protein without Interfering in the Antioxidant Enzyme Activation," *Int. J. Mol. Sci.* 2024, 25, 5: 3001. doi: 10.3390/ijms25053001

105. Fertig TE et al., "Beyond the injection site: identifying the cellular targets of mRNA vaccines," J *Cell Ident* 2024, 3, 1. doi: 10.47570/joci.2024.004

106. Fertig TE et al., “Vaccine mRNA Can Be Detected in Blood at 15 Days Post Vaccination,” *Biomedicines* 2022, 10, 7: 1538. doi: 10.3390/biomedicines10071538

107. Fontes-Dantas FL, “SARS-CoV-2 Spike Protein Induces TLR4-Mediated Long-Term Cognitive Dysfunction Recapitulating Post-COVID-19 Syndrome in Mice,” *Cell Reports* 2023, 42, 3: 112189. doi: 10.1016/j.celrep.2023.112189

108. Forsyth CB et al., “The SARS-CoV-2 S1 spike protein promotes MAPK and NF-kB activation in human lung cells and inflammatory cytokine production in human lung and intestinal epithelial cells,” *Microorganisms* 2022, 10, 10: 1996. doi: https://doi.org/10.3390/microorganisms10101996

109. Forte E, “Circulating spike protein may contribute to myocarditis after COVID-19 vaccination,” *Nat. Cardiovasc. Res.* 2023, 2: 100. doi: 10.1038/s44161-023-00222-0

110. Foster K et al., “Abstract 111: Cerebrovascular Effects Of Pre/post-losartan Treatment In Humanized ACE2 Knock-in Mice After SARS-CoV-2 Spike Protein Injection,” *Stroke* 2023, 54. doi: https://doi.org/10.1161/str.54.suppl_1.111

111. Frank MG et al., “Exploring the immunogenic properties of SARS-CoV-2 structural proteins: PAMP:TLR signaling in the mediation of the neuroinflammatory and neurologic sequelae of COVID-19,” *Brain Behav Immun* 2023, 111. doi: https://doi.org/10.1016/j.bbi.2023.04.009

112. Frank MG et al., “SARS-CoV-2 Spike S1 Subunit Induces Neuroinflammatory, Microglial and Behavioral Sickness Responses: Evidence of PAMP-Like Properties,” *Brain Behav. Immun.* 2022, 100: 267277. doi: 10.1016/j.bbi.2021.12.007

113. Frank MG et al., “SARS-CoV-2 S1 subunit produces a protracted priming of the neuroinflammatory, physiological, and behavioral responses to a remote immune challenge: A role for corticosteroids,” *Brain Behav. Immun.* 2024, 121: 87-103. doi: https://doi.org/10.1016/j.bbi.2024.07.034

114. Fraser ME at al., “SARS-CoV-2 Spike Protein and Viral RNA Persist in the Lung of Patients With Post-COVID Lung Disease (abstract),” *Am J Respir Crit Care Med* 2024, 209: A4193. doi: 10.1164/ajrccm-conference.2024.209.1_MeetingAbstracts.A4193

115. Freeborn J, “Misfolded Spike Protein Could Explain Complicated COVID-19 Symptoms,” Medical News Today, May 26, 2022,

https://www.medicalnewstoday.com/articles/misfolded-spike-protein-could-explain-complicated-covid-19-symptoms

116. Freitas RS et al., "SARS-CoV-2 Spike antagonizes innate antiviral immunity by targeting interferon regulatory factor 3," *Front Cell Infect Microbiol.* 2021, 11: 789462. doi: https://doi.org/10.3389/fcimb.2021.789462

117. Frühbeck G et al., "FNDC4 and FNDC5 reduce SARS-CoV-2 entry points and spike glycoprotein S1-induced pyroptosis, apoptosis, and necroptosis in human adipocytes," *Cell Mol Immunol.* 2021, 18, 10: 2457–9. doi: https://doi.org/10.1038/s41423-021-00762-0

118. Gamblicher T et al., "SARS-CoV-2 spike protein is present in both endothelial and eccrine cells of a chilblain-like skin lesion," *J Eur Acad Dermatol Venereol.* 2020, 1, 10: e187-e189. doi: https://doi.org/10.1111/jdv.16970

119. Gao X et al., "Spike-Mediated ACE2 Down-Regulation Was Involved in the Pathogenesis of SARS-CoV-2 Infection," *Journal of Infection* 2022, 85, 4: 418–427. doi: 10.1016/j.jinf.2022.06.030

120. Gasparello J et al., "Aged Garlic Extract (AGE) and Its Constituent S-Allyl-Cysteine (SAC) Inhibit the Expression of Pro-Inflammatory Genes Induced in Bronchial Epithelial IB3-1 Cells by Exposure to the SARS-CoV-2 Spike Protein and the BNT162b2 Vaccine," *Molecules* 2024, 29, 24: 5938. doi: 10.3390/molecules29245938

121. Gasparello J et al., "Assessing the interaction between hemoglobin and the receptor binding domain of SARS-CoV-2 spike protein through MARTINI coarse-grained molecular dynamics," *Int. J. Biol. Macromol.,* 2023, 253: 127088. doi: 10.1016/j.ijbiomac.2023.127088

122. Gasparello J et al., "In vitro induction of interleukin-8 by SARS-CoV-2 Spike protein is inhibited in bronchial epithelial IB3-1 cells by a miR-93-5p agomiR," *Int. Immunopharmacol.* 2021, 101: 108201. doi: 10.1016/j.intimp.2021.108201

123. Gasparello J et al., "Sulforaphane inhibits the expression of interleukin-6 and interleukin-8 induced in bronchial epithelial IB3-1 cells by exposure to the SARS-CoV-2 Spike protein," *Phytomedicine* 2021, 87: 153583. doi: https://doi.org/10.1016/j.phymed.2021.153583

124. Gawaz A et al., "SARS-CoV-2–Induced Vasculitic Skin Lesions Are Associated with Massive Spike Protein Depositions in Autophagosomes," *J Invest Dermatol.* 2024, 144, 2: 369-377.e4. doi: https://doi.org/10.1016/j.jid.2023.07.018

125. Ghazanfari D et al., “Mechanistic insights into SARS-CoV-2 spike protein induction of the chemokine CXCL10,” *Sci. Rep.* 2024, 14: 11179. doi: https://doi.org/10.1038/s41598-024-61906-6

126. Giannotta G et al., “COVID-19 mRNA Vaccines: The Molecular Basis of Some Adverse Events,” *Vaccines* 2023, 11, 4: 747. doi: 10.3390/vaccines11040747

127. Goh D et al., “Case report: Persistence of residual antigen and RNA of the SARS-CoV-2 virus in tissues of two patients with long COVID,” *Front. Immunol.* 2022, 13 (Sec. Viral Immunology). doi: https://doi.org/10.3389/fimmu.2022.939989

128. Golob-Schwarzl N et al., “SARS-CoV-2 spike protein functionally interacts with primary human conjunctival epithelial cells to induce a pro-inflammatory response,” *Eye* 2022, 36: 2353–5. doi: 10.1038/s41433-022-02066-7

129. Gracie NP et al., “Cellular signalling by SARS-CoV-2 spike protein,” *Microbiology Australia* 2024, 45, 1: 13-17. doi: https://doi.org/10.1071/MA24005

130. Greenberger JS et al., “SARS-CoV-2 Spike Protein Induces Oxidative Stress and Senescence in Mouse and Human Lung,” *In Vivo* 2024, 38, 4: 1546-1556; doi: https://doi.org/10.21873/invivo.13605

131. Grishma K and Das Sarma, “The Role of Coronavirus Spike Protein in Inducing Optic Neuritis in Mice: Parallels to the SARS-CoV-2 Virus,” *J Neuroophthalmol* 2024, 44, 3: 319-329. doi: 10.1097/WNO.0000000000002234

132. Grobbelaar LM et al., “SARS-CoV-2 Spike Protein S1 Induces Fibrin(ogen) Resistant to Fibrinolysis: Implications for Microclot Formation in COVID-19,” *Biosicence Reports* 2021, 41, 8: BSR20210611. doi: 10.1042/BSR20210611

133. Gu T et al., “Cytokine Signature Induced by SARS-CoV-2 Spike Protein in a Mouse Model,” *Front. Immunol.* 2021 (Sec. Inflammation). doi: https://doi.org/10.3389/fimmu.2020.621441

134. Gultom M et al., “Sustained Vascular Inflammatory Effects of SARS-CoV-2 Spike Protein on Human Endothelial Cells,” *Inflammation* 2024. doi: https://doi.org/10.1007/s10753-024-02208-x

135. Guo X et al., “Regulation of proinflammatory molecules and tissue factor by SARS-CoV-2 spike protein in human placental cells: implications for SARS-CoV-2 pathogenesis in pregnant women,” *Front Immunol* 2022, 13: 876555–876555. doi: https://doi.org/10.3389/fimmu.2022.876555

136. Guo Y and V Kanamarlapudi, “Molecular Analysis of SARS-CoV-2 Spike Protein-Induced Endothelial Cell Permeability and vWF Secretion,” *Int. J. Mol. Sci.* 2023, 24, 6: 5664. doi: https://doi.org/10.3390/ijms24065664

137. Gussow AB et al., “Genomic Determinants of Pathogenicity in SARS-CoV-2 and Other Human Coronaviruses,” *PNAS* 2020, 117, 26: 15193–15199. doi: https://doi.org/10.1073/pnas.2008176117

138. Halma MTJ et al., “Exploring autophagy in treating SARS-CoV-2 spike protein-related pathology,” *Endocrinol Metab (EnM)* 2024, 14: 100163. doi: https://doi.org/10.1016/j.endmts.2024.100163

139. Halma MTJ et al., “Strategies for the Management of Spike Protein-Related Pathology,” *Microorganisms* 2023, 11, 5: 1308, doi: 10.3390/microorganisms11051308

140. Hano S et al., “A case of persistent, confluent maculopapular erythema following a COVID-19 mRNA vaccination is possibly associated with the intralesional spike protein expressed by vascular endothelial cells and eccrine glands in the deep dermis,” *J Dermatol* 2023, 50, 9: 1208-1212. doi: 10.1111/1346-8138.16816

141. Hasan MZ et al., “SARS-CoV-2 infection induces adaptive NK cell responses by spike protein-mediated induction of HLA-E expression,” *Emerg Microbes Infect*. 2024, 13: 2361019. doi: https://doi.org/10.1080/22221751.2024.2361019

142. Heath SP et al., “SARS-CoV-2 Spike Protein Exacerbates Thromboembolic Cerebrovascular Complications in Humanized ACE2 Mouse Model,” *Transl Stroke Res.* 2024. doi: https://doi.org/10.1007/s12975-024-01301-5

143. Heil M, “Self-DNA driven inflammation in COVID-19 and after mRNA-based vaccination: lessons for non-COVID-19 pathologies,” *Front. Immunol.,* 2023, 14. doi: https://doi.org/10.3389/fimmu.2023.1259879

144. Hillard P et al., “Abstract WP400: SARS-CoV-2 Spike Protein Accelerates Alzheimer’s Disease-Related Dementia Through Increased Cerebrovascular Inflammation in hACE2 Mice,” *Stroke* 2025, 56. doi: 10.1161/str.56.suppl_1.WP400

145. Huang X et al., “Sars-Cov-2 Spike Protein-Induced Damage of hiPSC-Derived Cardiomyocytes,” *Adv. Biol.* 2022, 6, 7: e2101327. doi: 10.1002/adbi.202101327

146. Hulscher N et al., “Autopsy findings in cases of fatal COVID-19 vaccine-induced myocarditis,” *ESC Heart Failure* 2024. doi: https://doi.org/10.1002/ehf2.14680

147. Huynh TV et al., “Spike Protein Impairs Mitochondrial Function in Human Cardiomyocytes: Mechanisms Underlying Cardiac Injury in COVID-19,” *Cells* 2023, 12, 877. doi: https://doi.org/10.3390/cells12060877

148. Huynh TV et al., “Spike Protein of SARS-CoV-2 Activates Cardiac Fibrogenesis through NLRP3 Inflammasomes and NF-κB Signaling,” *Cells* 2024, 13, 16: 1331: doi: https://doi.org/10.3390/cells13161331

149. Iba T and JH Levy, “The roles of platelets in COVID-19-associated coagulopathy and vaccine-induced immune thrombotic thrombocytopenia,” *Trends Cardiovasc Med*. 2022, 32, 1: 1-9. doi: https://doi.org/10.1016/j.tcm.2021.08.012

150. Idrees D and Vijay Kumar, “SARS-CoV-2 Spike Protein Interactions with Amyloidogenic Proteins: Potential Clues to Neurodegeneration,” *Biochem Biophys Res Commun*. 2021, 554 : 94–98, doi: 10.1016/j.bbrc.2021.03.100

151. Imig JD, “SARS-CoV-2 spike protein causes cardiovascular disease independent of viral infection,” *Clin Sci (Lond)* 2022, 136, 6: 431–434. doi: 10.1042/CS20220028

152. Irrgang P et al., “Class switch toward noninflammatory, spike-specific IgG4 antibodies after repeated SARS-CoV-2 mRNA vaccination,” *Sci. Immunol.* 2022, 8, 79. doi: 10.1126/sciimmunol.ade2798

153. Jabi MSA et al., “Abstract 53: Covid-19 Spike-protein Causes Cerebrovascular Rarefaction And Deteriorates Cognitive Functions In A Mouse Model Of Humanized ACE2,” *Stroke* 2022, 53. doi: 10.1161/str.53.suppl_1.53

154. Jana S et al., “Cell-free hemoglobin does not attenuate the effects of SARS-CoV-2 spike protein S1 subunit in pulmonary endothelial cells,” *Int. J. Mol. Sci.* 2021, 22, 16: 9041. doi: https://doi.org/10.3390/ijms22169041

155. Jiang Q et al., “SARS-CoV-2 spike S1 protein induces microglial NLRP3-dependent neuroinflammation and cognitive impairment in mice,” *Exp. Neurol.* 2025, 383: 115020. doi: https://doi.org/10.1016/j.expneurol.2024.115020

156. Johnson EL et al., “The S1 spike protein of SARS-CoV-2 upregulates the ERK/MAPK signaling pathway in DC-SIGN-expressing THP-1 cells,” *Cell Stress Chaperones* 2024, 29, 2: 227-234. doi: 10.1016/j.cstres.2024.03.002

157. Jugler C et al, “SARS-CoV-2 Spike Protein-Induced Interleukin 6 Signaling Is Blocked by a Plant-Produced Anti-Interleukin 6 Receptor Monoclonal Antibody,” *Vaccines* 2021, 9, 11: 1365. https://doi.org/10.3390/vaccines9111365

158. Kammala AK et al., “In vitro mRNA-S maternal vaccination induced altered immune regulation at the maternal-fetal interface,” *Am. J. Reprod. Immunol.* 2024, 91, 5: e13861. doi: https://doi.org/10.1111/aji.13861

159. Kanduc D, “From Anti-SARS-CoV-2 Immune Responses to COVID-19 via Molecular Mimicry,” *Antibodies* 2020, 9, 3: 33. doi: 10.3390/antib9030033

160. Kanduc D and Y Shoenfeld, “Molecular mimicry between SARS-CoV-2 spike glycoprotein and mammalian proteomes: implications for the vaccine,” *Immunol Res* 2020, 68: 310-313. doi: https://doi.org/10.1007/s12026-020-09152-6

161. Karrow NA et al., “Maternal COVID-19 Vaccination and Its Potential Impact on Fetal and Neonatal Development,” *Vaccines* 2021, 9: 1351. doi: https://doi.org/10.3390/vaccines9111351

162. Karwaciak I et al., “Nucleocapsid and Spike Proteins of the Coronavirus Sars-Cov-2 Induce Il6 in Monocytes and Macrophages—Potential Implications for Cytokine Storm Syndrome,” *Vaccines* 2021, 9(1), 54: 1–10. doi: https://doi.org/10.3390/vaccines9010054

163. Kato Y et al., “TRPC3-Nox2 Protein Complex Formation Increases the Risk of SARS-CoV-2 Spike Protein-Induced Cardiomyocyte Dysfunction through ACE2 Upregulation,” *Int. J. Mol. Sci.* 2023, 24, 1: 102. doi: https://doi.org/10.3390/ijms24010102

164. Kawano H et al., “Fulminant Myocarditis 24 Days after Coronavirus Disease Messenger Ribonucleic Acid Vaccination,” *Intern. Med.* 2022, 61, 15: 2319-2325. doi: https://doi.org/10.2169/internalmedicine.9800-22

165. Kawata D et al., “Diverse pro-inflammatory ability of mutated spike protein derived from variant strains of SARS-CoV-2,” *Cytokine* 2024, 178: 156592. doi: https://doi.org/10.1016/j.cyto.2024.156592

166. Kempuraj D et al., “Long COVID elevated MMP-9 and release from microglia by SARS-CoV-2 Spike protein,” *Transl. Neurosci.* 2024, 15: 20220352. doi: https://doi.org/10.1515/tnsci-2022-0352

167. Ken W et al., “Low dose radiation therapy attenuates ACE2 depression and inflammatory cytokines induction by COVID-19 viral spike protein in human bronchial epithelial cells,” *Int J Radiat Biol.* 2022, 98, 10: 1532-1541. doi: https://doi.org/10.1080/09553002.2022.2055806

168. Kent SJ et al., “Blood Distribution of SARS-CoV-2 Lipid Nanoparticle mRNA Vaccine in Humans,” *ACS Nano* 2024, 18, 39: 27077-27089. doi: https://doi.org/10.1021/acsnano.4c11652

169. Khaddaj-Mallat R et al., “SARS-CoV-2 deregulates the vascular and immune functions of brain pericytes via Spike protein,” *Neurobiol. Dis.* 2021, 161, 105561. doi: https://doi.org/10.1016/j.nbd.2021.105561

170. Khan S et al., “SARS-CoV-2 Spike Protein Induces Inflammation via TLR2-Dependent Activation of the NF-κB Pathway,” *eLife* 2021, 10: e68563. doi: https://doi.org/10.7554/elife.68563

171. Kim ES et al., “Spike Proteins of SARS-CoV-2 Induce Pathological Changes in Molecular Delivery and Metabolic Function in the Brain Endothelial Cells,” *Viruses,* 2021, 13, 10. doi: https://doi.org/10.3390/v13102021

172. Kim MJ et al., “The SARS-CoV-2 spike protein induces lung cancer migration and invasion in a TLR2-dependent manner,” *Cancer Commun* (London), 2023, 44, 2: 273–277. doi: https://doi.org/10.1002/cac2.12485

173. Kim SY et al., “Characterization of heparin and severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) spike glycoprotein binding interactions,” *Antivir Res.* 2020, 181: 104873. doi: https://doi.org/10.1016/j.antiviral.2020.104873

174. Kircheis R, “Coagulopathies after Vaccination against SARS-CoV-2 May Be Derived from a Combined Effect of SARS-CoV-2 Spike Protein and Adenovirus Vector-Triggered Signaling Pathways,” *Int. J. Mol. Sci.* 2021, 22, 19: 10791. https://doi.org/10.3390/ijms221910791

175. Kircheis R and O Planz, “Could a Lower Toll-like Receptor (TLR) and NF-κB Activation Due to a Changed Charge Distribution in the Spike Protein Be the Reason for the Lower Pathogenicity of Omicron?” *Int. J. Mol. Sci.* 2022, 23, 11: 5966. doi: https://doi.org/10.3390/ijms23115966

176. Ko CJ et al., “Discordant anti-SARS-CoV-2 spike protein and RNA staining in cutaneous perniotic lesions suggests endothelial deposition of cleaved spike protein,” *J. Cutan Pathol* 2021, 48, 1: 47–52. doi: 10.1111/cup.13866

177. Kowarz E et al., “Vaccine-induced COVID-19 mimicry syndrome,” *eLife* 2022, 11: e74974. doi: https://doi.org/10.7554/eLife.74974

178. Krauson AM et al., “Duration of SARS-CoV-2 mRNA vaccine persistence and factors associated with cardiac involvement in recently vaccinated patients,” *npj Vaccines,* 8, 141. doi: https://doi.org/10.1038/s41541-023-00742-7

179. Kucia M et al., “An evidence that SARS-Cov-2/COVID-19 spike protein (SP) damages hematopoietic stem/progenitor cells in the mechanism of pyroptosis in Nlrp3 inflammasome-dependent manner,” *Leukemia* 2021, 35: 3026-3029. doi: https://doi.org/10.1038/s41375-021-01332-z

180. Kuhn CC et al., “Direct Cryo-ET observation of platelet deformation induced by SARS-CoV-2 spike protein,” *Nat. Commun.* (2023) 14, 620. doi: https://doi.org/10.1038/s41467-023-36279-5

181. Kulkoviene G et al., “Differential Mitochondrial, Oxidative Stress and Inflammatory Responses to SARS-CoV-2 Spike Protein Receptor Binding Domain in Human Lung Microvascular, Coronary Artery Endothelial and Bronchial Epithelial Cells,” *Int. J. Mol. Sci.* 2024, 25, 6: 3188. doi: 10.3390/ijms25063188

182. Kumar N et al., “SARS-CoV-2 spike protein S1-mediated endothelial injury and pro-inflammatory state Is amplified by dihydrotestosterone and prevented by mineralocorticoid antagonism,” *Viruses* 2021, 13, 11: 2209. doi: https://doi.org/10.3390/v13112209

183. Kyriakopoulos AM et al., “Mitogen Activated Protein Kinase (MAPK) Activation, p53, and Autophagy Inhibition Characterize the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Spike Protein Induced Neurotoxicity,” *Cureus* 2022, 14, 12: e32361. doi: 10.7759/cureus.32361

184. Lamprinou M et al., “COVID-19 vaccines adverse events: potential molecular mechanisms,” *Immunol. Res.* 2023, 71: 356-372. doi: https://doi.org/10.1007/s12026-023-09357-5

185. Lazebnik Y, “Cell fusion as a link between the SARS-CoV-2 spike protein, COVID-19 complications, and vaccine side effects,” *Oncotarget* 2021, 12, 25: 2476-2488. doi: https://doi.org/10.18632/oncotarget.28088

186. Lee AR et al., “SARS-CoV-2 spike protein promotes inflammatory cytokine activation and aggravates rheumatoid arthritis,” *Cell Commun Signal.* 2023, 21, 1: 44. doi: https://doi.org/10.1186/s12964-023-01044-0

187. Lehmann KJ, “Impact of SARS-CoV-2 Spikes on Safety of Spike-Based COVID-19 Vaccinations,” *Immunome Res.* 2024, 20, 2: 1000267. doi: 10.35248/1745-7580.24.20.267

188. Lehmann KJ, "SARS-CoV-2-Spike Interactions with the Renin-Angiotensin-Aldosterone System – Consequences of Adverse Reactions of Vaccination," *J Biol Today's World* 2023, 12/4: 001-013. doi: https://doi.org/10.31219/osf.io/27g5h

189. Lehmann KJ, "Suspected Causes of the Specific Intolerance Profile of Spike-Based Covid-19 Vaccines," *Med. Res. Arch* 2024, 12, 9. doi: 10.18103/mra.v12i9.5704

190. Lei Y et al., "SARS-CoV-2 Spike Protein Impairs Endothelial Function via Downregulation of ACE 2," *Circ. Res.* 2021, 128, 9: 1323–1326. doi: https://doi.org/10.1161/CIRCRESAHA.121.318902

191. Lesgard JF et al., "Toxicity of SARS-CoV-2 Spike Protein from the Virus and Produced from COVID-19 mRNA or Adenoviral DNA Vaccines," *Arch Microbiol Immun* 2023, 7, 3: 121-138. doi: 10.26502/ami.936500110

192. Letarov AV et al., "Free SARS-CoV-2 Spike Protein S1 Particles May Play a Role in the Pathogenesis of COVID-19 Infection," *Biochemistry* (Moscow) 2021, 86: 257–261. doi: https://doi.org/10.1134/S0006297921030032

193. Li C. et al., "Intravenous Injection of Coronavirus Disease 2019 (COVID-19) MRNA Vaccine Can Induce Acute Myopericarditis in Mouse Model," *Clin. Infect. Dis.* 2022, 74, 11: 1933-1950. doi: https://doi.org/10.1093/cid/ciab707

194. Li F et al., "SARS-CoV-2 Spike Promotes Inflammation and Apoptosis Through Autophagy by ROS-Suppressed PI3K/AKT/mTOR Signaling," *Biochim Biophys Acta BBA - Mol Basis Dis* 2021, 1867: 166260. doi: 10.1016/j.bbadis.2021.166260

195. Li K et al., "SARS-CoV-2 Spike protein promotes vWF secretion and thrombosis via endothelial cytoskeleton-associated protein 4 (CKAP4)," *Signal Transduct Targ Ther* 2022, 7, 332. doi: https://doi.org/10.1038/s41392-022-01183-9

196. Li T et al., "Platelets Mediate Inflammatory Monocyte Activation by SARS-CoV-2 Spike Protein," *J. Clin. Invest.* 2022, 132, 4: e150101. doi: 10.1172/JCI150101

197. Li Z et al., "SARS-CoV-2 pathogenesis in the gastrointestinal tract mediated by Spike-induced intestinal inflammation," *Precis. Clin. Med.* 2024, 7, 1: pbad034. doi: https://doi.org/10.1093/pcmedi/pbad034

198. Liang S et al., "SARS-CoV-2 spike protein induces IL-18-mediated cardiopulmonary inflammation via reduced mitophagy," *Signal Transduct Target Ther* 2023, 8, 103. doi: https://doi.org/10.1038/s41392-023-01368-w

199. Lin X et al., “Transplacental transmission of the COVID-19 vaccine messenger RNA: evidence from placental, maternal, and cord blood analyses postvaccination,” *Am J Obstet Gynecol* 2024, 92, 4: e13934. doi: https://doi.org/10.1111/aji.13934

200. Lin Z, “More than a key—the pathological roles of SARS-CoV-2 spike protein in COVID-19 related cardiac injury,” *Sports Med Health Sci* 2023, 6, 3: 209-220. doi: https://doi.org/10.1016/j.smhs.2023.03.004

201. Liu T et al., “RS-5645 attenuates inflammatory cytokine storm induced by SARS-CoV-2 spike protein and LPS by modulating pulmonary microbiota,” *Int J Biol Sci.* 2021, 17, 13: 3305–3319. doi: 10.7150/ijbs.63329

202. Liu X et al., “SARS-CoV-2 spike protein-induced cell fusion activates the cGAS-STING pathway and the interferon response,” *Sci Signal.* 2022, 15, 729: eabg8744. doi: 10.1126/scisignal.abg8744

203. Liu Y et al., “The recombinant spike S1 protein induces injury and inflammation in co-cultures of human alveolar epithelial cells and macrophages,” *PLoS ONE* 2025, 20, 2: e0318881. doi: https://doi.org/10.1371/journal.pone.0318881

204. Loh D, “The potential of melatonin in the prevention and attenuation of oxidative hemolysis and myocardial injury from cd147 SARS-CoV-2 spike protein receptor binding,” *Melatonin Research* 2020, 3, 3: 380-416. doi: 10.32794/mr11250069

205. Loh JT et al., “Dok3 restrains neutrophil production of calprotectin during TLR4 sensing of SARS-CoV-2 spike protein,” *Front. Immunol.* 2022, 13 (Sec. Molecular Innate Immunity). doi: https://doi.org/10.3389/fimmu.2022.996637

206. Lu J and PD Sun, “High affinity binding of SARS-CoV-2 spike protein enhances ACE2 carboxypeptidase activity,” *J. Biol. Chem* 2020, 295, 52: p18579-18588. doi: 10.1074/jbc.RA120.015303

207. Luchini A et al., “Lipid bilayer degradation induced by SARS-CoV-2 spike protein as revealed by neutron reflectometry,” *Sci. Rep.* 2021, 11: 14867. doi: https://doi.org/10.1038/s41598-021-93996-x

208. Luo Y et al., “SARS-Cov-2 spike induces intestinal barrier dysfunction through the interaction between CEACAM5 and Galectin-9,” *Front. Immunol.,* 2024, 15. doi: https://doi.org/10.3389/fimmu.2024.1303356

209. Lykhmus O et al., “Immunization with 674–685 fragment of SARS-Cov-2 spike protein induces neuroinflammation and impairs episodic memory of

mice," *Biochem. Biophys. Res. Commun.* 2022, 622: 57–63. doi: https://doi.org/10.1016/j.bbrc.2022.07.016

210. Ma G et al., "SARS-CoV-2 Spike protein S2 subunit modulates γ-secretase and enhances amyloid-β production in COVID-19 neuropathy," *Cell Discov* 2022, 8, 99. doi: https://doi.org/10.1038/s41421-022-00458-3

211. Maeda Y et al., "Differential Ability of Spike Protein of SARS-CoV-2 Variants to Downregulate ACE2," *Int. J. Mol. Sci.* 2024, 25, 2: 1353. doi: 10.3390/ijms25021353

212. Magen E et al., "Clinical and Molecular Characterization of a Rare Case of BNT162b2 mRNA COVID-19 Vaccine-Associated Myositis," *Vaccines* 2022, 10: 1135. doi: https://doi.org/10.3390/vaccines10071135

213. Magro C et al., "Disruption of the blood-brain barrier is correlated with spike endocytosis by ACE2 + endothelia in the CNS microvasculature in fatal COVID-19. Scientific commentary on 'Detection of blood-brain barrier disruption in brains of patients with COVID-19, but no evidence of brain penetration by SARS-CoV-2'," *Acta Neuropathol.* 2024, 147, 1: 47. doi: 10.1007/s00401-023-02681-y

214. Magro C et al., "The histologic and molecular correlates of COVID-19 vaccine-induced changes in the skin," *Clin. Dermatol.* 2021, 39, 6: 966-984. doi: https://doi.org/10.1016/j.clindermatol.2021.07.011

215. Marrone L et al., "Tirofiban prevents the effects of SARS-CoV-2 spike protein on macrophage activation and endothelial cell death," *Heliyon* 2024, 10, 15: e35341. doi: 10.1016/j.heliyon.2024.e35341

216. Martin-Navarro L et al., "In situ detection of vaccine mRNA in the cytoplasm of hepatocytes during COVID-19 vaccine-related hepatitis," *J. Hepatol.* 2023, 78, 1: e20-e22. doi: 10.1016/j.jhep.2022.08.039

217. Martinez-Marmol R et al., "SARS-CoV-2 infection and viral fusogens cause neuronal and glial fusion that compromises neuronal activity," *Sci. Adv.* 2023, 9, 23. doi: 10.1126/sciadv.adg2248

218. Matschke J et al., "Neuropathology of patients with COVID-19 in Germany: a post-mortem case series," *Lancet Neurol.* 2020, 19, 11: 919-929. doi: 10.1016/S1474-4422(20)30308-2

219. Matsuzawa Y et al., "Impact of Renin–Angiotensin–Aldosterone System Inhibitors on COVID-19," *Hypertens Res.* 2022, 45, 7: 1147–1153. doi: https://doi.org/10.1038/s41440-022-00922-3

220. Maugeri M et al.. “Linkage between endosomal escape of LNP-mRNA and loading into EVs for transport to other cells,” *Nat Commun* 2019, 10: 4333. doi: https://doi.org/10.1038/s41467-019-12275-6

221. Maugeri N et al., “Unconventional CD147-Dependent Platelet Activation Elicited by SARS-CoV-2 in COVID-19,” *J. Thromb. Haemost.* 2021, 20, 2: 434–448. doi: https://doi.org/10.1111/jth.15575

222. Mayordomo-Colunga J et al., “SARS-CoV-2 spike protein in intestinal cells of a patient with coronavirus disease 2019 multisystem inflammatory syndrome,” *J Pediatr.* 2022, 243: 214-18e215. doi: https://doi.org/10.1016/j.jpeds.2021.11.058

223. Mercado-Gómez M et al., “The spike of SARS-CoV-2 promotes metabolic rewiring in hepatocytes,” *Commun. Biol.* 2022, 5, 827. doi: 10.1038/s42003-022-03789-9

224. Meyer K et al., “SARS-CoV-2 Spike Protein Induces Paracrine Senescence and Leukocyte Adhesion in Endothelial Cells,” *J. Virol.* 2021, 95, e0079421. doi: https://doi.org/10.1128/jvi.00794-21

225. Miller GM et al., “SARS-CoV-2 and SARS-CoV-2 Spike protein S1 subunit Trigger Proinflammatory Response in Macrophages in the Absence of Productive Infection,” *J. Immunol.* 2023, 210 (1_Supplement): 71.30. doi: https://doi.org/10.4049/jimmunol.210.Supp.71.30

226. Mishra R and AC Banerjea, “SARS-CoV-2 Spike targets USP33-IRF9 axis via exosomal miR-148a to activate human microglia,” *Front. Immunol.* 2021, 12: 656700. doi: https://doi.org/10.3389/fimmu.2021.656700

227. Montezano AC et al., “SARS-CoV-2 spike protein induces endothelial inflammation via ACE2 independently of viral replication,” *Sci Rep.* 2023, 13, 1: 14086. doi: https://doi.org/10.1038/s41598-023-41115-3

228. Mörz M, “A Case Report: Multifocal Necrotizing Encephalitis and Myocarditis after BNT162b2 mRNA Vaccination against COVID-19,” *Vaccines* 2022, 10, 10: 1651. doi: https://doi.org/10.3390/vaccines10101651

229. Moutal A et al., “SARS-CoV-2 Spike protein co-opts VEGF-A/Neuropilin-1 receptor signaling to induce analgesia,” *Pain* 2020, 162, 1: 243–252. doi: 10.1097/j.pain.0000000000002097

230. Munavilli GG et al., “COVID-19/SARS-CoV-2 virus spike protein-related delayed inflammatory reaction to hyaluronic acid dermal fillers: a challenging clinical

conundrum in diagnosis and treatment," *Arch. Dermatol. Res.* 2022, 314: 1-15. doi: https://doi.org/10.1007/s00403-021-02190-6

231. Nahalka J, "1-L Transcription of SARS-CoV-2 Spike Protein S1 Subunit," *Int. J. Mol. Sci.* 2024, 25, 8: 4440. doi: https://doi.org/10.3390/ijms25084440

232. Nascimento RR et al., "SARS-CoV-2 Spike protein triggers gut impairment since mucosal barrier to innermost layers: From basic science to clinical relevance," *Mucosal Immunol.* 2024, 17, 4: 565-583. doi: 10.1016/j.mucimm.2024.03.00

233. Nguyen V, "The Spike Protein of SARS-CoV-2 Impairs Lipid Metabolism and Increases Susceptibility to Lipotoxicity: Implication for a Role of Nrf2," *Cells* 2022, 11, 12: 1916. doi: https://doi.org/10.3390/cells11121916

234. Niu C et al., "SARS-CoV-2 spike protein induces the cytokine release syndrome by stimulating T cells to produce more IL-2," *Front. Immunol.* 2024, 15: 1444643. doi: https://doi.org/10.3389/fimmu.2024.1444643

235. Norris B et al., "Evaluation of Glutathione in Spike Protein of SARS-CoV-2 Induced Immunothrombosis and Cytokine Dysregulation," *Antioxidants* 2024, 13, 3: 271. doi: https://doi.org/10.3390/antiox13030271

236. Nunez-Castilla J et al., "Potential autoimmunity resulting from molecular mimicry between SARS-CoV-2 spike and human proteins," *Viruses* 2022, 14, 7: 1415. doi: https://doi.org/10.3390/v14071415

237. Nuovo JG et al., "Endothelial Cell Damage Is the Central Part of COVID-19 and a Mouse Model Induced by Injection of the S1 Subunit of the Spike Protein," *Ann. Diagn. Pathol.* 2021, 51, 151682. doi: 10.1016/j.anndiagpath.2020.151682

238. Nyein CM et al., "Severe *de novo* liver injury after Moderna vaccination – not always autoimmune hepatitis," *J. Hepatol.* 2022, 77, 2: 556-558. doi: 10.1016/j.jhep.2022.03.041

239. Nyström S, "Amyloidogenesis of SARS-CoV-2 Spike Protein," *J. Am. Chem. Soc.* 2022, 144, 8945–8950. doi: https://doi.org/10.1021/jacs.2c03925

240. O'Brien BCV et al., "SARS-CoV-2 spike ectodomain targets α7 nicotinic acetylcholine receptors," *J. Biol. Chem.* 2023, 299, 5: 104707. doi: 10.1016/j.jbc.2023.104707

241. Ogata AF et al., "Circulating Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Vaccine Antigen Detected in the Plasma of mRNA-1273 Vaccine Recipients," *Clin. Infect. Dis.* 2022, 75, 4: 715–718. doi: 10.1093/cid/ciab465

242. Ogata AF et al., “Ultra-Sensitive Serial Profiling of SARS-CoV-2 Antigens and Antibodies in Plasma to Understand Disease Progression in COVID-19 Patients with Severe Disease,” *Clin. Chem.* 2020, 66, 12: 1562-1572. doi: https://doi.org/10.1093/clinchem/hvaa213

243. Oh J et al., “SARS-CoV-2 Spike Protein Induces Cognitive Deficit and Anxiety-Like Behavior in Mouse via Non-cell Autonomous Hippocampal Neuronal Death,” *Scientific Reports* 2022, 12, 5496. doi: 10.1038/s41598-022-09410-7

244. Oka N et al., “SARS-CoV-2 S1 protein causes brain inflammation by reducing intracerebral acetylcholine production,” *iScience* 2023, 26, 6: 106954. doi: 10.1016/j.isci.2023.106954

245. Olajide OA et al., “Induction of Exaggerated Cytokine Production in Human Peripheral Blood Mononuclear Cells by a Recombinant SARS-CoV-2 Spike Glycoprotein S1 and Its Inhibition by Dexamethasone,” *Inflammation* 2021, 44: 1865–1877. doi: https://doi.org/10.1007/s10753-021-01464-5

246. Olajide OA et al., “SARS-CoV-2 spike glycoprotein S1 induces neuroinflammation in BV-2 microglia,” *Mol. Neurobiol.* 2022, 59: 445-458. doi: https://doi.org/10.1007/s12035-021-02593-6

247. Oliveira ASF et al., “A potential interaction between the SARS-CoV-2 spike protein and nicotinic acetylcholine receptors,” *Biophys. J.* 2021, 120, 6: 983-993. doi: 10.1016/j.bpj.2021.01.037

248. Onnis A et al., “SARS-CoV-2 Spike protein suppresses CTL-mediated killing by inhibiting immune synapse assembly,” *J Exp Med* 2023, 220, 2: e20220906. doi: https://doi.org/10.1084/jem.20220906

249. Ota N et al., “Expression of SARS-CoV-2 spike protein in cerebral Arteries: Implications for hemorrhagic stroke Post-mRNA vaccination,” *J. Clin. Neurosci.* 2025, 136: 111223. doi: https://doi.org/10.1016/j.jocn.2025.111223

250. Palestra F et al., “SARS-CoV-2 Spike Protein Activates Human Lung Macrophages,” *Int. J. Mol. Sci.* 2023, 24, 3: 3036. doi: 10.3390/ijms24033036

251. Pallas RM, “Innate and adaptative immune mechanisms of COVID-19 vaccines. Serious adverse events associated with SARS-CoV-2 vaccination: A systematic review,” *Vacunas* (English ed.) 2024, 25, 2: 285.e1-285.e94. doi: https://doi.org/10.1016/j.vacune.2024.05.002

252. Panigrahi S et al., “SARS-CoV-2 Spike Protein Destabilizes Microvascular Homeostasis,” *Microbiol Spectr.* 2021, 9, 3: e0073521. doi: https://doi.org/10.1128/Spectrum.00735-21

253. Parcial ALN et al., “SARS-CoV-2 Is Persistent in Placenta and Causes Macroscopic, Histopathological, and Ultrastructural Changes,” *Viruses* 2022, 14, 9: 1885. doi: https://doi.org/10.3390/v14091885

254. Park C et al., “Murine alveolar Macrophages Rapidly Accumulate intranasally Administered SARS-CoV-2 Spike Protein leading to neutrophil Recruitment and Damage,” *Elife* 2024, 12: RP86764. doi: https://doi.org/10.7554/eLife.86764.3

255. Park YJ et al., “D-dimer and CoV-2 spike-immune complexes contribute to the production of PGE2 and proinflammatory cytokines in monocytes,” *PLoS Pathog.* 2022, 18, 4: e1010468. doi: https://doi.org/10.1371/journal.ppat.1010468

256. Park YJ et al., “Pyrogenic and inflammatory mediators are produced by polarized M1 and M2 macrophages activated with D-dimer and SARS-CoV-2 spike immune complexes,” *Cytokine* 2024, 173: 156447. doi: 10.1016/j.cyto.2023.156447

257. Parry PL et al., “‘Spikeopathy’: COVID-19 Spike Protein Is Pathogenic, from Both Virus and Vaccine mRNA,” *Biomedicine* 2023, 11, 8: 2287. doi: https://doi.org/10.3390/biomedicines11082287

258. Passariello M et al., “Interactions of Spike-RBD of SARS-CoV-2 and Platelet Factor 4: New Insights in the Etiopathogenesis of Thrombosis,” *Int. J. Mol. Sci.* 2021, 22, 16: 8562. doi: https://doi.org/10.3390/ijms22168562

259. Pateev I et al., “Biodistribution of RNA Vaccines and of Their Products: Evidence from Human and Animal Studies,” *Biomedicines* 2024, 12, 1: 59. doi: https://doi.org/10.3390/biomedicines12010059

260. Patra T et al., “SARS-CoV-2 spike protein promotes IL-6 trans-signaling by activation of angiotensin II receptor signaling in epithelial cells,” *PLoS Pathog.* 2020, 16: e1009128. doi: https://doi.org/10.1371/journal.ppat.1009128

261. Patterson BK et al., “Detection of S1 spike protein in CD16+ monocytes up to 245 days in SARS-CoV-2-negative post-COVID-19 vaccine syndrome (PCVS) individuals,” *Hum Vaccin Immunother.* 2025, 21, 1: 2494934. doi: https://doi.org/10.1080/21645515.2025.2494934

262. Patterson BK et al., “Persistence of SARS CoV-2 S1 Protein in CD16+ Monocytes in Post-Acute Sequelae of COVID-19 (PASC) up to 15 Months Post-Infection,” *Front. Immunol.* 12 (Sec. Viral Immunology). doi: 10.3389/fimmu.2021.746021

263. Peluso MJ et al., “Plasma-based antigen persistence in the post-acute phase of COVID-19,” *Lancet* 2024, 24, 6: E345-E347. doi: 10.1016/S1473-3099(24)00211-1

264. Peluso MJ et al., “SARS-CoV-2 and mitochondrial proteins in neural-derived exosomes of COVID-19,” *Ann Neurol* 2022, 91, 6: 772-781. doi: https://doi.org/10.1002/ana.26350

265. Pence B, “Recombinant SARS-CoV-2 Spike Protein Mediates Glycolytic and Inflammatory Activation in Human Monocytes,” *Innov Aging* 2020, 4, sp. 1: 955. doi: https://doi.org/10.1093/geroni/igaa057.3493

266. Perico L et al., “SARS-CoV-2 and the spike protein in endotheliopathy,” *Trends Microbiol.* 2024, 32, 1: 53-67. doi: 10.1016/j.tim.2023.06.004

267. Perico L et al., “SARS-CoV-2 Spike Protein 1 Activates Microvascular Endothelial Cells and Complement System Leading to Platelet Aggregation,” *Front. Immunol.* 2022, 13, 827146. doi: https://doi.org/10.3389/fimmu.2022.827146

268. Petrlova J et al., “SARS-CoV-2 spike protein aggregation is triggered by bacterial lipopolysaccharide,” *FEBS Lett.* 2022, 596: 2566–2575. doi: https://doi.org/10.1002/1873-3468.14490

269. Petrosino S and N Matende, “Elimination/Neutralization of COVID-19 Vaccine-Produced Spike Protein: Scoping Review,” *Mathews Journal of Nutrition & Dietetics* 2024, 7, 2. doi: https://doi.org/10.30654/MJND.10034

270. Petrovszki D et al., “Penetration of the SARS-CoV-2 Spike Protein across the Blood-Brain Barrier, as Revealed by a Combination of a Human Cell Culture Model System and Optical Biosensing,” *Biomedicines* 2022, 10, 1: 188. doi: https://doi.org/10.3390/biomedicines10010188

271. Petruk G et al., “SARS-CoV-2 spike protein binds to bacterial lipopolysaccharide and boosts proinflammatory activity,” *J. Mol. Cell Biol.* (2020) 12: 916-932. doi: https://doi.org/10.1093/jmcb/mjaa067

272. Polykretis P et al., “Autoimmune Inflammatory Reactions Triggered by the COVID-19 Genetic Vaccines in Terminally Differentiated Tissues,” *Autoimmunity* 2023, 56: 2259123. doi: https://doi.org/10.1080/08916934.2023.2259123

273. Posa A, “Spike protein-related proteinopathies: A focus on the neurological side of spikeopathies,” *Ann Anat. - Anatomischer Anzeiger* 2025, 260: 152662. doi: https://doi.org/10.1016/j.aanat.2025.152662

274. Prieto-Villalobos J et al., “SARS-CoV-2 spike protein S1 activates Cx43 hemichannels and disturbs intracellular Ca^{2+} dynamics,” *Biol Res.* 2023, 56, 1: 56. doi: https://doi.org/10.1186/s40659-023-00468-9

275. Puthia MTL et al., “Experimental model of pulmonary inflammation induced by SARS-CoV-2 spike protein and endotoxin,” *ACS Pharmacol Transl Sci*. 2022, 5, 3: 141–8. doi: https://doi.org/10.1021/acsptsci.1c00219

276. Raghavan S et al., “SARS-CoV-2 Spike Protein Induces Degradation of Junctional Proteins That Maintain Endothelial Barrier Integrity,” *Front. Cardiovasc. Med*. 2021, *8*, 687783. doi: https://doi.org/10.3389/fcvm.2021.687783

277. Rahman M et al., “Differential Effect of SARS-CoV-2 Spike Glycoprotein 1 on Human Bronchial and Alveolar Lung Mucosa Models: Implications for Pathogenicity,” *Viruses* 2021, 13, 12: 2537. doi: 10.3390/v13122537

278. Rajah MM et al., “SARS-CoV-2 Alpha, Beta, and Delta variants display enhanced spike-mediated syncytia formation,” *EMBO J.* 2021, 40: e108944. doi: https://doi.org/10.15252/embj.2021108944

279. Ratajczak MZ et al., “SARS-CoV-2 Entry Receptor ACE2 Is Expressed on Very Small $CD45^{-}$ Precursors of Hematopoietic and Endothelial Cells and in Response to Virus Spike Protein Activates the Nlrp3 Inflammasome,” *Stem Cell Rev Rep.* 2021, 17, 1: 266-277. doi: https://doi.org/10.1007/s12015-020-10010-z

280. Rhea EM et al., “The S1 protein of SARS-CoV-2 crosses the blood–brain barrier in mice,” *Nature Neuroscience* 2021, 24, 3: 368–378. doi: https://doi.org/10.1038/s41593-020-00771-8

281. Rivas MN et al., “COVID-19–associated multisystem inflammatory syndrome in children (MIS-C): A novel disease that mimics toxic shock syndrome—the superantigen hypothesis,” *J Allergy Clin Immunol* 2021, 147, 1: 57-59. doi: 10.1016/j.jaci.2020.10.008

282. Rivas MN et al., “Multisystem Inflammatory Syndrome in Children and Long COVID: The SARS-CoV-2 Viral Superantigen Hypothesis,” *Front Immunol.* 2022, 13 (Sec. Molecular Innate Immunity) doi: 10.3389/fimmu.2022.941009

283. Robles JP et al., “The Spike Protein of SARS-CoV-2 Induces Endothelial Inflammation through Integrin α5β1 and NF-κB Signaling,” *J. Biol. Chem*. 2022, 298, 3: 101695. doi: https://doi.org/10.1016/j.jbc.2022.101695

284. Roden AC et al., “Comparison of In Situ Hybridization, Immunohistochemistry, and Reverse Transcription–Droplet Digital Polymerase Chain Reaction for Severe

Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Testing in Tissue," *Arch Pathol Lab Med* 2021, 145, 7: 785–796. doi: 10.5858/arpa.2021-0008-SA

285. Rodriguez Y et al., "Autoinflammatory and autoimmune conditions at the crossroad of COVID-19," *J. Autoimmun*. 2020, 114: 102506. doi: https://doi.org/10.1016/j.jaut.2020.102506

286. Rong Z et al., "Persistence of spike protein at the skull-meninges-brain axis may contribute to the neurological sequelae of COVID-19," *Cell Host Microbe* 2024, 26: S1931-3128(24)00438-4. doi: 10.1016/j.chom.2024.11.007

287. Ropa J et al., "Human Hematopoietic Stem, Progenitor, and Immune Cells Respond Ex Vivo to SARS-CoV-2 Spike Protein," *Stem Cell Rev Rep*. 2021, 17, 1: 253-265. doi: https://doi.org/10.1007/s12015-020-10056-z

288. Rotoli BM et al., "Endothelial cell activation by SARS-CoV-2 spike S1 protein: A crosstalk between endothelium and innate immune cells," *Biomedicines* 2021, 9, 9: 1220. doi: https://doi.org/10.3390/biomedicines9091220

289. Roy A et al., "Ultradiluted *Eupatorium perfoliatum* Prevents and Alleviates SARS-CoV-2 Spike Protein-Induced Lung Pathogenesis by Regulating Inflammatory Response and Apoptosis," *Diseases* 2025, 13, 2: 36. doi: 10.3390/diseases13020036

290. Roytenberg R et al., "Thymidine phosphorylase mediates SARS-CoV-2 spike protein enhanced thrombosis in K18-hACE2TG mice," *Thromb. Res*. 2024, 244, 8: 109195. doi: 10.1016/j.thromres.2024.109195

291. Ruben ML et al., "The SARS-CoV-2 spike protein subunit S1 induces COVID-19-like acute lung injury in K18-hACE2 transgenic mice and barrier dysfunction in human endothelial cells," *Am J Physiol Lung Cell Mol Physiol*. 2021, 321, 2: L477-L484. doi: https://doi.org/10.1152/ajplung.00223.2021

292. Russo A, et al., "Implication of COVID-19 on Erythrocytes Functionality: Red Blood Cell Biochemical Implications and Morpho-Functional Aspects," *Int. J. Mol. Sci*. 2022, 23, 4: 2171. doi: https://doi.org/10.3390/ijms23042171

293. Ryu JK et al., "Fibrin drives thromboinflammation and neuropathology in COVID-19," *Nature* 2024, 633: 905-913. doi: https://doi.org/10.1038/s41586-024-07873-4

294. Rzymski P and Andrzej Fal, "To aspirate or not to aspirate? Considerations for the COVID-19 vaccines," *Pharmacol. Rep* 2022, 74: 1223–1227. doi: https://doi.org/10.1007/s43440-022-00361-4

295. Saadi F et al., “Spike glycoprotein is central to coronavirus pathogenesis-parallel between m-CoV and SARS-CoV-2,” *Ann Neurosci.* 2021, 28 (3-4): 201–218. doi: https://doi.org/10.1177/09727531211023755

296. Sacco K et al., “Immunopathological signatures in multisystem inflammatory syndrome in children and pediatric COVID-19,” *Nat. Med.* 2022, 28: 1050-1062. doi: https://doi.org/10.1038/s41591-022-01724-3

297. Samsudin S et al., “SARS-CoV-2 spike protein as a bacterial lipopolysaccharide delivery system in an overzealous inflammatory cascade,” *J. Mol. Biol.* 2022, 14, 9: mjac058. doi: https://doi.org/10.1093/jmcb/mjac058

298. Sandelius A et al., “Biodistribution of lipid nanoparticle, eGFP mRNA and translated protein following subcutaneous administration in mouse,” *Bioanalysis* 2024, 16, 14: 721-733. doi: https://doi.org/10.1080/17576180.2024.2360361

299. Sano H et al., “A case of persistent, confluent maculopapular erythema following a COVID-19 mRNA vaccination is possibly associated with the intralesional spike protein expressed by vascular endothelial cells and eccrine glands in the deep dermis,” *J. Dermatol.* 2023, 50: 1208–1212. doi: 10.1111/1346-8138.16816

300. Sano S et al., “SARS-CoV-2 spike protein found in the acrosyringium and eccrine gland of repetitive miliaria-like lesions in a woman following mRNA vaccination,” *J. Dermatol.* 2024, 51, 9: e293-e295. doi: 10.1111/1346-8138.17204

301. Santonja C et al., “COVID-19 chilblain-like lesion: immunohistochemical demonstration of SARS-CoV-2 spike protein in blood vessel endothelium and sweat gland epithelium in a polymerase chain reaction-negative patient,” *Br J Dermatol*. 2020, 183, 4: 778-780. doi: https://doi.org/10.1111/bjd.19338

302. Satta S et al., “An engineered nano-liposome-human ACE2 decoy neutralizes SARS-CoV-2 Spike protein-induced inflammation in both murine and human macrophages,” *Theranostics* 2022, 12, 6: 2639–2657. doi: 10.7150/thno.66831

303. Sattar S et al., “Nuclear translocation of spike mRNA and protein is a novel feature of SARS-CoV-2,” 2023 *Front. Microbiol.* 2023, 14 (Sec. Virology). doi: https://doi.org/10.3389/fmicb.2023.1073789

304. Scheim DE, “A Deadly Embrace: Hemagglutination Mediated by SARS-CoV-2 Spike Protein at its 22 N-Glycosylation Sites, Red Blood Cell Surface Sialoglycoproteins, and Antibody,” *Int. J. Mol. Sci.* 2022, 23, 5, 2558. doi: https://doi.org/10.3390/ijms23052558

305. Scheim DE et al., “Sialylated Glycan Bindings from SARS-CoV-2 Spike Protein to Blood and Endothelial Cells Govern the Severe Morbidities of COVID-19,” *Int. J. Mol. Schi.* 2023, 24, 23:17039. doi: https://doi.org/10.3390/ijms242317039

306. Scholkmann F and CA May, “COVID-19, post-acute COVID-19 syndrome (PACS, ‘long COVID’) and post-COVID-19 vaccination syndrome (PCVS, ‘post-COVIDvac-syndrome’): Similarities and differences,” *Pathol Res Pract.* 2023, 246: 154497. doi: https://doi.org/10.1016/j.prp.2023.154497

307. Schreckenberg R et al., “Cardiac side effects of RNA-based SARS-CoV-2 vaccines: Hidden cardiotoxic effects of mRNA-1273 and BNT162b2 on ventricular myocyte function and structure,” *Br. J. Pharmacol.* 2024, 181, 3: 345-361. doi: https://doi.org/10.1111/bph.16262

308. Schroeder JT and AP Bieneman, “The S1 Subunit of the SARS-CoV-2 Spike protein activates human monocytes to produce cytokines linked to COVID-19: relevance to galectin-3,” *Front Immunol.* 2022, 13: 831763. doi: 10.3389/fimmu.2022.831763

309. Schultheiss C et al., “Liquid biomarkers of macrophage dysregulation and circulating spike protein illustrate the biological heterogeneity in patients with post-acute sequelae of COVID-19,” *J Med Virol* 2023, 95, 1: e28364. doi: https://doi.org/10.1002/jmv.28364

310. Schwartz L et al., “Toxicity of the spike protein of COVID-19 is a redox shift phenomenon: A novel therapeutic approach,” *Free Rad. Biol. Med.* 2023, 206: 106–110. doi: https://doi.org/10.1016/j.freeradbiomed.2023.05.034

311. Sebastio AI et al., “CuMV VLPs containing the RBM from SARS-CoV-2 spike protein drive dendritic cell activation and Th1 polarization,” *Pharmaceutics* 2023, 15, 3: 825. doi: https://doi.org/10.3390/pharmaceutics15030825

312. Segura-Villalobos D et al., “Jacareubin inhibits TLR4-induced lung inflammatory response caused by the RBD domain of SARS-CoV-2 Spike protein,” *Pharmacol. Rep.* 2022, 74: 1315–1325. doi: https://doi.org/10.1007/s43440-022-00398-5

313. Semmarath W et al., “Cyanidin-3-O-glucoside and Peonidin-3-O-glucoside-Rich Fraction of Black Rice Germ and Bran Suppresses Inflammatory Responses from SARS-CoV-2 Spike Glycoprotein S1-Induction In Vitro in A549 Lung Cells and THP-1 Macrophages via Inhibition of the NLRP3 Inflammasome Pathway,” *Nutrients* 2022, 14, 13: 2738. doi: https://doi.org/10.3390/nu14132738

314. Sharma VK et al., “Nanocurcumin Potently Inhibits SARS-CoV-2 Spike Protein-Induced Cytokine Storm by Deactivation of MAPK/NF-κB Signaling in Epithelial Cells,” *ACS Appl. Bio Mater.* 2022, 5, 2: 483–491. doi: 10.1021/acsabm.1c00874

315. Shirato K and Takako Kizaki, “SARS-CoV-2 Spike Protein S1 Subunit Induces Pro-inflammatory Responses via Toll-Like Receptor 4 Signaling in Murine and Human Macrophages,” *Heliyon* 2021, 7, 2: e06187. doi: 10.1016/j.heliyon.2021.e06187

316. Singh N and Anuradha Bharara Singh, “S2 Subunit of SARS-nCoV-2 Interacts with Tumor Suppressor Protein p53 and BRCA: An in Silico Study,” *Transl. Oncol.* 2020, 13, 10: 100814, doi: https://doi.org/10.1016/j.tranon.2020.100814

317. Singh RD, “The spike protein of sars-cov-2 induces heme oxygenase-1: pathophysiologic implications,” *Biochim Biophys Acta, Mol Basis Dis* 2022, 1868, 3: 166322. doi: https://doi.org/10.1016/j.bbadis.2021.166322

318. Sirsendu J et al., “Cell-Free Hemoglobin Does Not Attenuate the Effects of SARS-CoV-2 Spike Protein S1 Subunit in Pulmonary Endothelial Cells,” *Int. J. Mol. Sci.*, 2021, 22, 16: 9041. doi: https://doi.org/10.3390/ijms22169041

319. Soares CD et al., “Oral vesiculobullous lesions as an early sign of COVID-19: immunohistochemical detection of SARS-CoV-2 spike protein,” *Br. J. Dermatol.* 2021, 184, 1: e6. doi: https://doi.org/10.1111/bjd.19569

320. Solis O et al., “The SARS-CoV-2 spike protein binds and modulates estrogen receptors,” *Sci. Adv.* 2022, 8, 48: eadd4150. doi: 10.1126/sciadv.add4150

321. Solopov PA et al., “Alcohol increases lung angiotensin-converting enzyme 2 expression and exacerbates severe acute respiratory syndrome coronavirus 2 spike protein subunit 1-induced acute lung injury in K18-hACE2 transgenic mice,” *Am J Pathol* 2022, 192, 7: 990-1000. doi: 10.1016/j.ajpath.2022.03.012

322. Solopov PA et al., “KVX-053, a protein tyrosine phosphatase 4A3 inhibitor, ameliorates SARS-CoV-2 spike protein subunit 1–induced acute lung injury in mice,” *J. Pharmacol. Exp. Ther.* 2025, 392, 3: 100022. doi: https://doi.org/10.1124/jpet.124.002154

323. Stern B et al., “SARS-CoV-2 spike protein induces endothelial dysfunction in 3D engineered vascular networks,” *J. Biomed. Mater. Res. A.* 2023, 112, 4: 524-533. doi: https://doi.org/10.1002/jbm.a.37543

324. Sui Y et al., “SARS-CoV-2 Spike Protein Suppresses ACE2 and Type I Interferon Expression in Primary Cells From Macaque Lung Bronchoalveolar Lavage,” *Front. Immunol.* 2021, 12. doi: https://doi.org/10.3389/fimmu.2021.658428

325. Sun Q et al., “SARS-coV-2 spike protein S1 exposure increases susceptibility to angiotensin II-induced hypertension in rats by promoting central neuroinflammation and oxidative stress,” *Neurochem. Res.* 2023, 48, 3016–3026. doi: https://doi.org/10.1007/s11064-023-03949-1

326. Sung PS et al., “CLEC5A and TLR2 Are Critical in SARS-CoV-2-Induced NET Formation and Lung Inflammation,” *J. Biomed. Sci.* 2002, 29, 52. doi: https://doi.org/10.1186/s12929-022-00832-z

327. Suprewicz L et al., “Blood-brain barrier function in response to SARS-CoV-2 and its spike protein,” *Neurol. Neurochir Pol.* 2023, 57: 14–25. doi: 10.5603/PJNNS.a2023.0014

328. Suprewicz L et al., “Recombinant human plasma gelsolin reverses increased permeability of the blood-brain barrier induced by the spike protein of the SARS-CoV-2 virus,” *J Neuroinflamm.* 2022, 19, 1: 282. doi: 10.1186/s12974-022-02642-4

329. Suzuki YJ et al., “SARS-CoV-2 spike protein-mediated cell signaling in lung vascular cells,” *Vascul. Pharmacol.* 2021, 137: 106823. doi: https://doi.org/10.1016/j.vph.2020.106823

330. Suzuki YJ and SG Gychka, “SARS-CoV-2 Spike Protein Elicits Cell Signaling in Human Host Cells: Implications for Possible Consequences of COVID-19 Vaccines,” *Vaccines* 2021, 9, 1, 36. doi: https://doi.org/10.3390/vaccines9010036

331. Swank Z, et al. “Persistent Circulating Severe Acute Respiratory Syndrome Coronavirus 2 Spike Is Associated With Post-acute Coronavirus Disease 2019 Sequelae,” *Clin. Infect. Dis.* 2023, 76, 3: e487–e490. doi: 10.1093/cid/ciac722

332. Tetz G and Victor Tetz, “Prion-Like Domains in Spike Protein of SARS-CoV-2 Differ across Its Variants and Enable Changes in Affinity to ACE2,” *Microorganisms* 2022, 10, 2: 280. doi: https://doi.org/10.3390/microorganisms10020280

333. Theobald SJ et al., “Long-lived macrophage reprogramming drives spike protein-mediated inflammasome activation in COVID-19,” *EMBO Mol. Med.* 2021, 13: e14150. doi: https://doi.org/10.15252/emmm.202114150

334. Theoharides TC, “Could SARS-CoV-2 Spike Protein Be Responsible for Long-COVID Syndrome?” *Mol. Neurobiol.* 2022, 59, 3: 1850–1861. doi: https://doi.org/10.1007/s12035-021-02696-0

335. Theoharides TC and P Conti, “Be Aware of SARS-CoV-2 Spike Protein: There Is More Than Meets the Eye,” *J. Biol. Reg. Homeost. Agents* 2021, 35, 3: 833–838 doi: 10.23812/THEO_EDIT_3_21

336. Theuerkauf SA et al., “Quantitative assays reveal cell fusion at minimal levels of SARS-CoV-2 spike protein and fusion from without,” *iScience* 2021, 24, 3: 102170. doi: https://doi.org/10.1016/j.isci.2021.102170

337. Tillman TS et al., “SARS-CoV-2 Spike Protein Downregulates Cell Surface alpha7nAChR through a Helical Motif in the Spike Neck,” *ACS Chem. Neurosci.* 2023, 14, 4: 689–698. doi: 10.1021/acschemneuro.2c00610

338. Trougakos IP et al., “Adverse Effects of COVID-19 mRNA Vaccines: The Spike Hypothesis,” *Trends Mol. Med.* 2022, 28, 7: 542–554. doi: 10.1016/j.molmed.2022.04.007

339. Tsilioni I et al., “Nobiletin and Eriodictyol Suppress Release of IL-1β, CXCL8, IL-6, and MMP-9 from LPS, SARS-CoV-2 Spike Protein, and Ochratoxin A-Stimulated Human Microglia,” *Int. J. Mol. Sci.* 2025, 26, 2: 636. doi: 10.3390/ijms26020636

340. Tsilioni S et al., “Recombinant SARS-CoV-2 spike protein and its receptor binding domain stimulate release of different pro-inflammatory mediators via activation of distinct receptors on human microglia cells,” *Mol Neurobiol.* 2023, 60, 11: 6704–14. doi: 10.1007/s12035-023-03493-7

341. Tsilioni S et al., “Recombinant SARS-CoV-2 Spike Protein Stimulates Secretion of Chymase, Tryptase, and IL-1beta from Human Mast Cells, Augmented by IL-33,” *Int. J. Mol. Sci.* 2023, 24, 11: 9487. doi: https://doi.org/10.3390/ijms24119487

342. Tu TH et al., “The identification of a SARs-CoV2 S2 protein derived peptide with super-antigen-like stimulatory properties on T-cells,” *Commun. Biol.* 2025, 8, 14. doi: https://doi.org/10.1038/s42003-024-07350-8

343. Tyrkalska SD et al., “Differential proinflammatory activities of spike proteins of SARS-CoV-2 variants of concern,” *Sci. Adv.* 2022, 8, 37: eabo0732. doi: 10.1126/sciadv.abo0732

344. Vargas-Castro R et al., “Calcitriol prevents SARS-CoV spike-induced inflammation in human trophoblasts through downregulating *ACE2* and *TMPRSS2* expression,” *J Steroid Biochem Mol Biol* 2025, 245: 106625. doi: https://doi.org/10.1016/j.jsbmb.2024.106625

345. Vettori M et al., “Effects of Different Types of Recombinant SARS-CoV-2 Spike Protein on Circulating Monocytes’ Structure,” *Int. J. Mol. Sci.* 2023, 24, 11: 9373. doi: https://doi.org/10.3390/ijms24119373

346. Villacampa A et al., “SARS-CoV-2 S protein activates NLRP3 inflammasome and deregulates coagulation factors in endothelial and immune cells,” *Cell Commun. Signal.* 2024, 22, 38. doi: https://doi.org/10.1186/s12964-023-01397-6

347. Visvabharathy L et al., “Case report: Treatment of long COVID with a SARS-CoV-2 antiviral and IL-6 blockade in a patient with rheumatoid arthritis and SARS-CoV-2 antigen persistence,” *Front. Med.* 2022, 9 (Sec. Infectious Diseases – Surveillance). doi: https://doi.org/10.3389/fmed.2022.1003103

348. Vojdani A and D Kharrazian, “Potential antigenic cross-reactivity between SARS-CoV-2 and human tissue with a possible link to an increase in autoimmune diseases,” *Clin Immunol.* 2020, 217: 108480. doi: 10.1016/j.clim.2020.108480

349. Vojdani A et al., “Reaction of Human Monoclonal Antibodies to SARS-CoV-2 Proteins With Tissue Antigens: Implications for Autoimmune Diseases,” *Front. Immunol.* 2021, 11 (Sec. Autoimmune and Autoinflammatory Disorders). doi: https://doi.org/10.3389/fimmu.2020.617089

350. Wang J et al., “SARS-CoV-2 Spike Protein S1 Domain Accelerates α-Synuclein Phosphorylation and Aggregation in Cellular Models of Synucleinopathy,” *Mol Neurobiol.* 2024, 61, 4:2446-2458. doi: 10.1007/s12035-023-03726-9

351. Wu H et al., “Molecular evidence suggesting the persistence of residual SARS-CoV-2 and immune responses in the placentas of pregnant patients recovered from COVID-19,” *Cell Prolif.* 2021, 54, 9: e13091. doi: 10.1111/cpr.13091

352. Wu ML et al., “Mast cell activation triggered by SARS-CoV-2 causes inflammation in brain microvascular endothelial cells and microglia,” *Front. Cell. Infect. Microbiol.,* 2024, 14. doi: https://doi.org/10.3389/fcimb.2024.1358873

353. Yang K et al., “SARS-CoV-2 spike protein receptor-binding domain perturbates intracellular calcium homeostasis and impairs pulmonary vascular endothelial cells,” *Signal Transduct. Target. Ther.* 2023, 8, 276. doi: https://doi.org/10.1038/s41392-023-01556-8

354. Yamamoto M et al., “Persistent varicella zoster virus infection following mRNA COVID-19 vaccination was associated with the presence of encoded spike protein in the lesion,” *J. Cutan Immunol. Allergy.* 2022:1–6. doi: https://doi.org/10.1002/cia2.12278

355. Yeung-Luk BH et al., “SARS-CoV-2 infection alters mitochondrial and cytoskeletal function in human respiratory epithelial cells mediated by expression of spike protein,” *mBio* 2023, 14, 4: e00820-23. doi: 10.1128/mbio.00820-23

356. Yilmaz A et al., “Differential proinflammatory responses of colon epithelial cells to SARS-CoV-2 spike protein and *Pseudomonas aeruginosa* lipopolysaccharide,” *Turk J Biochem*. 2024. doi: https://doi.org/10.1515/tjb-2024-0144

357. Yonker LM et al., “Circulating Spike Protein Detected in Post–COVID-19 mRNA Vaccine Myocarditis,” *Circulation* 2023, 147, 11. doi: https://doi.org/10.1161/CIRCULATIONAHA.122.061025

358. Yonker LM et al., “Multisystem inflammatory syndrome in children is driven by zonulin-dependent loss of gut mucosal barrier,” *J Clin Invest*. 2021, 131, 14: e149633. doi: https://doi.org/10.1172/JCI149633

359. Youn JY et al., “Therapeutic application of estrogen for COVID-19: Attenuation of SARS-CoV-2 spike protein and IL-6 stimulated, ACE2-dependent NOX2 activation, ROS production and MCP-1 upregulation in endothelial cells,” *Redox Biol*. 2021, 46: 102099. doi: https://doi.org/10.1016/j.redox.2021.102099

360. Youn YJ et al., “Nucleocapsid and spike proteins of SARS-CoV-2 drive neutrophil extracellular trap formation,” *Immune Netw.* 2021, 21, 2: e16. doi: https://doi.org/10.4110/in.2021.21.e16

361. Yu J et al., “Direct activation of the alternative complement pathway by SARS-CoV-2 spike proteins is blocked by factor D inhibition,” *Blood* 2020, 136, 18: 2080–2089. doi: https://doi.org/10.1182/blood.2020008248

362. Zaki H and S Khan, “SARS-CoV-2 spike protein induces inflammatory molecules through TLR2 in macrophages and monocytes,” *J. Immunol*. 2021, 206 (1_supplement): 62.07. doi: https://doi.org/10.4049/jimmunol.206.Supp.62.07

363. Zaki H and S Khan, “TLR2 senses spike protein of SARS-CoV-2 to trigger inflammation,” *J. Immunol*. 2022, 208 (1_Supplement): 125.30. doi: https://doi.org/10.4049/jimmunol.208.Supp.125.30

364. Zekri-Nechar K et al., “Spike Protein Subunits of SARS-CoV-2 Alter Mitochondrial Metabolism in Human Pulmonary Microvascular Endothelial Cells: Involvement of Factor Xa,” *Dis. Markers* 2022: 1118195. doi: 10.1155/2022/1118195

365. Zeng FM et al., “SARS-CoV-2 spike spurs intestinal inflammation via VEGF production in enterocytes,” *EMBO Mol Med*. 2022, 14: e14844. doi: https://doi.org/10.15252/emmm.202114844

366. Zhang Q et al., “Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) membrane (M) and spike (S) proteins antagonize host type i interferon response,” *Front Cell Infect Microbiol* 2021, 11: 766922. doi: 10.3389/fcimb.2021.766922

367. Zhang RG et al., “SARS-CoV-2 spike protein receptor binding domain promotes IL-6 and IL-8 release via ATP/$P2Y_2$ and ERK1/2 signaling pathways in human bronchial epithelia,” *Mol. Immunol.* 2024, 167: 53-61. doi: 10.1016/j.molimm.2024.02.005

368. Zhang S et al., “SARS-CoV-2 Binds Platelet ACE2 to Enhance Thrombosis in COVID-19,” *J. Hematol. Oncol.* 2020, 13, 120. doi: 10.1186/s13045-020-00954-7

369. Zhang Z et al., “SARS-CoV-2 spike protein dictates syncytium-mediated lymphocyte elimination,” *Cell Death Differ.* 2021, 28: 2765–2777. doi: 10.1038/s41418-021-00782-3

370. Zhao Y et al., “SARS-CoV-2 spike protein interacts with and activates TLR4,” *Cell Res.* 2021, 31: 818–820. doi: https://doi.org/10.1038/s41422-021-00495-9

371. Zheng Y et al., “SARS-CoV-2 Spike Protein Causes Blood Coagulation and Thrombosis by Competitive Binding to Heparan Sulfate,” *Int. J. Biol. Macromol.* 2021, 193: 1124–1129, doi: https://doi.org/10.1016/j.ijbiomac.2021.10.112

372. Zhu G et al., “SARS-CoV-2 spike protein-induced host inflammatory response signature in human corneal epithelial cells,” *Mol. Med. Rep.* 2021, 24: 584. doi: https://doi.org/10.3892/mmr.2021.12223

373. Zhu W et al., “Prothrombotic antibodies targeting the spike protein's receptor-binding domain in severe COVID-19,” *Blood* 2025, 145, 6: 635-647. doi: https://doi.org/10.1182/blood.2024025010

374. Zollner A et al., “Postacute COVID-19 is Characterized by Gut Viral Antigen Persistence in Inflammatory Bowel Diseases,” *Gastroenterology* 2022, 163, 2: 495-506.e8. doi: https://doi.org/10.1053/j.gastro.2022.04.037

375. Zurlow M et al., “The anti-SARS-CoV-2 BNT162b2 vaccine suppresses mithramycin-induced erythroid differentiation and expression of embryo-fetal globin genes in human erythroleukemia K562 cells.” *Exp Cell Res* 2023, 433, 2: 113853. doi: https://doi.org/10.1016/j.yexcr.2023.113853

II. CATEGORIES

A. General/Overview

1. Acevedo-Whitehouse K and R Bruno, "Potential health risks of mRNA-based vaccine therapy: A hypothesis," *Med. Hypotheses* 2023, 171: 111015. doi: https://doi.org/10.1016/j.mehy.2023.111015

2. Almehdi AM et al., “SARS-CoV-2 Spike Protein: Pathogenesis, Vaccines, and Potential Therapies,” *Infection* 2021, 49, 5: 855–876. doi: https://doi.org/10.1007/s15010-021-01677-8

3. Baldari CT et al., “Emerging Roles of SARS-CoV-2 Spike-ACE2 in Immune Evasion and Pathogenesis,” *Trends Immunol.* 2023, 44, 6. doi: 10.1016/j.it.2023.04.001

4. Bansal S et al., “Cutting Edge: Circulating Exosomes with COVID Spike Protein Are Induced by BNT162b2 (Pfizer-BioNTech) Vaccination prior to Development of Antibodies: A Novel Mechanism for Immune Activation by mRNA Vaccines,” *J. Immunol.* 2021, 207, 10: 2405–2410. doi: 10.4049/jimmunol.2100637

5. Bellucci M et al., “Post-SARS-CoV-2 infection and post-vaccine-related neurological complications share clinical features and the same positivity to anti-ACE2 antibodies,” *Front. Immunol.* 2024, 15 (Sec. Multiple Sclerosis and Neuroimmunology). doi: https://doi.org/10.3389/fimmu.2024.1398028

6. Boros LG et al., “Long-lasting, biochemically modified mRNA, and its frameshifted recombinant spike proteins in human tissues and circulation after COVID-19 vaccination,” *Pharmacol Res Perspect* 2024, 12, 3: e1218. doi: 10.1002/prp2.1218

7. Brady M et al., “Spike protein multiorgan tropism suppressed by antibodies targeting SARS-CoV-2,” *Comm. Biol.* 2021, 4, 1318. doi: 10.1038/s42003-021-02856-x

8. Cari L et al., “Differences in the expression levels of SARS-CoV-2 spike protein in cells treated with mRNA-based COVID-19 vaccines: a study on vaccines from the real world,” *Vaccines* 2023, 11, 4: 879. doi: 10.3390/vaccines11040879

9. Cosentino M and Franca Marino, “Understanding the Pharmacology of COVID- 19 mRNA Vaccines: Playing Dice with the Spike?” *Int. J. Mol. Sci.* 2022, 23, 18: 10881. doi: https://doi.org/10.3390/ijms231810881

10. Fertig TE et al., “Beyond the injection site: identifying the cellular targets of mRNA vaccines,” J *Cell Ident* 2024, 3, 1. doi: 10.47570/joci.2024.004

11. Fertig TE et al., “Vaccine mRNA Can Be Detected in Blood at 15 Days Post Vaccination,” *Biomedicines* 2022, 10, 7: 1538. doi: 10.3390/biomedicines10071538

12. Giannotta G et al., “COVID-19 mRNA Vaccines: The Molecular Basis of Some Adverse Events,” *Vaccines* 2023, 11, 4: 747. doi: 10.3390/vaccines11040747

13. Gussow AB et al., “Genomic Determinants of Pathogenicity in SARS-CoV-2 and Other Human Coronaviruses,” *PNAS* 117, 2020, 26: 15193–15199. doi: https://doi.org/10.1073/pnas.2008176117

14. Halma MTJ et al., “Strategies for the Management of Spike Protein-Related Pathology,” *Microorganisms* 2023, 11, 5: 1308, doi: https://doi.org/10.3390/microorganisms11051308

15. Kent SJ et al., “Blood Distribution of SARS-CoV-2 Lipid Nanoparticle mRNA Vaccine in Humans,” *ACS Nano* 2024, 18, 39: 27077-27089. doi: 10.1021/acsnano.4c11652

16. Kowarz E et al., “Vaccine-induced COVID-19 mimicry syndrome,” *eLife* 2022, 11: e74974. doi: https://doi.org/10.7554/eLife.74974

17. Lamprinou M et al., “COVID-19 vaccines adverse events: potential molecular mechanisms,” *Immunol. Res.* 2023, 71: 356-372. doi: 10.1007/s12026-023-09357-5

18. Lehmann KJ, “Impact of SARS-CoV-2 Spikes on Safety of Spike-Based COVID-19 Vaccinations,” *Immunome Res.* 2024, 20, 2: 1000267. doi: 10.35248/1745-7580.24.20.267

19. Lehmann KJ, “Suspected Causes of the Specific Intolerance Profile of Spike-Based Covid-19 Vaccines,” *Med. Res. Arch* 2024, 12, 9. doi: 10.18103/mra.v12i9.5704

20. Lesgard JF et al., “Toxicity of SARS-CoV-2 Spike Protein from the Virus and Produced from COVID-19 mRNA or Adenoviral DNA Vaccines,” *Arch Microbiol Immun* 2023, 7, 3: 121- 138. doi: 10.26502/ami.936500110

21. Letarov AV et al., “Free SARS-CoV-2 Spike Protein S1 Particles May Play a Role in the Pathogenesis of COVID-19 Infection,” *Biochemistry* (Moscow) 2021, 86, 257–261. doi: https://doi.org/10.1134/S0006297921030032

22. Nuovo JG et al., “Endothelial Cell Damage Is the Central Part of COVID-19 and a Mouse Model Induced by Injection of the S1 Subunit of the Spike Protein,” *Ann. Diagn. Pathol.* 2021, 51, 151682. doi: 10.1016/j.anndiagpath.2020.151682

23. Pallas RM, “Innate and adaptative immune mechanisms of COVID-19 vaccines. Serious adverse events associated with SARS-CoV-2 vaccination: A systematic review,” *Vacunas* (English ed.) 2024, 25, 2: 285.e1-285.e94. doi: https://doi.org/10.1016/j.vacune.2024.05.002

24. Parry PL et al., “‘Spikeopathy’: COVID-19 Spike Protein Is Pathogenic, from Both Virus and Vaccine mRNA,” *Biomedicine* 2023, 11, 8: 2287. doi: https://doi.org/10.3390/biomedicines11082287

25. Pateev I et al., “Biodistribution of RNA Vaccines and of Their Products: Evidence from Human and Animal Studies,” *Biomedicines* 2024, 12, 1: 59. doi: https://doi.org/10.3390/biomedicines12010059

26. Peluso MJ et al., “Plasma-based antigen persistence in the post-acute phase of COVID-19,” *Lancet* 2024, 24, 6: E345-E347. doi: 10.1016/S1473-3099(24)00211-1

27. Rzymski P and Andrzej Fal, “To aspirate or not to aspirate? Considerations for the COVID-19 vaccines,” *Pharmacol. Rep* 2022, 74: 1223–1227. doi: https://doi.org/10.1007/s43440-022-00361-4

28. Saadi F et al., “Spike glycoprotein is central to coronavirus pathogenesis-parallel between m-CoV and SARS-CoV-2,” *Ann Neurosci.* 2021, 28 (3-4): 201–218. doi: https://doi.org/10.1177/09727531211023755

29. Sacco K et al., “Immunopathological signatures in multisystem inflammatory syndrome in children and pediatric COVID-19,” *Nat. Med.* 2022, 28: 1050-1062. doi: https://doi.org/10.1038/s41591-022-01724-3

30. Scholkmann F and CA May, “COVID-19, post-acute COVID-19 syndrome (PACS, ‘long COVID’) and post-COVID-19 vaccination syndrome (PCVS, ‘post-COVIDvac-syndrome’): Similarities and differences,” *Pathol Res Pract.* 2023, 246: 154497. doi: https://doi.org/10.1016/j.prp.2023.154497

31. Schwartz L et al., “Toxicity of the spike protein of COVID-19 is a redox shift phenomenon: A novel therapeutic approach,” *Free Rad. Biol. Med.* 2023, 206: 106–110. doi: https://doi.org/10.1016/j.freeradbiomed.2023.05.034

32. Swank Z, et al. “Persistent Circulating Severe Acute Respiratory Syndrome Coronavirus 2 Spike Is Associated With Post-acute Coronavirus Disease 2019 Sequelae,” *Clin. Infect. Dis* 2023, 76, 3: e487–e490. doi: 10.1093/cid/ciac722

33. Theoharides TC, “Could SARS-CoV-2 Spike Protein Be Responsible for Long-COVID Syndrome?” *Mol. Neurobiol.* 2022, 59, 3: 1850–1861. doi: 10.1007/s12035-021-02696-0

34. Theoharides TC and P. Conti, “Be Aware of SARS-CoV-2 Spike Protein: There Is More Than Meets the Eye,” *J Biol Reg Homeostat Agents* 2021, 35, 3: 833–838. doi: 10.23812/THEO_EDIT_3_21

35. Trougakos IP et al., “Adverse Effects of COVID-19 mRNA Vaccines: The Spike Hypothesis,” *Trends Mol Med.* 2022, 28, 7: 542–554. doi: 10.1016/j.molmed.2022.04.007

36. Tyrkalska SD et al., “Differential proinflammatory activities of spike proteins of SARS-CoV-2 variants of concern,” *Sci. Adv.* 2022, 8, 37: eabo0732. doi: 10.1126/sciadv.abo0732

B. ACE2

1. Aboudounya MM and RJ Heads, “COVID-19 and Toll-Like Receptor 4 (TLR4): SARS-CoV-2 May Bind and Activate TLR4 to Increase ACE2 Expression, Facilitating Entry and Causing Hyperinflammation,” *Mediators Inflamm.* 2021, 8874339. doi: https://doi.org/10.1155/2021/8874339

2. Aksenova AY et al., “The increased amyloidogenicity of Spike RBD and pH-dependent binding to ACE2 may contribute to the transmissibility and pathogenic properties of SARS-CoV-2 omicron as suggested by in silico study,” *Int J Mol Sci.* 2022, 23, 21: 13502. doi: https://doi.org/10.3390/ijms232113502

3. Angeli F et al., “COVID-19, vaccines and deficiency of ACE2 and other angiotensinases. Closing the loop on the ‘Spike effect’,” *Eur J. Intern. Med.* 2022, 103: 23–28. doi: 10.1016/j.ejim.2022.06.015

4. Baldari CT et al., “Emerging Roles of SARS-CoV-2 Spike-ACE2 in Immune Evasion and Pathogenesis,” *Trends Immunol.* 2023, 44, 6. doi: 10.1016/j.it.2023.04.001

5. Bellucci M et al., “Post-SARS-CoV-2 infection and post-vaccine-related neurological complications share clinical features and the same positivity to anti-ACE2 antibodies,” *Front. Immunol.* 2024, 15 (Sec. Multiple Sclerosis and Neuroimmunology). doi: https://doi.org/10.3389/fimmu.2024.1398028

6. Devaux CA and L. Camoin-Jau, “Molecular mimicry of the viral spike in the SARS-CoV-2 vaccine possibly triggers transient dysregulation of ACE2, leading to vascular and coagulation dysfunction similar to SARS-CoV-2 infection,” *Viruses* 2023, 15, 5: 1045. doi: https://doi.org/10.3390/v15051045

7. Foster K et al., “Abstract 111: Cerebrovascular Effects Of Pre/post-losartan Treatment In Humanized ACE2 Knock-in Mice After SARS-CoV-2 Spike Protein Injection,” *Stroke* 2023, 54. doi: https://doi.org/10.1161/str.54.suppl_1.11

8. Gao X et al., “Spike-Mediated ACE2 Down-Regulation Was Involved in the Pathogenesis of SARS-CoV-2 Infection,” *J. Infect.* 2022, 85, 4: 418–427. doi: 10.1016/j.jinf.2022.06.030

9. Jabi MSA et al., “Abstract 53: Covid-19 Spike-protein Causes Cerebrovascular Rarefaction And Deteriorates Cognitive Functions In A Mouse Model Of Humanized ACE2,” *Stroke* 2022, 53. doi: https://doi.org/10.1161/str.53.suppl_1.53

10. Kato Y et al., “TRPC3-Nox2 Protein Complex Formation Increases the Risk of SARS-CoV-2 Spike Protein-Induced Cardiomyocyte Dysfunction through ACE2 Upregulation,” *Int. J. Mol. Sci.* 2023, 24, 1: 102. doi: 10.3390/ijms24010102

11. Ken W et al., “Low dose radiation therapy attenuates ACE2 depression and inflammatory cytokines induction by COVID-19 viral spike protein in human bronchial epithelial cells,” *Int J Radiat Biol.* 2022, 98, 10: 1532-1541. doi: https://doi.org/10.1080/09553002.2022.2055806

12. Lei Y et al., “SARS-CoV-2 Spike Protein Impairs Endothelial Function via Downregulation of ACE 2,” *Circulation Research* 2021, 128, 9: 1323–1326. doi: https://doi.org/10.1161/CIRCRESAHA.121.318902

13. Lu J and PD Sun, “High affinity binding of SARS-CoV-2 spike protein enhances ACE2 carboxypeptidase activity,” *J. Biol. Chem* 2020, 295, 52: p18579-18588. doi: 10.1074/jbc.RA120.015303

14. Maeda Y et al., “Differential Ability of Spike Protein of SARS-CoV-2 Variants to Downregulate ACE2,” *Int. J. Mol. Sci.* 2024, 25, 2: 1353. doi: 10.3390/ijms25021353

15. Magro N et al., “Disruption of the blood-brain barrier is correlated with spike endocytosis by ACE2 + endothelia in the CNS microvasculature in fatal COVID-19. Scientific commentary on ‘Detection of blood-brain barrier disruption in brains of patients with COVID-19, but no evidence of brain penetration by SARS-CoV-2’,” *Acta Neuropathol.* 2024, 147, 1: 47. doi: https://doi.org/10.1007/s00401-023-02681-y

16. Montezano AC et al., “SARS-CoV-2 spike protein induces endothelial inflammation via ACE2 independently of viral replication,” *Sci Rep.* 2023, 13, 1: 14086. doi: https://doi.org/10.1038/s41598-023-41115-3

17. Satta S et al., “An engineered nano-liposome-human ACE2 decoy neutralizes SARS-CoV-2 Spike protein-induced inflammation in both murine and human macrophages,” *Theranostics* 2022, 12, 6: 2639–2657. doi: 10.7150/thno.66831

18. Solopov et al., “Alcohol increases lung angiotensin-converting enzyme 2 expression and exacerbates severe acute respiratory syndrome coronavirus 2 spike protein subunit 1-induced acute lung injury in K18-hACE2 transgenic mice,” *Am J Pathol* 2022, 192, 7: 990-1000. doi: 10.1016/j.ajpath.2022.03.012

19. Sui Y et al., “SARS-CoV-2 Spike Protein Suppresses ACE2 and Type I Interferon Expression in Primary Cells From Macaque Lung Bronchoalveolar Lavage,” *Front. Immunol.* 2021, 12. doi: https://doi.org/10.3389/fimmu.2021.658428

20. Tetz G and Victor Tetz, “Prion-Like Domains in Spike Protein of SARS-CoV-2 Differ across Its Variants and Enable Changes in Affinity to ACE2,” *Microorganisms* 2025, 10, 2: 280. doi: https://doi.org/10.3390/microorganisms10020280

21. Vargas-Castro R et al., “Calcitriol prevents SARS-CoV spike-induced inflammation in human trophoblasts through downregulating *ACE2* and *TMPRSS2* expression,” *J Steroid Biochem Mol Biol* 2025, 245: 106625. doi: 10.1016/j.jsbmb.2024.106625

22. Youn JY et al., “Therapeutic application of estrogen for COVID-19: Attenuation of SARS-CoV-2 spike protein and IL-6 stimulated, ACE2-dependent NOX2 activation, ROS production and MCP-1 upregulation in endothelial cells,” *Redox Biol.* 2021, 46: 102099. doi: https://doi.org/10.1016/j.redox.2021.102099

23. Zhang S et al., “SARS-CoV-2 Binds Platelet ACE2 to Enhance Thrombosis in COVID-19,” *J. Hematol. Oncol.* 2020, 13, 120: 120. doi: 10.1186/s13045-020-00954-7

C. Amyloid, prion-like properties

1. Aksenova AY et al., “The increased amyloidogenicity of Spike RBD and pH-dependent binding to ACE2 may contribute to the transmissibility and pathogenic properties of SARS-CoV-2 omicron as suggested by in silico study,” *Int. J. Mol. Sci.* 2022, 23, 21: 13502. doi: https://doi.org/10.3390/ijms232113502

2. Cao S et al., “Spike Protein Fragments Promote Alzheimer’s Amyloidogenesis,” *ACS Appl. Mater. Interfaces* 2023, 15, 34: 40317-40329. doi: 10.1021/acsami.3c09815

3. Chakrabarti SS et al., “Rapidly Progressive Dementia with Asymmetric Rigidity Following ChAdOx1 nCoV-19 Vaccination,” *Aging Dis.* 2022, 13, 3: 633-636. doi: 10.14336/AD.2021.1102

4. Freeborn J, “Misfolded Spike Protein Could Explain Complicated COVID-19 Symptoms,” Medical News Today, May 26, 2022, https://www.medicalnewstoday.com/articles/misfolded-spike-protein-could-explain-complicated-covid-19-symptoms

5. Idrees D and Vijay Kumar, “SARS-CoV-2 Spike Protein Interactions with Amyloidogenic Proteins: Potential Clues to Neurodegeneration,” *Biochemical and Biophysical Research Communications* 2021, 554 : 94–98. doi: https://doi.org/10.1016/j.bbrc.2021.03.100

6. Hillard P et al., “Abstract WP400: SARS-CoV-2 Spike Protein Accelerates Alzheimer’s Disease-Related Dementia Through Increased Cerebrovascular Inflammation in hACE2 Mice,” *Stroke* 2025, 56. doi: https://doi.org/10.1161/str.56.suppl_1.WP400

7. Ma G et al., “SARS-CoV-2 Spike protein S2 subunit modulates γ-secretase and enhances amyloid-β production in COVID-19 neuropathy,” *Cell Discov* 2022, 8, 99. doi: https://doi.org/10.1038/s41421-022-00458-3

8. Nahalka J, “1-L Transcription of SARS-CoV-2 Spike Protein S1 Subunit,” *Int. J. Mol. Sci.* 2024, 25, 8: 4440. doi: https://doi.org/10.3390/ijms25084440

9. Nyström S, “Amyloidogenesis of SARS-CoV-2 Spike Protein,” *J. Am. Chem. Soc.* 2022, 144, 8945–8950. doi: https://doi.org/10.1021/jacs.2c03925

10. Petrlova J et al., “SARS-CoV-2 spike protein aggregation is triggered by bacterial lipopolysaccharide,” *FEBS Lett.* 2022, 596:2566–2575. doi: https://doi.org/10.1002/1873-3468.14490

11. Petruk G et al., “SARS-CoV-2 spike protein binds to bacterial lipopolysaccharide and boosts proinflammatory activity,” *J. Mol. Cell Biol.* 2020, 12: 916-932. doi: https://doi.org/10.1093/jmcb/mjaa067

12. Rong Z et al., “Persistence of spike protein at the skull-meninges-brain axis may contribute to the neurological sequelae of COVID-19,” *Cell Host Microbe* 2024, 26: S1931-3128(24)00438-4. doi: 10.1016/j.chom.2024.11.007

13. Tetz G and Victor Tetz, “Prion-Like Domains in Spike Protein of SARS-CoV-2 Differ across Its Variants and Enable Changes in Affinity to ACE2,” *Microorganisms* 2022, 10, 2: 280, doi: https://doi.org/10.3390/microorganisms10020280

14. Wang J et al., “SARS-CoV-2 Spike Protein S1 Domain Accelerates α-Synuclein Phosphorylation and Aggregation in Cellular Models of Synucleinopathy,” *Mol Neurobiol.* 2024, 61, 4: 2446-2458. doi: 10.1007/s12035-023-03726-9

D. Autoimmune

1. Anft M et al., “Effect of immunoadsorption on clinical presentation and immune alterations in COVID-19–induced and/or aggravated ME/CFS,” *Mol. Ther.* 2025, 33, 6: 2886-2899. doi: 10.1016/j.ymthe.2025.01.007

2. Chen Y et al., “New-onset autoimmune phenomena post-COVID-19 vaccination,” *Immunology* 2022, 165, 4: 386-401. doi: https://doi.org/10.1111/imm.13443

3. Cheng MY et al., “Clinical Research into Central Nervous System Inflammatory Demyelinating Diseases Related to COVID-19 Vaccines,” *Diseases* 2024, 12, 3: 60. doi: https://doi.org/10.3390/diseases12030060

4. Diaz M et al., “SARS-CoV-2 spike peptide analysis reveals a highly conserved region that elicits potentially pathogenic autoantibodies: implications to pan-coronavirus vaccine development,” *Front. Immunol.* 2025, 16 (Sec. B Cell Biology). doi: https://doi.org/10.3389/fimmu.2025.1488388

5. Elrashdy F et al., “Autoimmunity roots of the thrombotic events after COVID-19 vaccination,” *Autoimmun. Rev.* 2021, 20, 11: 102941. doi: 10.1016/j.autrev.2021.102941

6. Heil M, “Self-DNA driven inflammation in COVID-19 and after mRNA-based vaccination: lessons for non-COVID-19 pathologies,” *Front. Immunol.,* 2023, 14. doi: https://doi.org/10.3389/fimmu.2023.1259879

7. Kanduc D, “From Anti-SARS-CoV-2 Immune Responses to COVID-19 via Molecular Mimicry,” *Antibodies* 2020, 9, 3: 33. doi: https://doi.org/10.3390/antib9030033

8. Kanduc D and Y Shoenfeld, “Molecular mimicry between SARS-CoV-2 spike glycoprotein and mammalian proteomes: implications for the vaccine,” *Immunol Res* 2020, 68: 310-313. doi: https://doi.org/10.1007/s12026-020-09152-6

9. Lee AR et al., “SARS-CoV-2 spike protein promotes inflammatory cytokine activation and aggravates rheumatoid arthritis,” *Cell Commun Signal.* 2023, 21, 1: 44. doi: https://doi.org/10.1186/s12964-023-01044-0

10. Nunez-Castilla J et al., “Potential autoimmunity resulting from molecular mimicry between SARS-CoV-2 spike and human proteins,” *Viruses* 2022, 14, 7: 1415. doi: https://doi.org/10.3390/v14071415

11. Polykretis P et al., “Autoimmune Inflammatory Reactions Triggered by the COVID-19 Genetic Vaccines in Terminally Differentiated Tissues,” *Autoimmunity* 2023, 56: 2259123. doi: https://doi.org/10.1080/08916934.2023.2259123

12. Rodriguez Y et al., “Autoinflammatory and autoimmune conditions at the crossroad of COVID-19,” *J. Autoimmun.* 2020, 114: 102506. doi: 10.1016/j.jaut.2020.102506

13. Vojdani A and D Kharrazian, “Potential antigenic cross-reactivity between SARS-CoV-2 and human tissue with a possible link to an increase in autoimmune diseases,” *Clin Immunol.* 2020, 217: 108480. doi: 10.1016/j.clim.2020.108480

14. Vojdani A et al., “Reaction of Human Monoclonal Antibodies to SARS-CoV-2 Proteins With Tissue Antigens: Implications for Autoimmune Diseases,” *Front. Immunol.* 2021, 11 (Sec. Autoimmune and Autoinflammatory Disorders). doi: https://doi.org/10.3389/fimmu.2020.617089

E. Blood pressure/hypertension

1. Angeli F et al., “The spike effect of acute respiratory syndrome coronavirus 2 and coronavirus disease 2019 vaccines on blood pressure,” *Eur J Intern Med.* 2023, 109: 12-21. doi: 10.1016/j.ejim.2022.12.004

2. Sun Q et al., “SARS-coV-2 spike protein S1 exposure increases susceptibility to angiotensin II-induced hypertension in rats by promoting central neuroinflammation and oxidative stress,” *Neurochem. Res.* 2023, 48, 3016–3026. doi: https://doi.org/10.1007/s11064-023-03949-1

F. CD147

1. Avolio E et al., “The SARS-CoV-2 Spike Protein Disrupts Human Cardiac Pericytes Function through CD147 Receptor-Mediated Signalling: A Potential Non-infective Mechanism of COVID-19 Microvascular Disease,” *Clin. Sci.* 2021, 135, 24: 2667–2689. doi: https://doi.org/10.1042/CS20210735

2. Loh D, “The potential of melatonin in the prevention and attenuation of oxidative hemolysis and myocardial injury from cd147 SARS-CoV-2 spike protein receptor binding,” *Melatonin Research* 2020, 3, 3: 380-416. doi: 10.32794/mr11250069

3. Maugeri N et al., “Unconventional CD147-Dependent Platelet Activation Elicited by SARS-CoV-2 in COVID-19,” *J. Thromb. Haemost.* 2021, 20, 2: 434–448. doi: 10.1111/jth.15575

G. Cell membrane permeability, barrier dysfunction

1. Asandei A et al., “Non-Receptor-Mediated Lipid Membrane Permeabilization by the SARS-CoV-2 Spike Protein S1 Subunit,” *ACS Appl. Mater. Interfaces* 2020, 12, 50: 55649–55658. doi: https://doi.org/10.1021/acsami.0c17044

2. Biancatelli RMLC, et al. “The SARS-CoV-2 spike protein subunit S1 induces COVID-19-like acute lung injury in Kappa18-hACE2 transgenic mice and barrier dysfunction in human endothelial cells,” *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2021, 321: L477–L484. doi: https://doi.org/10.1152/ajplung.00223.2021

3. Biering SB et al., “SARS-CoV-2 Spike Triggers Barrier Dysfunction and Vascular Leak via Integrins and TGF-β Signaling,” *Nat. Commun.* 2022, 13: 7630. doi: https://doi.org/10.1038/s41467-022-34910-5

4. Buzhdygan TP et al., “The SARS-CoV-2 Spike Protein Alters Barrier Function in 2D Static and 3D Microfluidic in-Vitro Models of the Human Blood-Brain Barrier,” *Neurobiol. Dis.* 2020, 146: 105131. doi: 10.1016/j.nbd.2020.105131

5. Cappalletti G et al., “iPSC-derived human cortical organoids display profound alterations of cellular homeostasis following SARS-CoV-2 infection and Spike protein exposure,” *FASEB J* 2025 39, 4: e70396. doi: 10.1096/fj.202401604RRR

6. Chaves JCS et al., “Differential Cytokine Responses of *APOE3* and *APOE4* Blood–brain Barrier Cell Types to SARS-CoV-2 Spike Proteins,” *J. Neuroimmune Pharmacol.* 2024, 19, 22. doi: https://doi.org/10.1007/s11481-024-10127-9

7. Correa Y et al., “SARS-CoV-2 spike protein removes lipids from model membranes and interferes with the capacity of high-density lipoprotein to exchange lipids,” *J. Colloid Interface Sci.* 2021, 602: 732-739. doi: 10.1016/j.jcis.2021.06.056

8. DeOre BJ et al., “SARS-CoV-2 Spike Protein Disrupts Blood-Brain Barrier Integrity via RhoA Activation,” *J Neuroimmune Pharmacol.* 2021, 16, 4:722-728. Doi: https://doi.org/10.1007/s11481-021-10029-0

9. Fajloun Z et al., “COVID-19 and Ehlers-Danlos Syndrome: The Dangers of the Spike Protein of SARS-CoV-2,” *Infect. Disord. Drug Targets* 2023, 23, 3: 26-28. doi: https://doi.org/10.2174/1871526523666230104145108

10. Guo Y and V Kanamarlapudi, “Molecular Analysis of SARS-CoV-2 Spike Protein-Induced Endothelial Cell Permeability and vWF Secretion,” *Int. J. Mol. Sci.* 2023, 24, 6: 5664. doi: https://doi.org/10.3390/ijms24065664

11. Luchini A et al., “Lipid bilayer degradation induced by SARS-CoV-2 spike protein as revealed by neutron reflectometry,” *Sci. Rep.* 2021, 11: 14867. doi: https://doi.org/10.1038/s41598-021-93996-x

12. Luo Y et al., “SARS-Cov-2 spike induces intestinal barrier dysfunction through the interaction between CEACAM5 and Galectin-9,” *Front. Immunol.* 2024, 15. doi: https://doi.org/10.3389/fimmu.2024.1303356

13. Magro N et al., “Disruption of the blood-brain barrier is correlated with spike endocytosis by ACE2 + endothelia in the CNS microvasculature in fatal COVID-19. Scientific commentary on ‘Detection of blood-brain barrier disruption in brains of

patients with COVID-19, but no evidence of brain penetration by SARS-CoV-2'," *Acta Neuropathol.* 2024, 147, 1: 47. doi: https://doi.org/10.1007/s00401-023-02681-y

14. Raghavan S et al., "SARS-CoV-2 Spike Protein Induces Degradation of Junctional Proteins That Maintain Endothelial Barrier Integrity," *Front. Cardiovasc. Med.* 2021, 8, 687783. doi: https://doi.org/10.3389/fcvm.2021.687783

15. Ruben ML et al., "The SARS-CoV-2 spike protein subunit S1 induces COVID-19-like acute lung injury in K18-hACE2 transgenic mice and barrier dysfunction in human endothelial cells," *Am J Physiol Lung Cell Mol Physiol.* 2021, 321, 2: L477-L484. doi: https://doi.org/10.1152/ajplung.00223.2021

16. Yang K et al., "SARS-CoV-2 spike protein receptor-binding domain perturbates intracellular calcium homeostasis and impairs pulmonary vascular endothelial cells," *Signal Transduct. Target. Ther.* 2023, 8, 276. doi: 10.1038/s41392-023-01556-8

H. Cerebral, cerebrovascular, neurologic, blood-brain barrier, cognitive

1. Bellucci M et al., "Post-SARS-CoV-2 infection and post-vaccine-related neurological complications share clinical features and the same positivity to anti-ACE2 antibodies," *Front. Immunol.* 2024, 15 (Sec. Multiple Sclerosis and Neuroimmunology). doi: https://doi.org/10.3389/fimmu.2024.1398028

2. Burnett FN et al., "SARS-CoV-2 Spike Protein Intensifies Cerebrovascular Complications in Diabetic hACE2 Mice through RAAS and TLR Signaling Activation," *Int. J. Mol. Sci.* 2023, 24, 22: 16394. doi: https://doi.org/10.3390/ijms242216394

3. Choi JY et al., "SARS-CoV-2 spike S1 subunit protein-mediated increase of beta-secretase 1 (BACE1) impairs human brain vessel cells," *Biochem. Biophys. Res. Commun.* 2022, 625, 20: 66-71. doi: https://doi.org/10.1016/j.bbrc.2022.07.113

4. Clough E et al., "Mitochondrial Dynamics in SARS-COV2 Spike Protein Treated Human Microglia: Implications for Neuro-COVID," *J. Neuroimmune Pharmacol.* 2021, 4, 16: 770–784. doi: https://doi.org/10.1007/s11481-021-10015-6

5. Coly M, et al., "Subacute monomelic radiculoplexus neuropathy following Comirnaty(c) (Pfizer-BioNTech COVID-19) vaccination: A case report," *Revue Neurologique* 2023, 179, 6: 636-639. doi: 10.1016/j.neurol.2023.02.063

6. DeOre BJ et al., "SARS-CoV-2 Spike Protein Disrupts Blood-Brain Barrier Integrity via RhoA Activation," *J Neuroimmune Pharmacol.* 2021, 16, 4: 722-728. Doi: https://doi.org/10.1007/s11481-021-10029-0

7. Erdogan MA, “Prenatal SARS-CoV-2 Spike Protein Exposure Induces Autism-Like Neurobehavioral Changes in Male Neonatal Rats,” *J Neuroimmune Pharmacol.* 2023, 18, 4: 573-591. doi: 10.1007/s11481-023-10089-4

8. Erickson MA et al., “Blood-brain barrier penetration of non-replicating SARS-CoV-2 and S1 variants of concern induce neuroinflammation which is accentuated in a mouse model of Alzheimer’s disease,” *Brain Behav Immun* 2023, 109: 251-268. doi: https://doi.org/10.1016/j.bbi.2023.01.010

9. Fontes-Dantas FL, “SARS-CoV-2 Spike Protein Induces TLR4-Mediated Long- Term Cognitive Dysfunction Recapitulating Post-COVID-19 Syndrome in Mice,” *Cell Reports* 2023, 42, 3: 112189. doi: https://doi.org/10.1016/j.celrep.2023.112189

10. Foster K et al., “Abstract 111: Cerebrovascular Effects Of Pre/post-losartan Treatment In Humanized ACE2 Knock-in Mice After SARS-CoV-2 Spike Protein Injection,” *Stroke* 2023, 54. doi: https://doi.org/10.1161/str.54.suppl_1.11

11. Frank MG et al., “Exploring the immunogenic properties of SARS-CoV-2 structural proteins: PAMP:TLR signaling in the mediation of the neuroinflammatory and neurologic sequelae of COVID-19,” *Brain Behav Immun* 2023, 111. doi: https://doi.org/10.1016/j.bbi.2023.04.009

12. Frank MG et al., “SARS-CoV-2 S1 subunit produces a protracted priming of the neuroinflammatory, physiological, and behavioral responses to a remote immune challenge: A role for corticosteroids,” *Brain Behav. Immun.* 2024, 121: 87-103. doi: https://doi.org/10.1016/j.bbi.2024.07.034

13. Heath SP et al., “SARS-CoV-2 Spike Protein Exacerbates Thromboembolic Cerebrovascular Complications in Humanized ACE2 Mouse Model,” *Transl Stroke Res.* 2024. doi: https://doi.org/10.1007/s12975-024-01301-5

14. Hillard P et al., “Abstract WP400: SARS-CoV-2 Spike Protein Accelerates Alzheimer’s Disease-Related Dementia Through Increased Cerebrovascular Inflammation in hACE2 Mice,” *Stroke* 2025, 56. doi: https://doi.org/10.1161/str.56.suppl_1.WP400

15. Jabi MSA et al., “Abstract 53: Covid-19 Spike-protein Causes Cerebrovascular Rarefaction And Deteriorates Cognitive Functions In A Mouse Model Of Humanized ACE2,” *Stroke* 2022, 53. doi: https://doi.org/10.1161/str.53.suppl_1.53

16. Khaddaj-Mallat R et al., “SARS-CoV-2 deregulates the vascular and immune functions of brain pericytes via Spike protein,” *Neurobiol. Dis.* 2021, 161, 105561. doi: https://doi.org/10.1016/j.nbd.2021.105561

17. Kim ES et al., “Spike Proteins of SARS-CoV-2 Induce Pathological Changes in Molecular Delivery and Metabolic Function in the Brain Endothelial Cells,” *Viruses* 2021, 13, 10: 2021. doi: https://doi.org/10.3390/v13102021

18. Lykhmus O et al., “Immunization with 674–685 fragment of SARS-Cov-2 spike protein induces neuroinflammation and impairs episodic memory of mice,” *Biochem. Biophys. Res. Commun.* 2022, 622: 57–63. doi: https://doi.org/10.1016/j.bbrc.2022.07.016

19. Magro N et al., “Disruption of the blood-brain barrier is correlated with spike endocytosis by ACE2 + endothelia in the CNS microvasculature in fatal COVID-19. Scientific commentary on ‘Detection of blood-brain barrier disruption in brains of patients with COVID-19, but no evidence of brain penetration by SARS-CoV-2’,” *Acta Neuropathol.* 2024, 147, 1: 47. doi: https://doi.org/10.1007/s00401-023-02681-y

20. Oh J et al., “SARS-CoV-2 Spike Protein Induces Cognitive Deficit and Anxiety-Like Behavior in Mouse via Non-cell Autonomous Hippocampal Neuronal Death,” *Scientific Reports* 2022, 12, 5496. doi: https://doi.org/10.1038/s41598-022-09410-7

21. Oka N et al., “SARS-CoV-2 S1 protein causes brain inflammation by reducing intracerebral acetylcholine production,” *iScience* 2023, 26, 6: 106954. doi: 10.1016/j.isci.2023.106954

22. Ota N et al., “Expression of SARS-CoV-2 spike protein in cerebral Arteries: Implications for hemorrhagic stroke Post-mRNA vaccination,” *J. Clin. Neurosci.* 2025, 136: 111223. doi: https://doi.org/10.1016/j.jocn.2025.111223

23. Peluso MJ et al., “SARS-CoV-2 and mitochondrial proteins in neural-derived exosomes of COVID-19,” *Ann Neurol* 2022, 91, 6: 772-781. doi: 10.1002/ana.26350

24. Petrovszki D et al., “Penetration of the SARS-CoV-2 Spike Protein across the Blood-Brain Barrier, as Revealed by a Combination of a Human Cell Culture Model System and Optical Biosensing,” *Biomedicines* 2022, 10, 1: 188. doi: https://doi.org/10.3390/biomedicines10010188

25. Posa A, “Spike protein-related proteinopathies: A focus on the neurological side of spikeopathies,” *Ann Anat. - Anatomischer Anzeiger* 2025, 260: 152662. doi: https://doi.org/10.1016/j.aanat.2025.152662

26. Rong Z et al., “Persistence of spike protein at the skull-meninges-brain axis may contribute to the neurological sequelae of COVID-19,” *Cell Host Microbe* 2024, 26: S1931-3128(24)00438-4. doi: 10.1016/j.chom.2024.11.007

27. Suprewicz L et al., “Blood-brain barrier function in response to SARS-CoV-2 and its spike protein,” *Neurol. Neurochir Pol.* 2023, 57: 14–25. doi: 10.5603/PJNNS.a2023.0014

28. Suprewicz L et al., “Recombinant human plasma gelsolin reverses increased permeability of the blood-brain barrier induced by the spike protein of the SARS-CoV-2 virus,” *J Neuroinflamm.* 2022, 19, 1: 282. doi: 10.1186/s12974-022-02642-4

29. Wu ML et al., “Mast cell activation triggered by SARS-CoV-2 causes inflammation in brain microvascular endothelial cells and microglia,” *Front. Cell. Infect. Microbiol.* 2024, 14. doi: https://doi.org/10.3389/fcimb.2024.1358873

I. Clinical pathology

1. Baumeier C et al., “Intramyocardial Inflammation after COVID-19 Vaccination: An Endomyocardial Biopsy-Proven Case Series,” *Int. J. Mol. Sci*. 2022, 23: 6940. doi: https://doi.org/10.3390/ijms23136940

2. Burkhardt A, “Pathology Conference: Vaccine-Induced Spike Protein Production in the Brain, Organs etc., now Proven,” Report24.news. 2022, https://report24.news/pathologie-konferenz-impfinduzierte-spike-produktion-in-gehirn-u-a-organen-nun-erwiesen/

3. Codoni G et al., “Histological and serological features of acute liver injury after SARS-CoV-2 vaccination,” *JHP Rep.* 2023, 5, 1: 100605. doi: 10.1016/j.jhepr.2022.100605

4. Craddock V et al., “Persistent circulation of soluble and extracellular vesicle-linked Spike protein in individuals with postacute sequelae of COVID-19,” *J Med. Virol.* 2023, 95, 2: e28568. doi: https://doi.org/10.1002/jmv.28568

5. De Michele M et al., “Evidence of SARS-CoV-2 Spike Protein on Retrieved Thrombi from COVID-19 Patients,” *J. Hematol. Oncol.* 2022, 15, 108. doi: https://doi.org/10.1186/s13045-022-01329-w

6. De Sousa PMB et al., “Fatal Myocarditis following COVID-19 mRNA Immunization: A Case Report and Differential Diagnosis Review,” *Vaccines* 2024, 12, 2: 194. doi: https://doi.org/10.3390/vaccines12020194

7. Gamblicher T et al., “SARS-CoV-2 spike protein is present in both endothelial and eccrine cells of a chilblain-like skin lesion,” *J Eur Acad Dermatol Venereol.* 2020, 1, 10: e187-e189. doi: https://doi.org/10.1111/jdv.16970

8. Gawaz A et al., “SARS-CoV-2–Induced Vasculitic Skin Lesions Are Associated with Massive Spike Protein Depositions in Autophagosomes,” *J Invest Dermatol.* 2024, 144, 2: 369-377.e4. doi: https://doi.org/10.1016/j.jid.2023.07.018

9. Hulscher N et al., “Autopsy findings in cases of fatal COVID-19 vaccine-induced myocarditis,” *ESC Heart Failure* 2024. doi: https://doi.org/10.1002/ehf2.14680

10. Ko CJ et al., “Discordant anti-SARS-CoV-2 spike protein and RNA staining in cutaneous perniotic lesions suggests endothelial deposition of cleaved spike protein,” *J. Cutan Pathol* 2021, 48, 1: 47–52. doi: https://doi.org/10.1111/cup.13866

11. Magen E et al., “Clinical and Molecular Characterization of a Rare Case of BNT162b2 mRNA COVID-19 Vaccine-Associated Myositis,” *Vaccines* 2022, 10: 1135. doi: https://doi.org/10.3390/vaccines10071135

12. Magro N et al., “Disruption of the blood-brain barrier is correlated with spike endocytosis by ACE2 + endothelia in the CNS microvasculature in fatal COVID-19. Scientific commentary on ‘Detection of blood-brain barrier disruption in brains of patients with COVID-19, but no evidence of brain penetration by SARS-CoV-2’,” *Acta Neuropathol.* 2024, 147, 1: 47. doi: https://doi.org/10.1007/s00401-023-02681-y

13. Matschke J et al., “Neuropathology of patients with COVID-19 in Germany: a post-mortem case series,” *Lancet Neurol.* 2020, 19, 11: 919-929. doi: 10.1016/S1474-4422(20)30308-2

14. Mörz M, “A Case Report: Multifocal Necrotizing Encephalitis and Myocarditis after BNT162b2 mRNA Vaccination against COVID-19,” *Vaccines* 2022, 10, 10: 1651. doi: https://doi.org/10.3390/vaccines10101651

15. Rong Z et al., “Persistence of spike protein at the skull-meninges-brain axis may contribute to the neurological sequelae of COVID-19,” *Cell Host Microbe* 2024, 26: S1931-3128(24)00438-4. doi: 10.1016/j.chom.2024.11.007

16. Sano H et al., “A case of persistent, confluent maculopapular erythema following a COVID-19 mRNA vaccination is possibly associated with the intralesional spike protein expressed by vascular endothelial cells and eccrine glands in the deep dermis,” *J. Dermatol.* 2023, 50: 1208–1212. doi: 10.1111/1346-8138.16816

17. Sano S et al., “SARS-CoV-2 spike protein found in the acrosyringium and eccrine gland of repetitive miliaria-like lesions in a woman following mRNA vaccination,” *J. Dermatol.* 2024, 51, 9: e293-e295. doi: https://doi.org/10.1111/1346-8138.17204

18. Santonja C et al., “COVID-19 chilblain-like lesion: immunohistochemical demonstration of SARS-CoV-2 spike protein in blood vessel endothelium and sweat

gland epithelium in a polymerase chain reaction-negative patient," *Br J Dermatol*. 2020, 183, 4: 778-780. doi: https://doi.org/10.1111/bjd.19338

19. Soares CD et al., "Oral vesiculobullous lesions as an early sign of COVID-19: immunohistochemical detection of SARS-CoV-2 spike protein," *Br. J. Dermatol.* 2021, 184, 1: e6. doi: https://doi.org/10.1111/bjd.19569

20. Wu H et al., "Molecular evidence suggesting the persistence of residual SARS-CoV-2 and immune responses in the placentas of pregnant patients recovered from COVID-19," *Cell Prolif.* 2021, 54, 9: e13091. doi: https://doi.org/10.1111/cpr.13091

21. Yamamoto M et al., "Persistent varicella zoster virus infection following mRNA COVID-19 vaccination was associated with the presence of encoded spike protein in the lesion," *J. Cutan Immunol. Allergy.* 2022: 1-6. doi: 10.1002/cia2.12278

22. Yonker LM et al., "Circulating Spike Protein Detected in Post–COVID-19 mRNA Vaccine Myocarditis," *Circulation* 2023, 147, 11. doi: https://doi.org/10.1161/CIRCULATIONAHA.122.061025

23. Yonker LM et al., "Multisystem inflammatory syndrome in children is driven by zonulin-dependent loss of gut mucosal barrier," *J Clin Invest*. 2021, 131, 14: e149633. doi: https://doi.org/10.1172/JCI149633

J. Clotting, platelets, hemoglobin

1. Al-Kuraishy HM et al., "Changes in the Blood Viscosity in Patients With SARS-CoV-2 Infection," *Front. Med.* 2022, 9: 876017. doi: 10.3389/fmed.2022.876017

2. Al-Kuraishy HM et al., "Hemolytic anemia in COVID-19," *Ann. Hematol.* 2022, 101: 1887–1895. doi: 10.1007/s00277-022-04907-7

3. Appelbaum K et al., "SARS-CoV-2 spike-dependent platelet activation in COVID-19 vaccine-induced thrombocytopenia," *Blood Adv.* 2022, 6: 2250–2253. doi: 10.1182/bloodadvances.2021005050

4. Becker RC et al., "The COVID-19 thrombus: distinguishing pathological, mechanistic, and phenotypic features and management," *J. Thromb. Thrombolysis* 2025, 58: 15-49. doi: https://doi.org/10.1007/s11239-024-03028-4

5. Boschi C et al., "SARS-CoV-2 Spike Protein Induces Hemagglutination: Implications for COVID-19 Morbidities and Therapeutics and for Vaccine Adverse Effects," *Int. J. Biol. Macromol.* 2022, 23, 24: 15480, doi: https://doi.org/10.3390/ijms232415480

6. Bye AP et al., “Aberrant glycosylation of anti-SARS-CoV-2 spike IgG is a prothrombotic stimulus for platelets,” *Blood* 2021, 138, 6: 1481–9. doi: https://doi.org/10.1182/blood.2021011871

7. Carnevale R et al., “Toll-Like Receptor 4-Dependent Platelet-Related Thrombosis in SARS-CoV-2 Infection,” *Circ. Res.* 2023, 132, 3: 290– 305. doi: https://doi.org/10.1161/CIRCRESAHA.122.321541

8. Cossenza LC et al., “Inhibitory effects of SARS-CoV-2 spike protein and BNT162b2 vaccine on erythropoietin-induced globin gene expression in erythroid precursor cells from patients with β-thalassemia,” *Exp. Hematol.* 2024, 129, 104128. doi: https://doi.org/10.1016/j.exphem.2023.11.002

9. De Michele M et al., “Vaccine-induced immune thrombotic thrombocytopenia: a possible pathogenic role of ChAdOx1 nCoV-19 vaccine-encoded soluble SARS-CoV-2 spike protein,” *Haematologica* 2022, 107, 7: 1687–92. doi: https://doi.org/10.3324/haematol.2021.280180

10. Elrashdy F et al., “Autoimmunity roots of the thrombotic events after COVID-19 vaccination,” *Autoimmun. Rev.* 2021, 20, 11: 102941. doi: 10.1016/j.autrev.2021.102941

11. Gasparello J et al., “Assessing the interaction between hemoglobin and the receptor binding domain of SARS-CoV-2 spike protein through MARTINI coarse-grained molecular dynamics,” *Int. J. Biol. Macromol.,* 2023, 253: 127088. doi: 10.1016/j.ijbiomac.2023.127088

12. Grobbelaar LM et al., “SARS-CoV-2 Spike Protein S1 Induces Fibrin(ogen) Resistant to Fibrinolysis: Implications for Microclot Formation in COVID-19,” *Biosicence Reports* 2021, 41, 8: BSR20210611. doi: https://doi.org/10.1042/BSR20210611

13. Heath SP et al., “SARS-CoV-2 Spike Protein Exacerbates Thromboembolic Cerebrovascular Complications in Humanized ACE2 Mouse Model,” *Transl Stroke Res.* 2024. doi: https://doi.org/10.1007/s12975-024-01301-5

14. Iba T and JH Levy, “The roles of platelets in COVID-19-associated coagulopathy and vaccine-induced immune thrombotic thrombocytopenia,” *Trends Cardiovasc Med.* 2022, 32, 1: 1-9. doi: https://doi.org/10.1016/j.tcm.2021.08.012

15. Huynh TV et al., “Spike Protein of SARS-CoV-2 Activates Cardiac Fibrogenesis through NLRP3 Inflammasomes and NF-κB Signaling,” *Cells* 2024, 13, 16: 1331. doi: https://doi.org/10.3390/cells13161331

16. Iba T and JH Levy, “The roles of platelets in COVID-19-associated coagulopathy and vaccine-induced immune thrombotic thrombocytopenia,” *Trends Cardiovasc Med.* 2022, 32, 1: 1-9. doi: https://doi.org/10.1016/j.tcm.2021.08.012

17. Jana S et al., “Cell-free hemoglobin does not attenuate the effects of SARS-CoV-2 spike protein S1 subunit in pulmonary endothelial cells,” *Int. J. Mol. Sci.* 2021, 22, 16: 9041. doi: https://doi.org/10.3390/ijms22169041

18. Kim SY et al., “Characterization of heparin and severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) spike glycoprotein binding interactions,” *Antivir Res.* 2020, 181: 104873. doi: https://doi.org/10.1016/j.antiviral.2020.104873

19. Kircheis R, “Coagulopathies after Vaccination against SARS-CoV-2 May Be Derived from a Combined Effect of SARS-CoV-2 Spike Protein and Adenovirus Vector-Triggered Signaling Pathways,” *Int. J. Mol. Sci.* 2021, 22, 19: 10791. doi: https://doi.org/10.3390/ijms221910791

20. Kuhn CC et al. “Direct Cryo-ET observation of platelet deformation induced by SARS-CoV-2 spike protein,” *Nat. Commun.* 2023, 14, 620. doi: https://doi.org/10.1038/s41467-023-36279-5

21. Li T et al., “Platelets Mediate Inflammatory Monocyte Activation by SARS-CoV-2 Spike Protein,” *J. Clin. Invest.* 2022, 132, 4: e150101. doi: 10.1172/JCI150101

22. Maugeri N et al., “Unconventional CD147-Dependent Platelet Activation Elicited by SARS-CoV-2 in COVID-19,” *J. Thromb. Haemost.* 2021, 20, 2: 434–448, doi: https://doi.org/10.1111/jth.15575

23. Ota N et al., “Expression of SARS-CoV-2 spike protein in cerebral Arteries: Implications for hemorrhagic stroke Post-mRNA vaccination,” *J. Clin. Neurosci.* 2025, 136: 111223. doi: https://doi.org/10.1016/j.jocn.2025.111223

24. Passariello M et al., “Interactions of Spike-RBD of SARS-CoV-2 and Platelet Factor 4: New Insights in the Etiopathogenesis of Thrombosis,” *Int. J. Mol. Sci.* 2021, 22, 16: 8562. doi: https://doi.org/10.3390/ijms22168562

25. Perico L et al., “SARS-CoV-2 Spike Protein 1 Activates Microvascular Endothelial Cells and Complement System Leading to Platelet Aggregation,” *Front. Immunol.* 2022, 13, 827146. doi: https://doi.org/10.3389/fimmu.2022.827146

26. Roytenberg R et al., “Thymidine phosphorylase mediates SARS-CoV-2 spike protein enhanced thrombosis in K18-hACE2TG mice,” *Thromb. Res.* 2024, 244, 8: 109195. doi: 10.1016/j.thromres.2024.109195

27. Russo A, et al., “Implication of COVID-19 on Erythrocytes Functionality: Red Blood Cell Biochemical Implications and Morpho-Functional Aspects,” *Int. J. Mol. Sci.* 2022, 23, 4: 2171. doi: https://doi.org/10.3390/ijms23042171

28. Ryu JK et al., “Fibrin drives thromboinflammation and neuropathology in COVID-19,” *Nature* 2024, 633: 905-913. doi: https://doi.org/10.1038/s41586-024-07873-4

29. Scheim, DE. “A Deadly Embrace: Hemagglutination Mediated by SARS-CoV-2 Spike Protein at its 22 N-Glycosylation Sites, Red Blood Cell Surface Sialoglycoproteins, and Antibody,” *Int. J. Mol. Sci.* 2022, 23, 5: 2558. doi: 10.3390/ijms23052558

30. Scheim DE et al., “Sialylated Glycan Bindings from SARS-CoV-2 Spike Protein to Blood and Endothelial Cells Govern the Severe Morbidities of COVID-19,” *Int. J. Mol. Sci.* 2023, 24, 23: 17039. doi: https://doi.org/10.3390/ijms242317039

31. Sirsendu J et al., “Cell-Free Hemoglobin Does Not Attenuate the Effects of SARS-CoV-2 Spike Protein S1 Subunit in Pulmonary Endothelial Cells,” *Int. J. Mol. Sci.*, 2021, 22, 16: 9041. doi: https://doi.org/10.3390/ijms22169041

32. Zhang S et al., “SARS-CoV-2 Binds Platelet ACE2 to Enhance Thrombosis in COVID-19,” *J. Hematol. Oncol.* 2020, 13, 120: 120. doi: 10.1186/s13045-020-00954-7

33. Zhang Z et al., “SARS-CoV-2 spike protein dictates syncytium-mediated lymphocyte elimination,” *Cell Death Differ.* 2021, 28, 2765–2777. doi: 10.1038/s41418-021-00782-3

34. Zheng Y et al., “SARS-CoV-2 Spike Protein Causes Blood Coagulation and Thrombosis by Competitive Binding to Heparan Sulfate,” *Int. J. Biol. Macromol.* 2021, 193: 1124–1129. doi: https://doi.org/10.1016/j.ijbiomac.2021.10.112

35. Zhu W et al., “Prothrombotic antibodies targeting the spike protein's receptor-binding domain in severe COVID-19,” *Blood* 2025, 145, 6: 635-647. doi: https://doi.org/10.1182/blood.2024025010

K. Cytokines, chemokines, inteferon, interleukins

1. Alghmadi A et al., “Altered Circulating Cytokine Profile Among mRNA-Vaccinated Young Adults: A Year-Long Follow-Up Study,” *Immun. Inflamm. Dis.* 2025, 13, 4: e70194. doi: https://doi.org/10.1002/iid3.70194

2. Ao Z et al., “SARS-CoV-2 Delta spike protein enhances the viral fusogenicity and inflammatory cytokine production,” *iScience* 2022, 25, 8: 104759. doi: 10.1016/j.isci.2022.104759

3. Azzarone B et al., “Soluble SARS-CoV-2 Spike glycoprotein: considering some potential pathogenic effects,” *Front. Immunol.* 2025, 16 (Sec. Cytokines and Soluble Mediators in Immunity). doi: https://doi.org/10.3389/fimmu.2025.1616106

4. Chaves JCS et al., “Differential Cytokine Responses of *APOE3* and *APOE4* Blood–brain Barrier Cell Types to SARS-CoV-2 Spike Proteins,” *J. Neuroimmune Pharmacol.* 2024, 19, 22. doi: https://doi.org/10.1007/s11481-024-10127-9

5. Chittasupho C et al., “Targeting spike glycoprotein S1 mediated by NLRP3 inflammasome machinery and the cytokine releases in A549 lung epithelial cells by nanocurcumin,” *Pharmaceuticals* (Basel) 2023, 16, 6: 862. doi: 10.3390/ph16060862

6. Das T et al., “N-glycosylation of the SARS-CoV-2 spike protein at Asn331 and Asn343 is involved in spike-ACE2 binding, virus entry, and regulation of IL-6,” *Microbiol. Immunol.* 2024, 68, 5: 165-178. doi: https://doi.org/10.1111/1348-0421.13121

7. Duarte C, “Age-dependent effects of the recombinant spike protein/SARS-CoV-2 on the M-CSF- and IL-34-differentiated macrophages in vitro,” *Biochem. Biophys. Res. Commun.* 2021, 546: 97–102. doi: https://doi.org/10.1016/j.bbrc.2021.01.104

8. Forsyth CB et al., “The SARS-CoV-2 S1 spike protein promotes MAPK and NF-kB activation in human lung cells and inflammatory cytokine production in human lung and intestinal epithelial cells,” *Microorganisms* 2022, 10, 10: 1996. doi: https://doi.org/10.3390/microorganisms10101996

9. Freitas RS et al., “SARS-CoV-2 Spike antagonizes innate antiviral immunity by targeting interferon regulatory factor 3,” *Front Cell Infect Microbiol.* 2021, 11: 789462. doi: https://doi.org/10.3389/fcimb.2021.789462

10. Gasparello J et al., “In vitro induction of interleukin-8 by SARS-CoV-2 Spike protein is inhibited in bronchial epithelial IB3-1 cells by a miR-93-5p agomiR,” *Int. Immunopharmacol.* 2021, 101: 108201. doi: 10.1016/j.intimp.2021.108201

11. Gasparello J et al., “Sulforaphane inhibits the expression of interleukin-6 and interleukin-8 induced in bronchial epithelial IB3-1 cells by exposure to the SARS-CoV-2 Spike protein,” *Phytomedicine* 2021, 87: 153583. doi: https://doi.org/10.1016/j.phymed.2021.153583

12. Ghazanfari D et al., “Mechanistic insights into SARS-CoV-2 spike protein induction of the chemokine CXCL10,” *Sci. Rep.* 2024, 14: 11179. doi: 10.1038/s41598-024-61906-6

13. Gracie NP et al., “Cellular signalling by SARS-CoV-2 spike protein,” *Microbiology Australia* 2024, 45, 1: 13-17. doi: https://doi.org/10.1071/MA24005

14. Gu T et al., “Cytokine Signature Induced by SARS-CoV-2 Spike Protein in a Mouse Model,” *Front. Immunol.,* 2021 (Sec. Inflammation). doi: 10.3389/fimmu.2020.621441

15. Jugler C et al, “SARS-CoV-2 Spike Protein-Induced Interleukin 6 Signaling Is Blocked by a Plant-Produced Anti-Interleukin 6 Receptor Monoclonal Antibody,” *Vaccines* 2021, *9*, 11: 1365. https://doi.org/10.3390/vaccines9111365

16. Kawata D et al., “Diverse pro-inflammatory ability of mutated spike protein derived from variant strains of SARS-CoV-2,” *Cytokine* 2024, 178: 156592. doi: https://doi.org/10.1016/j.cyto.2024.156592

17. Lee AR et al., “SARS-CoV-2 spike protein promotes inflammatory cytokine activation and aggravates rheumatoid arthritis,” *Cell Commun Signal.* 2023, 21, 1: 44. doi: https://doi.org/10.1186/s12964-023-01044-0

18. Liang S et al., “SARS-CoV-2 spike protein induces IL-18-mediated cardiopulmonary inflammation via reduced mitophagy,” *Signal Transduct Target Ther* 2023, 8, 103. doi: https://doi.org/10.1038/s41392-023-01368-w

19. Liu T et al., “RS-5645 attenuates inflammatory cytokine storm induced by SARS-CoV-2 spike protein and LPS by modulating pulmonary microbiota,” *Int. J. Biol. Sci.* 2021, 17, 13: 3305–3319. doi: 10.7150/ijbs.63329

20. Liu X et al., “SARS-CoV-2 spike protein-induced cell fusion activates the cGAS-STING pathway and the interferon response,” *Sci Signal.* 2022, 15, 729: eabg8744. doi: 10.1126/scisignal.abg8744

21. Niu C et al., “SARS-CoV-2 spike protein induces the cytokine release syndrome by stimulating T cells to produce more IL-2,” *Front. Immunol.* 2024, 15: 1444643. doi: https://doi.org/10.3389/fimmu.2024.1444643

22. Norris B et al., “Evaluation of Glutathione in Spike Protein of SARS-CoV-2 Induced Immunothrombosis and Cytokine Dysregulation,” *Antioxidants* 2024, 13, 3: 271. doi: https://doi.org/10.3390/antiox13030271

23. Olajide OA et al., “Induction of Exaggerated Cytokine Production in Human Peripheral Blood Mononuclear Cells by a Recombinant SARS-CoV-2 Spike Glycoprotein S1 and Its Inhibition by Dexamethasone,” *Inflammation* 2021, 44: 1865–1877. doi: https://doi.org/10.1007/s10753-021-01464-5

24. Park YJ et al., “D-dimer and CoV-2 spike-immune complexes contribute to the production of PGE2 and proinflammatory cytokines in monocytes,” *PLoS Pathog.*, 2022, 18, 4: e1010468. doi: https://doi.org/10.1371/journal.ppat.1010468

25. Patra T et al., “SARS-CoV-2 spike protein promotes IL-6 trans-signaling by activation of angiotensin II receptor signaling in epithelial cells,” *PLoS Pathog*. 2020, 16: e1009128. doi: https://doi.org/10.1371/journal.ppat.1009128

26. Samsudin S et al., “SARS-CoV-2 spike protein as a bacterial lipopolysaccharide delivery system in an overzealous inflammatory cascade,” *J. Mol. Biol.* 2022, 14, 9: mjac058. doi: https://doi.org/10.1093/jmcb/mjac058

27. Schroeder JT and AP Bieneman, “The S1 Subunit of the SARS-CoV-2 Spike protein activates human monocytes to produce cytokines linked to COVID-19: relevance to galectin-3,” *Front Immunol.* 2022, 13: 831763. doi: 10.3389/fimmu.2022.831763

28. Sebastio AI et al., “CuMV VLPs containing the RBM from SARS-CoV-2 spike protein drive dendritic cell activation and Th1 polarization,” *Pharmaceutics* 2023, 15, 3: 825. doi: https://doi.org/10.3390/pharmaceutics15030825

29. Sharma VK et al., “Nanocurcumin Potently Inhibits SARS-CoV-2 Spike Protein-Induced Cytokine Storm by Deactivation of MAPK/NF-κB Signaling in Epithelial Cells,” *ACS Appl. Bio Mater.* 2022, 5, 2: 483–491. doi: 10.1021/acsabm.1c00874

30. Sui Y et al., “SARS-CoV-2 Spike Protein Suppresses ACE2 and Type I Interferon Expression in Primary Cells From Macaque Lung Bronchoalveolar Lavage,” *Front. Immunol.* 2021, 12. doi: https://doi.org/10.3389/fimmu.2021.658428

31. Tsilioni I et al., “Nobiletin and Eriodictyol Suppress Release of IL-1β, CXCL8, IL-6, and MMP-9 from LPS, SARS-CoV-2 Spike Protein, and Ochratoxin A-Stimulated Human Microglia,” *Int. J. Mol. Sci.* 2025, 26, 2: 636. doi: 10.3390/ijms26020636

32. Tsilioni S et al., “Recombinant SARS-CoV-2 spike protein and its receptor binding domain stimulate release of different pro-inflammatory mediators via activation of distinct receptors on human microglia cells,” *Mol Neurobiol.* 2023, 60, 11: 6704–14. doi: 10.1007/s12035-023-03493-7

33. Tsilioni S et al., “Recombinant SARS-CoV-2 Spike Protein Stimulates Secretion of Chymase, Tryptase, and IL-1beta from Human Mast Cells, Augmented by IL-33,” *Int. J. Mol. Sci.* 2023, 24, 11: 9487. doi: https://doi.org/10.3390/ijms24119487

34. Youn JY et al., “Therapeutic application of estrogen for COVID-19: Attenuation of SARS-CoV-2 spike protein and IL-6 stimulated, ACE2-dependent NOX2 activation,

ROS production and MCP-1 upregulation in endothelial cells," *Redox Biol.* 2021, 46: 102099. doi: https://doi.org/10.1016/j.redox.2021.102099

35. Zhang Q et al., "Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) membrane (M) and spike (S) proteins antagonize host type i interferon response," *Front Cell Infect Microbiol* 2021, 11: 766922. doi: 10.3389/fcimb.2021.766922

36. Zhang RG et al., "SARS-CoV-2 spike protein receptor binding domain promotes IL-6 and IL-8 release via ATP/$P2Y_2$ and ERK1/2 signaling pathways in human bronchial epithelia," *Mol. Immunol.* 2024, 167: 53-61. doi: 10.1016/j.molimm.2024.02.005

L. Endothelial

1. Bhargavan B and GD Kanmogne, "SARS-CoV-2 spike proteins and cell–cell communication inhibits TFPI and induces thrombogenic factors in human lung microvascular endothelial cells and neutrophils: implications for COVID-19 coagulopathy pathogenesis," *Int. J. Mol. Sci.* 2022, 23, 18: 10436. doi: https://doi.org/10.3390/ijms231810436

2. Biancatelli RMLC, et al. "The SARS-CoV-2 spike protein subunit S1 induces COVID-19-like acute lung injury in Kappa18-hACE2 transgenic mice and barrier dysfunction in human endothelial cells," *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2021, 321: L477–L484. doi: https://doi.org/10.1152/ajplung.00223.2021

3. Castro-Robles B et al., "Distinct response patterns of endothelial markers to the BNT162b2 mRNA COVID-19 booster vaccine are associated with the spike-specific IgG antibody production," *Front. Immunol.* 2025, 15 (Sec. Vaccines and Molecular Therapeutics). doi: https://doi.org/10.3389/fimmu.2024.1471401

4. Du Preez HN et al., "COVID-19 vaccine adverse events: Evaluating the pathophysiology with an emphasis on sulfur metabolism and endotheliopathy," *Eur J Clin Invest.* 2024, 54, 10: e14296. doi: https://doi.org/10.1111/eci.14296

5. Gamblicher T et al., "SARS-CoV-2 spike protein is present in both endothelial and eccrine cells of a chilblain-like skin lesion," *J Eur Acad Dermatol Venereol.* 2020, 1, 10: e187-e189. doi: https://doi.org/10.1111/jdv.16970

6. Gultom M et al., "Sustained Vascular Inflammatory Effects of SARS-CoV-2 Spike Protein on Human Endothelial Cells," *Inflammation* 2024. doi: https://doi.org/10.1007/s10753-024-02208-x

7. Guo Y and V Kanamarlapudi, “Molecular Analysis of SARS-CoV-2 Spike Protein-Induced Endothelial Cell Permeability and vWF Secretion,” *Int. J. Mol. Sci.* 2023, 24, 6: 5664. doi: https://doi.org/10.3390/ijms24065664

8. Jana S et al., “Cell-free hemoglobin does not attenuate the effects of SARS-CoV-2 spike protein S1 subunit in pulmonary endothelial cells,” *Int. J. Mol. Sci.* 2021, 22, 16: 9041. doi: https://doi.org/10.3390/ijms22169041

9. Kulkoviene G et al., “Differential Mitochondrial, Oxidative Stress and Inflammatory Responses to SARS-CoV-2 Spike Protein Receptor Binding Domain in Human Lung Microvascular, Coronary Artery Endothelial and Bronchial Epithelial Cells,” *Int. J. Mol. Sci.* 2024, 25, 6: 3188. doi: https://doi.org/10.3390/ijms25063188

10. Marrone L et al., “Tirofiban prevents the effects of SARS-CoV-2 spike protein on macrophage activation and endothelial cell death,” *Heliyon*, 2024, 10, 15, e35341. doi: 10.1016/j.heliyon.2024.e35341

11. Meyer K et al., “SARS-CoV-2 Spike Protein Induces Paracrine Senescence and Leukocyte Adhesion in Endothelial Cells,” *J. Virol.* 2021, 95: e0079421. doi: https://doi.org/10.1128/jvi.00794-21

12. Montezano AC et al., “SARS-CoV-2 spike protein induces endothelial inflammation via ACE2 independently of viral replication,” *Sci Rep.* 2023, 13, 1: 14086. doi: https://doi.org/10.1038/s41598-023-41115-3

13. Nuovo JG et al., “Endothelial Cell Damage Is the Central Part of COVID-19 and a Mouse Model Induced by Injection of the S1 Subunit of the Spike Protein,” *Ann. Diagn. Pathol.* 2021, 51, 151682. doi: 10.1016/j.anndiagpath.2020.151682

14. Perico L et al., “SARS-CoV-2 and the spike protein in endotheliopathy,” *Trends Microbiol.* 2024, 32, 1: 53-67. doi: 10.1016/j.tim.2023.06.004

15. Perico L et al., “SARS-CoV-2 Spike Protein 1 Activates Microvascular Endothelial Cells and Complement System Leading to Platelet Aggregation,” *Front. Immunol.* 2022, 13, 827146. doi: https://doi.org/10.3389/fimmu.2022.827146

16. Raghavan S et al., “SARS-CoV-2 Spike Protein Induces Degradation of Junctional Proteins That Maintain Endothelial Barrier Integrity,” *Front. Cardiovasc. Med.* 2021, 8, 687783. doi: https://doi.org/10.3389/fcvm.2021.687783

17. Ratajczak MZ et al., “SARS-CoV-2 Entry Receptor ACE2 Is Expressed on Very Small $CD45^{-}$ Precursors of Hematopoietic and Endothelial Cells and in Response to Virus Spike Protein Activates the Nlrp3 Inflammasome,” *Stem Cell Rev Rep.* 2021, 17, 1: 266-277. doi: https://doi.org/10.1007/s12015-020-10010-z

18. Robles JP et al., “The Spike Protein of SARS-CoV-2 Induces Endothelial Inflammation through Integrin α5β1 and NF-κB Signaling,” *J. Biol. Chem.* 2022, 298, 3: 101695. doi: https://doi.org/10.1016/j.jbc.2022.101695

19. Rotoli BM et al., “Endothelial cell activation by SARS-CoV-2 spike S1 protein: A crosstalk between endothelium and innate immune cells,” *Biomedicines* 2021, 9, 9: 1220. doi: https://doi.org/10.3390/biomedicines9091220

20. Ruben ML et al., “The SARS-CoV-2 spike protein subunit S1 induces COVID-19-like acute lung injury in K18-hACE2 transgenic mice and barrier dysfunction in human endothelial cells,” *Am J Physiol Lung Cell Mol Physiol.* 2021, 321, 2: L477-L484. doi: https://doi.org/10.1152/ajplung.00223.2021

21. Sano H et al., “A case of persistent, confluent maculopapular erythema following a COVID-19 mRNA vaccination is possibly associated with the intralesional spike protein expressed by vascular endothelial cells and eccrine glands in the deep dermis,” *J. Dermatol.* 2023, 50: 1208–1212. doi: 10.1111/1346-8138.16816

22. Santonja C et al., “COVID-19 chilblain-like lesion: immunohistochemical demonstration of SARS-CoV-2 spike protein in blood vessel endothelium and sweat gland epithelium in a polymerase chain reaction-negative patient,” *Br J Dermatol*. 2020, 183, 4: 778-780. doi: https://doi.org/10.1111/bjd.19338

23. Scheim DE et al., “Sialylated Glycan Bindings from SARS-CoV-2 Spike Protein to Blood and Endothelial Cells Govern the Severe Morbidities of COVID-19,” *Int. J. Mol. Sci.* 2023, 24, 23: 17039. doi: https://doi.org/10.3390/ijms242317039

24. Sirsendu J et al., “Cell-Free Hemoglobin Does Not Attenuate the Effects of SARS-CoV-2 Spike Protein S1 Subunit in Pulmonary Endothelial Cells,” *Int. J. Mol. Sci.*, 2021, 22, 16: 9041. doi: https://doi.org/10.3390/ijms22169041

25. Stern B et al., “SARS-CoV-2 spike protein induces endothelial dysfunction in 3D engineered vascular networks,” *J. Biomed. Mater. Res. A.* 2023, 112, 4: 524-533. doi: https://doi.org/10.1002/jbm.a.37543

26. Villacampa A et al., “SARS-CoV-2 S protein activates NLRP3 inflammasome and deregulates coagulation factors in endothelial and immune cells,” *Cell Commun. Signal.* 2024, 22, 38. doi: https://doi.org/10.1186/s12964-023-01397-6

27. Wu ML et al., “Mast cell activation triggered by SARS-CoV-2 causes inflammation in brain microvascular endothelial cells and microglia,” *Front. Cell. Infect. Microbiol.* 2024, 14. doi: https://doi.org/10.3389/fcimb.2024.1358873

28. Yang K et al., “SARS-CoV-2 spike protein receptor-binding domain perturbates intracellular calcium homeostasis and impairs pulmonary vascular endothelial cells,” *Signal Transduct. Target. Ther.* 2023, 8, 276. doi: 10.1038/s41392-023-01556-8

29. Youn JY et al., “Therapeutic application of estrogen for COVID-19: Attenuation of SARS-CoV-2 spike protein and IL-6 stimulated, ACE2-dependent NOX2 activation, ROS production and MCP-1 upregulation in endothelial cells,” *Redox Biol.* 2021, 46: 102099. doi: https://doi.org/10.1016/j.redox.2021.102099

30. Zekri-Nechar K et al., “Spike Protein Subunits of SARS-CoV-2 Alter Mitochondrial Metabolism in Human Pulmonary Microvascular Endothelial Cells: Involvement of Factor Xa,” *Dis. Markers* 2022, 1118195. doi: https://doi.org/10.1155/2022/1118195

M. Gastrointestinal

1. Forsyth CB et al., “The SARS-CoV-2 S1 spike protein promotes MAPK and NF-kB activation in human lung cells and inflammatory cytokine production in human lung and intestinal epithelial cells,” *Microorganisms* 2022, 10, 10: 1996. doi: https://doi.org/10.3390/microorganisms10101996

2. Li Z et al., “SARS-CoV-2 pathogenesis in the gastrointestinal tract mediated by Spike-induced intestinal inflammation,” *Precis. Clin. Med.* 2024, 7, 1: pbad034. doi: https://doi.org/10.1093/pcmedi/pbad034

3. Luo Y et al., “SARS-Cov-2 spike induces intestinal barrier dysfunction through the interaction between CEACAM5 and Galectin-9,” *Front. Immunol.* 2024, 15. doi: https://doi.org/10.3389/fimmu.2024.1303356

4. Nascimento RR et al., “SARS-CoV-2 Spike protein triggers gut impairment since mucosal barrier to innermost layers: From basic science to clinical relevance,” *Mucosal Immunol.* 2024, 17, 4: 565-583. doi: 10.1016/j.mucimm.2024.03.00

5. Yilmaz A et al., “Differential proinflammatory responses of colon epithelial cells to SARS-CoV-2 spike protein and *Pseudomonas aeruginosa* lipopolysaccharide,” *Turk J Biochem.* 2024. doi: https://doi.org/10.1515/tjb-2024-0144

6. Yonker LM et al., “Multisystem inflammatory syndrome in children is driven by zonulin-dependent loss of gut mucosal barrier,” *J Clin Invest*. 2021, 131, 14: e149633. doi: https://doi.org/10.1172/JCI149633

7. Zeng FM et al., “SARS-CoV-2 spike spurs intestinal inflammation via VEGF production in enterocytes,” *EMBO Mol Med*. 2022, 14: e14844. doi: https://doi.org/10.15252/emmm.202114844

8. Zollner A et al., “Postacute COVID-19 is Characterized by Gut Viral Antigen Persistence in Inflammatory Bowel Diseases,” *Gastroenterology* 2022, 163, 2: 495-506.e8. doi: https://doi.org/10.1053/j.gastro.2022.04.037

N. Immune dysfunction

1. Baldari CT et al., “Emerging Roles of SARS-CoV-2 Spike-ACE2 in Immune Evasion and Pathogenesis,” *Trends Immunol.* 2023, 44, 6. doi: 10.1016/j.it.2023.04.001

2. Bocquet-Garcon A, “Impact of the SARS-CoV-2 Spike Protein on the Innate Immune System: A Review,” *Cureus* 2024, 16, 3: e57008. doi: 10.7759/cureus.57008

3. Delgado JF et al., “SARS-CoV-2 spike protein vaccine-induced immune imprinting reduces nucleocapsid protein antibody response in SARS-CoV-2 infection,” *J. Immunol. Res.* 2022: 8287087. doi: https://doi.org/10.1155/2022/8287087

4. Freitas RS et al., “SARS-CoV-2 Spike antagonizes innate antiviral immunity by targeting interferon regulatory factor 3,” *Front Cell Infect Microbiol.* 2021, 11: 789462. doi: https://doi.org/10.3389/fcimb.2021.789462

5. Irrgang P et al., “Class switch toward noninflammatory, spike-specific IgG4 antibodies after repeated SARS-CoV-2 mRNA vaccination,” *Sci. Immunol.* 2022, 8, 79. doi: 10.1126/sciimmunol.ade2798

6. Kim MJ et al., “The SARS-CoV-2 spike protein induces lung cancer migration and invasion in a TLR2-dependent manner,” *Cancer Commun* (London), 2023, 44, 2: 273–277. doi: https://doi.org/10.1002/cac2.12485

7. Onnis A et al., “SARS-CoV-2 Spike protein suppresses CTL-mediated killing by inhibiting immune synapse assembly,” *J Exp Med* 2023, 220, 2: e20220906. doi: https://doi.org/10.1084/jem.20220906

8. Tu TH et al., “The identification of a SARs-CoV2 S2 protein derived peptide with super-antigen-like stimulatory properties on T-cells,” *Commun. Biol.* 2025, 8, 14. doi: https://doi.org/10.1038/s42003-024-07350-8

O. Macrophages, monocytes, neutrophils

1. Ahn WM et al., “SARS-CoV-2 Spike Protein Stimulates Macropinocytosis in Murine and Human Macrophages via PKC-NADPH Oxidase Signaling,” *Antioxidants* 2024, 13, 2: 175. doi: https://doi.org/10.3390/antiox13020175

2. Ait-Belkacem I et al., “SARS-CoV-2 spike protein induces a differential monocyte activation that may contribute to age bias in COVID-19 severity,” *Sci. Rep.* 2022, 12: 20824. doi: https://doi.org/10.1038/s41598-022-25259-2

3. Barhoumi T et al., “SARS-CoV-2 coronavirus Spike protein-induced apoptosis, inflammatory, and oxidative stress responses in THP-1-like-macrophages: potential role of angiotensin-converting enzyme inhibitor (perindopril),” *Front. Immunol.* 2021, 12: 728896. doi: https://doi.org/10.3389/fimmu.2021.728896

4. Bortolotti D et al., “SARS-CoV-2 Spike 1 Protein Controls Natural Killer Cell Activation via the HLA-E/NKG2A Pathway,” *Cells* 2020, 9, 9: 1975. doi: https://doi.org/10.3390/cells9091975

5. Cao X et al., “Spike protein of SARS-CoV-2 activates macrophages and contributes to induction of acute lung inflammation in male mice,” *FASEB J.* 2021, 35, e21801. doi: https://doi.org/10.1096/fj.202002742RR

6. Chiok K et al., “Proinflammatory Responses in SARS-CoV-2 and Soluble Spike Glycoprotein S1 Subunit Activated Human Macrophages,” *Viruses* 2023, 15, 3: 754. doi: https://doi.org/10.3390/v15030754

7. Cory TJ et al., “Metformin Suppresses Monocyte Immunometabolic Activation by SARS-CoV-2 Spike Protein Subunit 1,” *Front. Immunol.* 2021, 12 (Sec. Cytokines and Soluble Mediators in Immunity): 733921. doi: 10.3389/fimmu.2021.733921

8. Del Re A et al., “Ultramicronized Palmitoylethanolamide Inhibits NLRP3 Inflammasome Expression and Pro-Inflammatory Response Activated by SARS-CoV-2 Spike Protein in Cultured Murine Alveolar Macrophages,” *Metabolites* 2021, 11, 9: 592. doi: https://doi.org/10.3390/metabo11090592

9. Duarte C, “Age-dependent effects of the recombinant spike protein/SARS-CoV-2 on the M-CSF- and IL-34-differentiated macrophages in vitro,” *Biochem. Biophys. Res. Commun.* 2021, 546: 97–102. doi: https://doi.org/10.1016/j.bbrc.2021.01.104

10. Fajloun Z et al., “Unveiling the Role of SARS-CoV-2 or mRNA Vaccine Spike Protein in Macrophage Activation Syndrome (MAS),” *Infect. Disord. Drug Targets* 2025, 25, 2: E220724232138. doi: https://doi.org/10.2174/0118715265341206240722050403

11. Karwaciak I et al., “Nucleocapsid and Spike Proteins of the Coronavirus Sars-Cov-2 Induce Il6 in Monocytes and Macrophages—Potential Implications for Cytokine Storm Syndrome,” *Vaccines* 2021, 9, 1, 54: 1–10. doi: 10.3390/vaccines9010054

12. Li T et al., “Platelets Mediate Inflammatory Monocyte Activation by SARS-CoV-2 Spike Protein,” *J. Clin. Invest.* 2022, 132, 4: e150101. doi: 10.1172/JCI150101

13. Liu Y et al., “The recombinant spike S1 protein induces injury and inflammation in co-cultures of human alveolar epithelial cells and macrophages,” *PLoS ONE* 2025, 20, 2: e0318881. doi: https://doi.org/10.1371/journal.pone.0318881

14. Loh JT et al., “Dok3 restrains neutrophil production of calprotectin during TLR4 sensing of SARS-CoV-2 spike protein,” *Front. Immunol.* 2022, 13 (Sec. Molecular Innate Immunity). doi: https://doi.org/10.3389/fimmu.2022.996637

15. Marrone L et al., “Tirofiban prevents the effects of SARS-CoV-2 spike protein on macrophage activation and endothelial cell death,” *Heliyon,* 2024, 10, 15: e35341. doi: 10.1016/j.heliyon.2024.e35341

16. Miller GM et al., “SARS-CoV-2 and SARS-CoV-2 Spike protein S1 subunit Trigger Proinflammatory Response in Macrophages in the Absence of Productive Infection,” *J. Immunol.* 2023, 210 (1_Supplement): 71.30. doi: 10.4049/jimmunol.210.Supp.71.30

17. Onnis A et al., “SARS-CoV-2 Spike protein suppresses CTL-mediated killing by inhibiting immune synapse assembly,” *J Exp Med* 2023, 220, 2: e20220906. doi: https://doi.org/10.1084/jem.20220906

18. Palestra F et al. “SARS-CoV-2 Spike Protein Activates Human Lung Macrophages,” *Int. J. Mol. Sci.* 2023, 24, 3: 3036. doi: 10.3390/ijms24033036

19. Park C et al., “Murine alveolar Macrophages Rapidly Accumulate intranasally Administered SARS-CoV-2 Spike Protein leading to neutrophil Recruitment and Damage,” *Elife* 2024, 12, RP86764. doi: https://doi.org/10.7554/eLife.86764.3

20. Park YJ et al., “D-dimer and CoV-2 spike-immune complexes contribute to the production of PGE2 and proinflammatory cytokines in monocytes,” *PLoS Pathog.* 2022, 18, 4: e1010468. doi: https://doi.org/10.1371/journal.ppat.1010468

21. Park YJ et al., “Pyrogenic and inflammatory mediators are produced by polarized M1 and M2 macrophages activated with D-dimer and SARS-CoV-2 spike immune complexes,” *Cytokine* 2024, 173: 156447. doi: 10.1016/j.cyto.2023.156447

22. Patterson BK et al., “Detection of S1 spike protein in CD16+ monocytes up to 245 days in SARS-CoV-2-negative post-COVID-19 vaccine syndrome (PCVS) individuals,” *Hum Vaccin Immunother.* 2025, 21, 1: 2494934. doi: 10.1080/21645515.2025.2494934

23. Patterson BK et al., “Persistence of SARS CoV-2 S1 Protein in CD16+ Monocytes in Post-Acute Sequelae of COVID-19 (PASC) up to 15 Months Post-Infection,” *Front. Immunol.* 12 (Sec. Viral Immunology). doi: 10.3389/fimmu.2021.746021

24. Pence B, “Recombinant SARS-CoV-2 Spike Protein Mediates Glycolytic and Inflammatory Activation in Human Monocytes,” *Innov Aging* 2020, 4, sp. 1: 955. doi: https://doi.org/10.1093/geroni/igaa057.3493

25. Satta S et al., “An engineered nano-liposome-human ACE2 decoy neutralizes SARS-CoV-2 Spike protein-induced inflammation in both murine and human macrophages,” *Theranostics* 2022, 12, 6: 2639–2657. doi: 10.7150/thno.66831

26. Schroeder JT and AP Bieneman, “The S1 Subunit of the SARS-CoV-2 Spike protein activates human monocytes to produce cytokines linked to COVID-19: relevance to galectin-3,” *Front Immunol.* 2022, 13: 831763. doi: 10.3389/fimmu.2022.831763

27. Shirato K and Takako Kizaki, “SARS-CoV-2 Spike Protein S1 Subunit Induces Pro-inflammatory Responses via Toll-Like Receptor 4 Signaling in Murine and Human Macrophages,” *Heliyon* 2021, 7, 2: e06187. doi: 10.1016/j.heliyon.2021.e06187

28. Theobald SJ et al., “Long-lived macrophage reprogramming drives spike protein-mediated inflammasome activation in COVID-19,” *EMBO Mol. Med.* 2021, 13: e14150. doi: https://doi.org/10.15252/emmm.202114150

29. Vettori M et al., “Effects of Different Types of Recombinant SARS-CoV-2 Spike Protein on Circulating Monocytes’ Structure,” *Int. J. Mol. Sci.* 2023, 24, 11: 9373. doi: https://doi.org/10.3390/ijms24119373

30. Youn YJ et al., “Nucleocapsid and spike proteins of SARS-CoV-2 drive neutrophil extracellular trap formation,” *Immune Netw.* 2021, 21, 2: e16. doi: https://doi.org/10.4110/in.2021.21.e16

31. Zaki H and S Khan, “SARS-CoV-2 spike protein induces inflammatory molecules through TLR2 in macrophages and monocytes,” *J. Immunol.* 2021, 206 (1_supplement): 62.07. doi: https://doi.org/10.4049/jimmunol.206.Supp.62.07

P. MAPK/NF-kB

1. Arjsri P et al., “Hesperetin from root extract of *Clerodendrum petasites* S. Moore inhibits SARS-CoV-2 spike protein S1 subunit-induced Nlrp3 inflammasome in A549 lung cells via modulation of the Akt/Mapk/Ap-1 pathway,” *Int. J. Mol. Sci.* 2022, 23, 18: 10346. doi: https://doi.org/10.3390/ijms231810346

2. Bhattacharyya S and JK Tobacman, “SARS-CoV-2 spike protein-ACE2 interaction increases carbohydrate sulfotransferases and reduces N-acetylgalactosamine-4-sulfatase by p38 MAPK,” *Signal Transduct Target Ther* 2024, 9, 39. doi: https://doi.org/10.1038/s41392-024-01741-3

3. Forsyth CB *et al.*, “The SARS-CoV-2 S1 spike protein promotes MAPK and NF-kB activation in human lung cells and inflammatory cytokine production in human lung and intestinal epithelial cells,” *Microorganisms* 2022, 10, 10: 1996. doi: https://doi.org/10.3390/microorganisms10101996

4. Johnson EL et al., “The S1 spike protein of SARS-CoV-2 upregulates the ERK/MAPK signaling pathway in DC-SIGN-expressing THP-1 cells,” *Cell Stress Chaperones* 2024, 29, 2: 227-234. doi: https://doi.org/10.1016/j.cstres.2024.03.002

5. Khan S et al., “SARS-CoV-2 Spike Protein Induces Inflammation via TLR2-Dependent Activation of the NF-κB Pathway,” *eLife* 2021, 10: e68563. doi: 10.7554/elife.68563

6. Kircheis R and O Planz, “Could a Lower Toll-like Receptor (TLR) and NF-κB Activation Due to a Changed Charge Distribution in the Spike Protein Be the Reason for the Lower Pathogenicity of Omicron?” *Int. J. Mol. Sci.* 2022, 23, 11: 5966. doi: https://doi.org/10.3390/ijms23115966

7. Kyriakopoulos AM et al., “Mitogen Activated Protein Kinase (MAPK) Activation, p53, and Autophagy Inhibition Characterize the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Spike Protein Induced Neurotoxicity,” *Cureus* 2022, 14, 12: e32361. doi: 10.7759/cureus.32361

8. Robles JP et al., “The Spike Protein of SARS-CoV-2 Induces Endothelial Inflammation through Integrin α5β1 and NF-κB Signaling,” *J. Biol. Chem.* 2022, 298, 3: 101695. doi: https://doi.org/10.1016/j.jbc.2022.101695

9. Sharma VK et al., “Nanocurcumin Potently Inhibits SARS-CoV-2 Spike Protein-Induced Cytokine Storm by Deactivation of MAPK/NF-κB Signaling in Epithelial Cells,” *ACS Appl. Bio Mater.* 2022, 5, 2: 483–491. doi: 10.1021/acsabm.1c00874

10. Bhattacharyya S and JK Tobacman, “SARS-CoV-2 spike protein-ACE2 interaction increases carbohydrate sulfotransferases and reduces N-acetylgalactosamine-4-sulfatase by p38 MAPK,” *Signal Transduct Target Ther* 2024, 9, 39. doi: https://doi.org/10.1038/s41392-024-01741-3

Q. Mast cells

1. Cao JB et al., "Mast cell degranulation-triggered by SARS-CoV-2 induces tracheal-bronchial epithelial inflammation and injury," *Virol. Sin.* 2024, 39, 2: 309-318. doi: https://doi.org/10.1016/j.virs.2024.03.001

2. Fajloun Z et al., "SARS-CoV-2 or Vaccinal Spike Protein can Induce Mast Cell Activation Syndrome (MCAS)," *Infect Disord Drug Targets,* 2025, 25, 1: e300424229561. doi: 10.2174/0118715265319896240427045026

3. Tsilioni S et al., "Recombinant SARS-CoV-2 Spike Protein Stimulates Secretion of Chymase, Tryptase, and IL-1beta from Human Mast Cells, Augmented by IL-33," *Int. J. Mol. Sci.* 2023, 24, 11: 9487. doi: https://doi.org/10.3390/ijms24119487

4. Wu ML et al., "Mast cell activation triggered by SARS-CoV-2 causes inflammation in brain microvascular endothelial cells and microglia," *Front. Cell. Infect. Microbiol.,* 2024, 14. doi: https://doi.org/10.3389/fcimb.2024.1358873

R. Microglia

1. Alves V et al., "SARS-CoV-2 Spike protein alters microglial purinergic signaling *Front. Immunol.* 2023, 14: 1158460. doi: 10.3389/fimmu.2023.1158460

2. Chang MH et al., "SARS-CoV-2 Spike Protein 1 Causes Aggregation of α-Synuclein via Microglia-Induced Inflammation and Production of Mitochondrial ROS: Potential Therapeutic Applications of Metformin," *Biomedicines* 2024, 12, 6: 1223. doi: https://doi.org/10.3390/biomedicines12061223

3. Clough E et al., "Mitochondrial Dynamics in SARS-COV2 Spike Protein Treated Human Microglia: Implications for Neuro-COVID," *J. Neuroimmune Pharmacol.* 2021, 16, 4: 770–784. doi: https://doi.org/10.1007/s11481-021-10015-6

4. Frank MG et al., "SARS-CoV-2 Spike S1 Subunit Induces Neuroinflammatory, Microglial and Behavioral Sickness Responses: Evidence of PAMP-Like Properties," *Brain Behav. Immun.* 2022, 100: 267277. doi: 10.1016/j.bbi.2021.12.007

5. Kempuraj D et al., "Long COVID elevated MMP-9 and release from microglia by SARS-CoV-2 Spike protein," *Transl. Neurosci.* 2024, 15: 20220352. doi: https://doi.org/10.1515/tnsci-2022-0352

6. Mishra R and AC Banerjea, “SARS-CoV-2 Spike targets USP33-IRF9 axis via exosomal miR-148a to activate human microglia,” *Front. Immunol.* 2021, 12: 656700. doi: https://doi.org/10.3389/fimmu.2021.656700

7. Olajide OA et al., “SARS-CoV-2 spike glycoprotein S1 induces neuroinflammation in BV-2 microglia,” *Mol. Neurobiol.* 2022, 59: 445-458. doi: 10.1007/s12035-021-02593-6

8. Tsilioni I et al., “Nobiletin and Eriodictyol Suppress Release of IL-1β, CXCL8, IL-6, and MMP-9 from LPS, SARS-CoV-2 Spike Protein, and Ochratoxin A-Stimulated Human Microglia,” *Int. J. Mol. Sci.* 2025, 26, 2: 636. doi: https://doi.org/10.3390/ijms26020636

9. Tsilioni S et al., “Recombinant SARS-CoV-2 spike protein and its receptor binding domain stimulate release of different pro-inflammatory mediators via activation of distinct receptors on human microglia cells,” *Mol Neurobiol.* 2023, 60, 11: 6704–14. doi: 10.1007/s12035-023-03493-7

10. Wu ML et al., “Mast cell activation triggered by SARS-CoV-2 causes inflammation in brain microvascular endothelial cells and microglia,” *Front. Cell. Infect. Microbiol.* 2024, 14. doi: https://doi.org/10.3389/fcimb.2024.1358873

S. Microvascular

1. Avolio E et al., “The SARS-CoV-2 Spike Protein Disrupts Human Cardiac Pericytes Function through CD147 Receptor-Mediated Signalling: A Potential Non-infective Mechanism of COVID-19 Microvascular Disease,” *Clin. Sci.* 2021, 135, 24: 2667–2689. doi: https://doi.org/10.1042/CS20210735

2. Bhargavan B and GD Kanmogne, “SARS-CoV-2 spike proteins and cell–cell communication inhibits TFPI and induces thrombogenic factors in human lung microvascular endothelial cells and neutrophils: implications for COVID-19 coagulopathy pathogenesis,” *Int. J. Mol. Sci.* 2022, 23, 18: 10436. doi: https://doi.org/10.3390/ijms231810436

3. Kulkoviene G et al., “Differential Mitochondrial, Oxidative Stress and Inflammatory Responses to SARS-CoV-2 Spike Protein Receptor Binding Domain in Human Lung Microvascular, Coronary Artery Endothelial and Bronchial Epithelial Cells,” *Int. J. Mol. Sci.* 2024, 25, 6: 3188. doi: https://doi.org/10.3390/ijms25063188

4. Magro N et al., “Disruption of the blood-brain barrier is correlated with spike endocytosis by ACE2 + endothelia in the CNS microvasculature in fatal COVID-19. Scientific commentary on ‘Detection of blood-brain barrier disruption in brains of

patients with COVID-19, but no evidence of brain penetration by SARS-CoV-2'," *Acta Neuropathol.* 2024, 147, 1: 47. doi: https://doi.org/10.1007/s00401-023-02681-y

5. Panigrahi S et al., "SARS-CoV-2 Spike Protein Destabilizes Microvascular Homeostasis," *Microbiol Spectr.* 2021, 9, 3: e0073521. doi: 10.1128/Spectrum.00735-21

6. Perico L et al., "SARS-CoV-2 Spike Protein 1 Activates Microvascular Endothelial Cells and Complement System Leading to Platelet Aggregation," *Front. Immunol.* 2022, 13, 827146. doi: https://doi.org/10.3389/fimmu.2022.827146

7. Wu ML et al., "Mast cell activation triggered by SARS-CoV-2 causes inflammation in brain microvascular endothelial cells and microglia," *Front. Cell. Infect. Microbiol.* 2024, 14. doi: https://doi.org/10.3389/fcimb.2024.1358873

8. Zekri-Nechar K et al., "Spike Protein Subunits of SARS-CoV-2 Alter Mitochondrial Metabolism in Human Pulmonary Microvascular Endothelial Cells: Involvement of Factor Xa," *Dis. Markers* 2022: 1118195. doi: https://doi.org/10.1155/2022/1118195

T. MIS-C, pediatric

1. Chang A et al., "Recovery from antibody-mediated biliary ductopenia and multiorgan inflammation after COVID-19 vaccination," *NPJ Vaccines* 2024, 9, 75. doi: https://doi.org/10.1038/s41541-024-00861-9

2. Colmenero I et al., "SARS-CoV-2 endothelial infection causes COVID-19 chilblains: histopathological, immunohistochemical and ultrastructural study of seven paediatric cases," *Br J Dermatol.* 2020, 183: 729-737. doi: https://doi.org/10.1111/bjd.19327/

3. Dadonite B et al., "SARS-CoV-2 neutralizing antibody specificities differ dramatically between recently infected infants and immune-imprinted individuals," *J. Virol.* 2025, 99, 4. doi: https://doi.org/10.1128/jvi.00109-25

4. De Sousa PMB et al., "Fatal Myocarditis following COVID-19 mRNA Immunization: A Case Report and Differential Diagnosis Review," *Vaccines* 2024, 12, 2: 194. doi: https://doi.org/10.3390/vaccines12020194

5. Mayordomo-Colunga J et al., "SARS-CoV-2 spike protein in intestinal cells of a patient with coronavirus disease 2019 multisystem inflammatory syndrome," *J Pediatr.* 2022, 243: 214-18e215. doi: https://doi.org/10.1016/j.jpeds.2021.11.058

6. Rivas MN et al., “COVID-19–associated multisystem inflammatory syndrome in children (MIS-C): A novel disease that mimics toxic shock syndrome—the superantigen hypothesis,” *J Allergy Clin Immunol* 2021, 147, 1: 57-59. doi: 10.1016/j.jaci.2020.10.008

7. Rivas MN et al., “Multisystem Inflammatory Syndrome in Children and Long COVID: The SARS-CoV-2 Viral Superantigen Hypothesis,” *Front Immunol.* 2022, 13 (Sec. Molecular Innate Immunity). doi: 10.3389/fimmu.2022.941009

8. Sacco K et al., “Immunopathological signatures in multisystem inflammatory syndrome in children and pediatric COVID-19,” *Nat. Med.* 2022, 28: 1050-1062. doi: https://doi.org/10.1038/s41591-022-01724-3

9. Yonker LM et al., “Multisystem inflammatory syndrome in children is driven by zonulin-dependent loss of gut mucosal barrier,” *J Clin Invest*. 2021, 131, 14: e149633. doi: https://doi.org/10.1172/JCI149633

U. Mitochondria/metabolism

1. Cao X et al., “The SARS-CoV-2 spike protein induces long-term transcriptional perturbations of mitochondrial metabolic genes, causes cardiac fibrosis, and reduces myocardial contractile in obese mice,” *Mol. Metab.* 2023, 74, 101756. doi: https://doi.org/10.1016/j.molmet.2023.101756

2. Chang MH et al., “SARS-CoV-2 Spike Protein 1 Causes Aggregation of α-Synuclein via Microglia-Induced Inflammation and Production of Mitochondrial ROS: Potential Therapeutic Applications of Metformin,” *Biomedicines* 2024, 12, 6: 1223. doi: https://doi.org/10.3390/biomedicines12061223

3. Clough E et al., “Mitochondrial Dynamics in SARS-COV2 Spike Protein Treated Human Microglia: Implications for Neuro-COVID,” *Journal of Neuroimmune Pharmacology* 2021, 16, 4: 770–784. doi: 10.1007/s11481-021-10015-6

4. Huynh TV et al., “Spike Protein Impairs Mitochondrial Function in Human Cardiomyocytes: Mechanisms Underlying Cardiac Injury in COVID-19,” *Cells* 2023, 12, 877. doi: https://doi.org/10.3390/cells12060877

5. Kulkoviene G et al., “Differential Mitochondrial, Oxidative Stress and Inflammatory Responses to SARS-CoV-2 Spike Protein Receptor Binding Domain in Human Lung Microvascular, Coronary Artery Endothelial and Bronchial Epithelial Cells,” *Int. J. Mol. Sci.* 2024, 25, 6: 3188. doi: https://doi.org/10.3390/ijms25063188

6. Mercado-Gómez M et al., “The spike of SARS-CoV-2 promotes metabolic rewiring in hepatocytes,” *Commun. Biol.* 2022, 5, 827. doi: 10.1038/s42003-022-03789-9

7. Nguyen V, “The Spike Protein of SARS-CoV-2 Impairs Lipid Metabolism and Increases Susceptibility to Lipotoxicity: Implication for a Role of Nrf2,” *Cells* 2022, 11, 12: 1916. doi: https://doi.org/10.3390/cells11121916

8. Yeung-Luk BH et al., “SARS-CoV-2 infection alters mitochondrial and cytoskeletal function in human respiratory epithelial cells mediated by expression of spike protein,” *mBio* 2023, 14, 4: e00820-23. doi: https://doi.org/10.1128/mbio.00820-23

9. Zekri-Nechar K et al., “Spike Protein Subunits of SARS-CoV-2 Alter Mitochondrial Metabolism in Human Pulmonary Microvascular Endothelial Cells: Involvement of Factor Xa,” *Dis. Markers* 2022, 1118195. doi: https://doi.org/10.1155/2022/1118195

V. Myocarditis/cardiac/cardiomyopathy

1. Abdi A et al., “Biomed Interaction of SARS-CoV-2 with cardiomyocytes: Insight into the underlying molecular mechanisms of cardiac injury and pharmacotherapy,” *Pharmacother.* 2022, 146: 112518. doi: 10.1016/j.biopha.2021.112518

2. Avolio E et al., “The SARS-CoV-2 Spike Protein Disrupts Human Cardiac Pericytes Function through CD147 Receptor-Mediated Signalling: A Potential Non-infective Mechanism of COVID-19 Microvascular Disease,” *Clin. Sci.* 2021, 135, 24: 2667–2689. doi: https://doi.org/10.1042/CS20210735

3. Baumeier C et al., “Intramyocardial Inflammation after COVID-19 Vaccination: An Endomyocardial Biopsy-Proven Case Series,” *Int. J. Mol. Sci*. 2022, 23: 6940. doi: https://doi.org/10.3390/ijms23136940

4. Bellavite P et al., “Immune response and molecular mechanisms of cardiovascular adverse effects of spike proteins from SARS-coV-2 and mRNA vaccines,” *Biomedicines* 2023, 11, 2: 451. doi: https://doi.org/10.3390/biomedicines11020451

5. Boretti A. “PQQ Supplementation and SARS-CoV-2 Spike Protein-Induced Heart Inflammation,” *Nat. Prod. Commun*. 2022, 17, 1934578x221080929. doi: https://doi.org/10.1177/1934578X221080929

6. Buoninfante A et al., “Myocarditis associated with COVID-19 vaccination,” *npj Vaccines* 2024, 122. doi: https://doi.org/10.1038/s41541-024-00893-1

7. Cao X et al., “The SARS-CoV-2 spike protein induces long-term transcriptional perturbations of mitochondrial metabolic genes, causes cardiac fibrosis, and

reduces myocardial contractile in obese mice," *Mol. Metab.* 2023, 74, 101756. doi: https://doi.org/10.1016/j.molmet.2023.101756

8. Clemens DJ et al., "SARS-CoV-2 spike protein-mediated cardiomyocyte fusion may contribute to increased arrhythmic risk in COVID-19," *PLoS One* 2023, 18, 3: e0282151. doi: https://doi.org/10.1371/journal.pone.0282151

9. De Sousa PMB et al., "Fatal Myocarditis following COVID-19 mRNA Immunization: A Case Report and Differential Diagnosis Review," *Vaccines* 2024, 12, 2: 194. doi: https://doi.org/10.3390/vaccines12020194

10. Forte E, "Circulating spike protein may contribute to myocarditis after COVID-19 vaccination," *Nat. Cardiovasc. Res.* 2023, 2: 100. doi: 10.1038/s44161-023-00222-0

11. Huang X et al., "Sars-Cov-2 Spike Protein-Induced Damage of hiPSC-Derived Cardiomyocytes," *Adv. Biol.* 2022, 6, 7: e2101327. doi: 10.1002/adbi.202101327

12. Hulscher N et al., "Autopsy findings in cases of fatal COVID-19 vaccine-induced myocarditis," *ESC Heart Failure* 2024. doi: https://doi.org/10.1002/ehf2.14680

13. Huynh TV et al., "Spike Protein Impairs Mitochondrial Function in Human Cardiomyocytes: Mechanisms Underlying Cardiac Injury in COVID-19," *Cells* 2023, 12, 877. doi: https://doi.org/10.3390/cells12060877

14. Huynh TV et al., "Spike Protein of SARS-CoV-2 Activates Cardiac Fibrogenesis through NLRP3 Inflammasomes and NF-κB Signaling," *Cells* 2024, 13, 16: 1331. doi: https://doi.org/10.3390/cells13161331

15. Imig JD, "SARS-CoV-2 spike protein causes cardiovascular disease independent of viral infection," *Clin Sci (Lond)* 2022, 136, 6: 431–434. doi: 10.1042/CS20220028

16. Kato Y et al., "TRPC3-Nox2 Protein Complex Formation Increases the Risk of SARS-CoV-2 Spike Protein-Induced Cardiomyocyte Dysfunction through ACE2 Upregulation," *Int. J. Mol. Sci.* 2023, 24, 1: 102. doi: 10.3390/ijms24010102

17. Kawano H et al., "Fulminant Myocarditis 24 Days after Coronavirus Disease Messenger Ribonucleic Acid Vaccination," *Intern. Med.* 2022, 61, 15: 2319-2325. doi: https://doi.org/10.2169/internalmedicine.9800-22

18. Li C. et al., "Intravenous Injection of Coronavirus Disease 2019 (COVID-19) MRNA Vaccine Can Induce Acute Myopericarditis in Mouse Model," *Clin. Infect. Dis.* 2022, 74, 11: 1933-1950. doi: https://doi.org/10.1093/cid/ciab707

19. Lin Z, "More than a key—the pathological roles of SARS-CoV-2 spike protein in COVID-19 related cardiac injury," *Sports Med Health Sci* 2023, 6, 3: 209-220. doi: https://doi.org/10.1016/j.smhs.2023.03.004

20. Rzymski P and Andrzej Fal, "To aspirate or not to aspirate? Considerations for the COVID-19 vaccines," *Pharmacol. Rep* 2022, 74: 1223–1227. doi: https://doi.org/10.1007/s43440-022-00361-4

21. Schreckenberg R et al., "Cardiac side effects of RNA-based SARS-CoV-2 vaccines: Hidden cardiotoxic effects of mRNA-1273 and BNT162b2 on ventricular myocyte function and structure," *Br. J. Pharmacol.* 2024, 181, 3: 345-361. doi: https://doi.org/10.1111/bph.16262

22. Yonker LM et al., "Circulating Spike Protein Detected in Post–COVID-19 mRNA Vaccine Myocarditis," *Circulation* 2023, 147, 11. doi: 10.1161/CIRCULATIONAHA.122.061025

W. NLRP3

1. Albornoz EA et al., "SARS-CoV-2 drives NLRP3 inflammasome activation in human microglia through spike protein," *Mol. Psychiatr.* 2023, 28: 2878–2893. doi: https://doi.org/10.1038/s41380-022-01831-0

2. Arjsri P et al., "Hesperetin from root extract of *Clerodendrum petasites* S. Moore inhibits SARS-CoV-2 spike protein S1 subunit-induced Nlrp3 inflammasome in A549 lung cells via modulation of the Akt/Mapk/Ap-1 pathway," *Int. J. Mol. Sci.* 2022, 23, 18: 10346. doi: https://doi.org/10.3390/ijms231810346

3. Chittasupho C et al., "Inhibition of SARS-CoV-2-Induced NLRP3 Inflammasome-Mediated Lung Cell Inflammation by Triphala-Loaded Nanoparticle Targeting Spike Glycoprotein S1," *Pharmaceutics* 2024, 16, 6: 751. doi: 10.3390/pharmaceutics16060751

4. Chittasupho C et al., "Targeting spike glycoprotein S1 mediated by NLRP3 inflammasome machinery and the cytokine releases in A549 lung epithelial cells by nanocurcumin," *Pharmaceuticals* (Basel) 2023, 16, 6: 862. doi: 10.3390/ph16060862

11. Corpetti C et al., "Cannabidiol inhibits SARS-Cov-2 spike (S) protein-induced cytotoxicity and inflammation through a PPARγ-dependent TLR4/NLRP3/Caspase-1 signaling suppression in Caco-2 cell line," *Phytother. Res.* 2021, 35, 12: 6893–6903. doi: https://doi.org/10.1002/ptr.7302

12. Del Re A et al., “Ultramicronized Palmitoylethanolamide Inhibits NLRP3 Inflammasome Expression and Pro-Inflammatory Response Activated by SARS-CoV-2 Spike Protein in Cultured Murine Alveolar Macrophages,” *Metabolites* 2021, 11, 9: 592. dsoi: https://doi.org/10.3390/metabo11090592

13. Dissook S et al., “Luteolin-rich fraction from Perilla frutescens seed meal inhibits spike glycoprotein S1 of SARS-CoV-2-induced NLRP3 inflammasome lung cell inflammation via regulation of JAK1/STAT3 pathway: A potential anti-inflammatory compound against inflammation-induced long-COVID,” *Front. Med.* 2023, 9: 1072056. doi: https://doi.org/10.3389/fmed.2022.1072056

14. Huynh TV et al., “Spike Protein of SARS-CoV-2 Activates Cardiac Fibrogenesis through NLRP3 Inflammasomes and NF-κB Signaling,” *Cells* 2024, 13, 16: 1331. doi: https://doi.org/10.3390/cells13161331

15. Jiang Q et al., “SARS-CoV-2 spike S1 protein induces microglial NLRP3-dependent neuroinflammation and cognitive impairment in mice,” *Exp. Neurol.* 2025, 383: 115020. doi: https://doi.org/10.1016/j.expneurol.2024.115020

16. Kucia M et al. “An evidence that SARS-Cov-2/COVID-19 spike protein (SP) damages hematopoietic stem/progenitor cells in the mechanism of pyroptosis in Nlrp3 inflammasome-dependent manner,” *Leukemia* 2021, 35: 3026-3029. doi: https://doi.org/10.1038/s41375-021-01332-z

17. Ratajczak MZ et al., “SARS-CoV-2 Entry Receptor ACE2 Is Expressed on Very Small $CD45^{-}$ Precursors of Hematopoietic and Endothelial Cells and in Response to Virus Spike Protein Activates the Nlrp3 Inflammasome,” *Stem Cell Rev Rep.* 2021, 17, 1: 266-277. doi: https://doi.org/10.1007/s12015-020-10010-z

18. Semmarath W et al., “Cyanidin-3-O-glucoside and Peonidin-3-O-glucoside-Rich Fraction of Black Rice Germ and Bran Suppresses Inflammatory Responses from SARS-CoV-2 Spike Glycoprotein S1-Induction In Vitro in A549 Lung Cells and THP-1 Macrophages via Inhibition of the NLRP3 Inflammasome Pathway,” *Nutrients* 2022, 14, 13: 2738. doi: https://doi.org/10.3390/nu14132738

19. Villacampa A et al., “SARS-CoV-2 S protein activates NLRP3 inflammasome and deregulates coagulation factors in endothelial and immune cells,” *Cell Commun. Signal.* 2024, 22, 38. doi: https://doi.org/10.1186/s12964-023-01397-6

X. Ocular, ophthalmic, conjunctival

1. Golob-Schwarzl N et al., “SARS-CoV-2 spike protein functionally interacts with primary human conjunctival epithelial cells to induce a pro-inflammatory response,” *Eye* 2022, 36: 2353–5. doi: https://doi.org/10.1038/s41433-022-02066-7

2. Grishma K and Das Sarma, “The Role of Coronavirus Spike Protein in Inducing Optic Neuritis in Mice: Parallels to the SARS-CoV-2 Virus,” *J Neuroophthalmol* 2024, 44, 3: 319-329. doi: 10.1097/WNO.0000000000002234

3. Zhu G et al., “SARS-CoV-2 spike protein-induced host inflammatory response signature in human corneal epithelial cells,” *Mol. Med. Rep.* 2021, 24: 584. doi: https://doi.org/10.3892/mmr.2021.12223

Y. Other cell signaling

1. Caohuy H et al., “Inflammation in the COVID-19 airway is due to inhibition of CFTR signaling by the SARS-CoV-2 spike protein,” *Sci. Rep.* 2024, 14: 16895. doi: https://doi.org/10.1038/s41598-024-66473-4

2. Choi JY et al., “SARS-CoV-2 spike S1 subunit protein-mediated increase of beta-secretase 1 (BACE1) impairs human brain vessel cells,” *Biochem. Biophys. Res. Commun.* 2022, 625, 20: 66-71. doi: https://doi.org/10.1016/j.bbrc.2022.07.113

3. Chrestia JF et al., “A Functional Interaction Between Y674-R685 Region of the SARS-CoV-2 Spike Protein and the Human α7 Nicotinic Receptor,” *Mol. Neurobiol.* 2022, 59: 676-690. doi: https://doi.org/10.1007/s12035-022-02947-8

4. Corpetti C et al., “Cannabidiol inhibits SARS-Cov-2 spike (S) protein-induced cytotoxicity and inflammation through a PPARγ-dependent TLR4/NLRP3/Caspase-1 signaling suppression in Caco-2 cell line,” *Phytother. Res.* 2021, 35, 12: 6893–6903. doi: https://doi.org/10.1002/ptr.7302

5. Gracie NP et al., “Cellular signalling by SARS-CoV-2 spike protein,” *Microbiology Australia* 2024, 45, 1: 13-17. doi: https://doi.org/10.1071/MA24005

6. Hasan MZ et al., “SARS-CoV-2 infection induces adaptive NK cell responses by spike protein-mediated induction of HLA-E expression,” *Emerg Microbes Infect*. 2024, 13: 2361019. doi: https://doi.org/10.1080/22221751.2024.2361019

7. Li F et al., “SARS-CoV-2 Spike Promotes Inflammation and Apoptosis Through Autophagy by ROS-Suppressed PI3K/AKT/mTOR Signaling,” *Biochim Biophys Acta BBA - Mol Basis Dis* 2021, 1867: 166260. doi: 10.1016/j.bbadis.2021.166260

8. Li K et al., “SARS-CoV-2 Spike protein promotes vWF secretion and thrombosis via endothelial cytoskeleton-associated protein 4 (CKAP4),” *Signal Transduct Targ Ther.* 2022, 7, 332. doi: https://doi.org/10.1038/s41392-022-01183-9

9. Moutal A et al., “SARS-CoV-2 Spike protein co-opts VEGF-A/Neuropilin-1 receptor signaling to induce analgesia,” *Pain* 2020, 162, 1: 243–252. doi: 10.1097/j.pain.0000000000002097

10. Munavilli GG et al., “COVID-19/SARS-CoV-2 virus spike protein-related delayed inflammatory reaction to hyaluronic acid dermal fillers: a challenging clinical conundrum in diagnosis and treatment,” *Arch. Dermatol. Res.* 2022, 314: 1-15. doi: https://doi.org/10.1007/s00403-021-02190-6

11. O’Brien BCV et al., “SARS-CoV-2 spike ectodomain targets α7 nicotinic acetylcholine receptors,” *J. Biol. Chem.* 2023, 299, 5: 104707. doi: 10.1016/j.jbc.2023.104707

12. Oliveira ASF et al., “A potential interaction between the SARS-CoV-2 spike protein and nicotinic acetylcholine receptors,” *Biophys. J.* 2021, 120, 6: 983-993. doi: 10.1016/j.bpj.2021.01.037

13. Prieto-Villalobos J et al., “SARS-CoV-2 spike protein S1 activates Cx43 hemichannels and disturbs intracellular Ca^{2+} dynamics,” *Biol Res.* 2023, 56, 1: 56. doi: https://doi.org/10.1186/s40659-023-00468-9

14. Rotoli BM et al., “Endothelial cell activation by SARS-CoV-2 spike S1 protein: A crosstalk between endothelium and innate immune cells,” *Biomedicines* 2021, 9, 9: 1220. doi: https://doi.org/10.3390/biomedicines9091220

15. Singh N and Anuradha Bharara Singh, “S2 Subunit of SARS-nCoV-2 Interacts with Tumor Suppressor Protein p53 and BRCA: An in Silico Study,” *Translational Oncology* 2020, 13, 10: 100814. doi: https://doi.org/10.1016/j.tranon.2020.100814

16. Singh RD, “The spike protein of sars-cov-2 induces heme oxygenase-1: pathophysiologic implications,” *Biochim Biophys Acta, Mol Basis Dis* 2022, 1868, 3: 166322. doi: https://doi.org/10.1016/j.bbadis.2021.166322

17. Solis O et al., “The SARS-CoV-2 spike protein binds and modulates estrogen receptors,” *Sci. Adv.* 2022, 8, 48: eadd4150. doi: 10.1126/sciadv.add4150

18. Suzuki YJ et al., “SARS-CoV-2 spike protein-mediated cell signaling in lung vascular cells,” *Vascul. Pharmacol.* 2021, 137: 106823. doi: 10.1016/j.vph.2020.106823

19. Suzuki YJ and SG Gychka, “SARS-CoV-2 Spike Protein Elicits Cell Signaling in Human Host Cells: Implications for Possible Consequences of COVID-19 Vaccines,” *Vaccines* 2021, 9, 1: 36. doi: https://doi.org/10.3390/vaccines9010036

20. Tillman TS et al., “SARS-CoV-2 Spike Protein Downregulates Cell Surface alpha7nAChR through a Helical Motif in the Spike Neck,” *ACS Chem. Neurosci.* 2023, 14, 4: 689–698. doi: 10.1021/acschemneuro.2c00610

Z. PASC, post COVID, long COVID

1. Bellucci M et al., “Post-SARS-CoV-2 infection and post-vaccine-related neurological complications share clinical features and the same positivity to anti-ACE2 antibodies,” *Front. Immunol.* 2024, 15 (Sec. Multiple Sclerosis and Neuroimmunology). doi: https://doi.org/10.3389/fimmu.2024.1398028

2. Craddock V et al., “Persistent circulation of soluble and extracellular vesicle-linked Spike protein in individuals with postacute sequelae of COVID-19,” *J Med. Virol.* 2023, 95, 2: e28568. doi: https://doi.org/10.1002/jmv.28568

3. De Melo BP et al., “SARS-CoV-2 Spike Protein and Long COVID—Part 1: Impact of Spike Protein in Pathophysiological Mechanisms of Long COVID Syndrome,” *Viruses* 2025, 17, 5: 617. doi: https://doi.org/10.3390/v17050617

4. Dissook S et al., “Luteolin-rich fraction from Perilla frutescens seed meal inhibits spike glycoprotein S1 of SARS-CoV-2-induced NLRP3 inflammasome lung cell inflammation via regulation of JAK1/STAT3 pathway: A potential anti-inflammatory compound against inflammation-induced long-COVID,” *Front. Med.* 2023, 9: 1072056. doi: https://doi.org/10.3389/fmed.2022.1072056

5. Frank MG et al., “Exploring the immunogenic properties of SARS-CoV-2 structural proteins: PAMP:TLR signaling in the mediation of the neuroinflammatory and neurologic sequelae of COVID-19,” *Brain Behav Immun* 2023, 111. doi: https://doi.org/10.1016/j.bbi.2023.04.009

6. Frank MG et al., “SARS-CoV-2 S1 subunit produces a protracted priming of the neuroinflammatory, physiological, and behavioral responses to a remote immune challenge: A role for corticosteroids,” *Brain Behav. Immun.* 2024, 121: 87-103. doi: https://doi.org/10.1016/j.bbi.2024.07.034

7. Fraser ME at al., “SARS-CoV-2 Spike Protein and Viral RNA Persist in the Lung of Patients With Post-COVID Lung Disease (abstract),” *Am J Respir Crit Care Med* 2024, 209: A4193. doi: 10.1164/ajrccm-conference.2024.209.1_MeetingAbstracts.A4193

8. Goh D et al., “Case report: Persistence of residual antigen and RNA of the SARS-CoV-2 virus in tissues of two patients with long COVID,” *Front. Immunol.* 2022, 13 (Sec. Viral Immunology). doi: https://doi.org/10.3389/fimmu.2022.939989

9. Halma MTJ et al., “Exploring autophagy in treating SARS-CoV-2 spike protein-related pathology,” *Endocrinol Metab (EnM)* 2024, 14: 100163. doi: https://doi.org/10.1016/j.endmts.2024.100163

10. Halma MTJ et al., “Strategies for the Management of Spike Protein-Related Pathology,” *Microorganisms* 2023, 11, 5: 1308. doi: 10.3390/microorganisms11051308

11. Hano S et al., “A case of persistent, confluent maculopapular erythema following a COVID-19 mRNA vaccination is possibly associated with the intralesional spike protein expressed by vascular endothelial cells and eccrine glands in the deep dermis,” *J Dermatol* 2023, 50, 9: 1208-1212. doi: 10.1111/1346-8138.16816

12. Kempuraj D et al., “Long COVID elevated MMP-9 and release from microglia by SARS-CoV-2 Spike protein,” *Transl. Neurosci.* 2024, 15: 20220352. doi: https://doi.org/10.1515/tnsci-2022-0352

13. Patterson BK et al., “Persistence of SARS CoV-2 S1 Protein in CD16+ Monocytes in Post-Acute Sequelae of COVID-19 (PASC) up to 15 Months Post-Infection,” *Front. Immunol.* 12 (Sec. Viral Immunology). doi: 10.3389/fimmu.2021.746021

14. Peluso MJ et al., “Plasma-based antigen persistence in the post-acute phase of COVID-19,” *Lancet* 2024, 24, 6: E345-E347. doi: 10.1016/S1473-3099(24)00211-1

15. Rong Z et al., “Persistence of spike protein at the skull-meninges-brain axis may contribute to the neurological sequelae of COVID-19,” *Cell Host Microbe* 2024, 26: S1931-3128(24)00438-4. doi: 10.1016/j.chom.2024.11.007

16. Scholkmann F and CA May, “COVID-19, post-acute COVID-19 syndrome (PACS, ‘long COVID’) and post-COVID-19 vaccination syndrome (PCVS, ‘post-COVIDvac-syndrome’): Similarities and differences,” *Pathol Res Pract.* 2023, 246: 154497. doi: https://doi.org/10.1016/j.prp.2023.154497

17. Schultheiss C et al., “Liquid biomarkers of macrophage dysregulation and circulating spike protein illustrate the biological heterogeneity in patients with post-acute sequelae of COVID-19,” *J Med Virol* 2023, 95, 1: e28364. doi: 10.1002/jmv.28364

18. Swank Z, et al. “Persistent Circulating Severe Acute Respiratory Syndrome Coronavirus 2 Spike Is Associated With Post-acute Coronavirus Disease 2019 Sequelae,” *Clin. Infect. Dis.* 2023, 76, 3: e487–e490. doi: 10.1093/cid/ciac722

19. Theoharides TC, “Could SARS-CoV-2 Spike Protein Be Responsible for Long-COVID Syndrome?” *Mol. Neurobiol.* 2022, 59, 3: 1850–1861. doi: 10.1007/s12035-021-02696-0

20. Visvabharathy L et al., “Case report: Treatment of long COVID with a SARS-CoV-2 antiviral and IL-6 blockade in a patient with rheumatoid arthritis and SARS-CoV-2 antigen persistence,” *Front. Med.* 2022, 9 (Sec. Infectious Diseases – Surveillance). doi: https://doi.org/10.3389/fmed.2022.1003103

21. Yamamoto M et al., “Persistent varicella zoster virus infection following mRNA COVID-19 vaccination was associated with the presence of encoded spike protein in the lesion,” *J. Cutan Immunol. Allergy.* 2022:1–6. doi: 10.1002/cia2.12278

22. Zollner A et al., “Postacute COVID-19 is Characterized by Gut Viral Antigen Persistence in Inflammatory Bowel Diseases,” *Gastroenterology* 2022, 163, 2: 495-506.e8. doi: https://doi.org/10.1053/j.gastro.2022.04.037

AA. Pregnancy

1. Erdogan MA, “Prenatal SARS-CoV-2 Spike Protein Exposure Induces Autism-Like Neurobehavioral Changes in Male Neonatal Rats,” *J Neuroimmune Pharmacol.* 2023, 18, 4: 573-591. doi: 10.1007/s11481-023-10089-4

2. Guo X et al., “Regulation of proinflammatory molecules and tissue factor by SARS-CoV-2 spike protein in human placental cells: implications for SARS-CoV-2 pathogenesis in pregnant women,” *Front. Immunol.* 2022, 13: 876555–876555. doi: https://doi.org/10.3389/fimmu.2022.876555

3. Kammala AK et al., “In vitro mRNA-S maternal vaccination induced altered immune regulation at the maternal-fetal interface,” *Am. J. Reprod. Immunol.* 2024, 91, 5: e13861. doi: https://doi.org/10.1111/aji.13861

4. Karrow NA et al., “Maternal COVID-19 Vaccination and Its Potential Impact on Fetal and Neonatal Development,” *Vaccines* 2021, 9: 1351. doi: 10.3390/vaccines9111351

5. Parcial ALN et al., “SARS-CoV-2 Is Persistent in Placenta and Causes Macroscopic, Histopathological, and Ultrastructural Changes,” *Viruses* 2022, 14, 9: 1885. doi: https://doi.org/10.3390/v14091885

6. Wu H et al., “Molecular evidence suggesting the persistence of residual SARS-CoV-2 and immune responses in the placentas of pregnant patients recovered from COVID-19,” *Cell Prolif.* 2021, 54, 9: e13091. doi: https://doi.org/10.1111/cpr.13091

7. Zurlow M et al., “The anti-SARS-CoV-2 BNT162b2 vaccine suppresses mithramycin-induced erythroid differentiation and expression of embryo-fetal globin genes in human erythroleukemia K562 cells.” *Exp Cell Res* 2023, 433, 2: 113853. doi: https://doi.org/10.1016/j.yexcr.2023.113853

BB. Pulmonary, respiratory

1. Bhargavan B and GD Kanmogne, “SARS-CoV-2 spike proteins and cell–cell communication inhibits TFPI and induces thrombogenic factors in human lung microvascular endothelial cells and neutrophils: implications for COVID-19 coagulopathy pathogenesis,” *Int. J. Mol. Sci.* 2022, 23, 18: 10436. doi: https://doi.org/10.3390/ijms231810436

2. Biancatelli RMLC et al., “The SARS-CoV-2 spike protein subunit S1 induces COVID-19-like acute lung injury in Kappa18-hACE2 transgenic mice and barrier dysfunction in human endothelial cells,” *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2021, 321: L477–L484. doi: https://doi.org/10.1152/ajplung.00223.2021

3. Cao JB et al., “Mast cell degranulation-triggered by SARS-CoV-2 induces tracheal-bronchial epithelial inflammation and injury,” *Virol. Sin.* 2024, 39, 2: 309-318. doi: https://doi.org/10.1016/j.virs.2024.03.001

4. Cao X et al., “Spike protein of SARS-CoV-2 activates macrophages and contributes to induction of acute lung inflammation in male mice,” *FASEB J.* 2021, 35, e21801. doi: https://doi.org/10.1096/fj.202002742RR

5. Caohuy H et al., “Inflammation in the COVID-19 airway is due to inhibition of CFTR signaling by the SARS-CoV-2 spike protein,” *Sci. Rep.* 2024, 14: 16895. doi: https://doi.org/10.1038/s41598-024-66473-4

6. Chittasupho C et al., “Inhibition of SARS-CoV-2-Induced NLRP3 Inflammasome-Mediated Lung Cell Inflammation by Triphala-Loaded Nanoparticle Targeting Spike Glycoprotein S1,” *Pharmaceutics* 2024, 16, 6: 751. doi: 10.3390/pharmaceutics16060751

7. Chittasupho C et al., “Targeting spike glycoprotein S1 mediated by NLRP3 inflammasome machinery and the cytokine releases in A549 lung epithelial cells by nanocurcumin,” *Pharmaceuticals* (Basel) 2023, 16, 6: 862. doi: 10.3390/ph16060862

8. DeVries A et al., “SARS-CoV-2 Spike Protein is Sufficient to Induce Enhanced Pro-inflammatory Transcriptional Responses in Nasal Epithelial Cells from Atopic Asthmatics,” *J Allergy Clin Immunol* 2025, 155, 2: AB85. doi: 10.1016/j.jaci.2024.12.271

9. Del Re A et al., “Intranasal delivery of PEA-producing *Lactobacillus paracasei F19* alleviates SARS-CoV-2 spike protein-induced lung injury in mice,” *Transl. Med. Commun.* 2024, 9, 9. doi: https://doi.org/10.1186/s41231-024-00167-x

10. Forsyth CB *et al.*, “The SARS-CoV-2 S1 spike protein promotes MAPK and NF-kB activation in human lung cells and inflammatory cytokine production in human lung and intestinal epithelial cells,” *Microorganisms* 2022, 10, 10: 1996. doi: https://doi.org/10.3390/microorganisms10101996

11. Fraser ME at al., “SARS-CoV-2 Spike Protein and Viral RNA Persist in the Lung of Patients With Post-COVID Lung Disease (abstract),” *Am J Respir Crit Care Med* 2024, 209: A4193. doi: 10.1164/ajrccm-conference.2024.209.1_MeetingAbstracts.A4193

12. Greenberger JS et al., “SARS-CoV-2 Spike Protein Induces Oxidative Stress and Senescence in Mouse and Human Lung,” *In Vivo* 2024, 38, 4: 1546-1556. doi: https://doi.org/10.21873/invivo.13605

13. Jana S et al., “Cell-free hemoglobin does not attenuate the effects of SARS-CoV-2 spike protein S1 subunit in pulmonary endothelial cells,” *Int. J. Mol. Sci.* 2021, 22, 16: 9041. doi: https://doi.org/10.3390/ijms22169041

14. Kulkoviene G et al., “Differential Mitochondrial, Oxidative Stress and Inflammatory Responses to SARS-CoV-2 Spike Protein Receptor Binding Domain in Human Lung Microvascular, Coronary Artery Endothelial and Bronchial Epithelial Cells,” *Int. J. Mol. Sci.* 2024, 25, 6: 3188. doi: https://doi.org/10.3390/ijms25063188

15. Liang S et al., “SARS-CoV-2 spike protein induces IL-18-mediated cardiopulmonary inflammation via reduced mitophagy,” *Signal Transduct Target Ther* 2023, 8, 103. doi: https://doi.org/10.1038/s41392-023-01368-w

16. Liu T et al., “RS-5645 attenuates inflammatory cytokine storm induced by SARS-CoV-2 spike protein and LPS by modulating pulmonary microbiota,” *Int J Biol Sci.* 2021, 17, 13: 3305–3319. doi: 10.7150/ijbs.63329

17. Liu Y et al., “The recombinant spike S1 protein induces injury and inflammation in co-cultures of human alveolar epithelial cells and macrophages,” *PLoS ONE* 2025, 20, 2: e0318881. doi: https://doi.org/10.1371/journal.pone.0318881

18. Palestra F et al. “SARS-CoV-2 Spike Protein Activates Human Lung Macrophages,” *Int. J. Mol. Sci.* 2023, 24, 3: 3036. doi: 10.3390/ijms24033036

19. Park C et al., “Murine alveolar Macrophages Rapidly Accumulate intranasally Administered SARS-CoV-2 Spike Protein leading to neutrophil Recruitment and Damage,” *Elife* 2024, 12: RP86764. doi: https://doi.org/10.7554/eLife.86764.3

20. Puthia MTL et al., “Experimental model of pulmonary inflammation induced by SARS-CoV-2 spike protein and endotoxin,” *ACS Pharmacol Transl Sci*. 2022, 5, 3: 141–8. doi: https://doi.org/10.1021/acsptsci.1c00219

21. Rahman M et al., “Differential Effect of SARS-CoV-2 Spike Glycoprotein 1 on Human Bronchial and Alveolar Lung Mucosa Models: Implications for Pathogenicity,” *Viruses* 2021, 13, 12: 2537. doi: https://doi.org/10.3390/v13122537

22. Roy A et al., “Ultradiluted *Eupatorium perfoliatum* Prevents and Alleviates SARS-CoV-2 Spike Protein-Induced Lung Pathogenesis by Regulating Inflammatory Response and Apoptosis,” *Diseases* 2025, 13, 2: 36. doi: 10.3390/diseases13020036

23. Ruben ML et al., “The SARS-CoV-2 spike protein subunit S1 induces COVID-19-like acute lung injury in K18-hACE2 transgenic mice and barrier dysfunction in human endothelial cells,” *Am J Physiol Lung Cell Mol Physiol.* 2021, 321, 2: L477-L484. doi: https://doi.org/10.1152/ajplung.00223.2021

24. Segura-Villalobos D et al., “Jacareubin inhibits TLR4-induced lung inflammatory response caused by the RBD domain of SARS-CoV-2 Spike protein,” *Pharmacol. Rep.* 2022, 74: 1315–1325. doi: https://doi.org/10.1007/s43440-022-00398-5

25. Semmarath W et al., “Cyanidin-3-O-glucoside and Peonidin-3-O-glucoside-Rich Fraction of Black Rice Germ and Bran Suppresses Inflammatory Responses from SARS-CoV-2 Spike Glycoprotein S1-Induction In Vitro in A549 Lung Cells and THP-1 Macrophages via Inhibition of the NLRP3 Inflammasome Pathway,” *Nutrients* 2022, 14, 13: 2738. doi: https://doi.org/10.3390/nu14132738

26. Sirsendu J et al., “Cell-Free Hemoglobin Does Not Attenuate the Effects of SARS-CoV-2 Spike Protein S1 Subunit in Pulmonary Endothelial Cells,” *Int. J. Mol. Sci.* 2021, 22, 16: 9041. doi: https://doi.org/10.3390/ijms22169041

27. Solopov et al., “Alcohol increases lung angiotensin-converting enzyme 2 expression and exacerbates severe acute respiratory syndrome coronavirus 2 spike protein subunit 1-induced acute lung injury in K18-hACE2 transgenic mice,” *Am J Pathol* 2022, 192, 7: 990-1000. doi: 10.1016/j.ajpath.2022.03.012

28. Sui Y et al., “SARS-CoV-2 Spike Protein Suppresses ACE2 and Type I Interferon Expression in Primary Cells From Macaque Lung Bronchoalveolar Lavage,” *Front. Immunol.* 2021, 12. doi: https://doi.org/10.3389/fimmu.2021.658428

29. Sung PS et al., “CLEC5A and TLR2 Are Critical in SARS-CoV-2-Induced NET Formation and Lung Inflammation,” *J. Biomed. Sci.* 2022, 29, 52. doi: https://doi.org/10.1186/s12929-022-00832-z

30. Suzuki YJ et al., “SARS-CoV-2 spike protein-mediated cell signaling in lung vascular cells,” *Vascul. Pharmacol.* 2021, 137: 106823. doi: 10.1016/j.vph.2020.106823

31. Yang K et al., “SARS-CoV-2 spike protein receptor-binding domain perturbates intracellular calcium homeostasis and impairs pulmonary vascular endothelial cells,” *Signal Transduct Target Ther.* 2023, 8, 276. doi: 10.1038/s41392-023-01556-8

32. Yeung-Luk BH et al., “SARS-CoV-2 infection alters mitochondrial and cytoskeletal function in human respiratory epithelial cells mediated by expression of spike protein,” *mBio* 2023, 14, 4: e00820-23. doi: https://doi.org/10.1128/mbio.00820-23

33. Zekri-Nechar K et al., “Spike Protein Subunits of SARS-CoV-2 Alter Mitochondrial Metabolism in Human Pulmonary Microvascular Endothelial Cells: Involvement of Factor Xa,” *Dis. Markers* 2022, 1118195. doi: https://doi.org/10.1155/2022/1118195

CC. Renin-Angiotensin-Aldosterone System

1. Burnett FN et al., “SARS-CoV-2 Spike Protein Intensifies Cerebrovascular Complications in Diabetic hACE2 Mice through RAAS and TLR Signaling Activation,” *Int. J. Mol. Sci.* 2023, 24, 22: 16394. doi: https://doi.org/10.3390/ijms242216394

2. Lehmann KJ, “SARS-CoV-2-Spike Interactions with the Renin-Angiotensin-Aldosterone System – Consequences of Adverse Reactions of Vaccination,” *J Biol Today’s World* 2023, 12/4: 001-013. doi: https://doi.org/10.31219/osf.io/27g5h

3. Matsuzawa Y et al., “Impact of Renin–Angiotensin–Aldosterone System Inhibitors on COVID-19,” *Hypertens. Res.* 2022, 45, 7: 1147–1153. doi: 10.1038/s41440-022-00922-3

DD. Senescence/aging

1. Duarte C, “Age-dependent effects of the recombinant spike protein/SARS-CoV-2 on the M-CSF- and IL-34-differentiated macrophages in vitro,” *Biochem. Biophys. Res. Commun.* 2021, 546: 97–102. doi: https://doi.org/10.1016/j.bbrc.2021.01.104

2. Greenberger JS et al., “SARS-CoV-2 Spike Protein Induces Oxidative Stress and Senescence in Mouse and Human Lung,” *In Vivo* 2024, 38, 4: 1546-1556. doi: https://doi.org/10.21873/invivo.13605

3. Meyer K et al., “SARS-CoV-2 Spike Protein Induces Paracrine Senescence and Leukocyte Adhesion in Endothelial Cells,” *J. Virol.* 2021, 95, e0079421. doi: https://doi.org/10.1128/jvi.00794-21

EE. Stem cells

1. Balzanelli MG et al., “The Role of SARS-CoV-2 Spike Protein in Long-term Damage of Tissues and Organs, the Underestimated Role of Retrotransposons and Stem Cells, a Working Hypothesis,” *Endocr Metab Immune Disord Drug Targets* 2025, 25, 2: 85-98. doi: 10.2174/0118715303283480240227113401

2. Kucia M et al. “An evidence that SARS-Cov-2/COVID-19 spike protein (SP) damages hematopoietic stem/progenitor cells in the mechanism of pyroptosis in Nlrp3 inflammasome-dependent manner,” *Leukemia* 2021, 35: 3026-3029. doi: https://doi.org/10.1038/s41375-021-01332-z

3. Ropa J et al., “Human Hematopoietic Stem, Progenitor, and Immune Cells Respond Ex Vivo to SARS-CoV-2 Spike Protein,” *Stem Cell Rev Rep.* 2021, 17, 1: 253-265. doi: https://doi.org/10.1007/s12015-020-10056-z

FF. Syncytia/cell fusion

1. Braga L et al., “Drugs that inhibit TMEM16 proteins block SARS-CoV-2 spike-induced syncytia,” *Nature* 2021, 594: 88–93. doi: 10.1038/s41586-021-03491-6

2. Cattin-Ortolá J et al., “Sequences in the cytoplasmic tail of SARS-CoV-2 Spike facilitate expression at the cell surface and syncytia formation,” *Nat Commun* 2021, 12, 1: 5333. doi: https://doi.org/10.1038/s41467-021-25589-1

3. Clemens DJ et al., “SARS-CoV-2 spike protein-mediated cardiomyocyte fusion may contribute to increased arrhythmic risk in COVID-19,” *PLoS One* 2023, 18, 3: e0282151. doi: https://doi.org/10.1371/journal.pone.0282151

4. Lazebnik Y, “Cell fusion as a link between the SARS-CoV-2 spike protein, COVID-19 complications, and vaccine side effects,” *Oncotarget* 2021, 12, 25: 2476-2488. doi: https://doi.org/10.18632/oncotarget.28088

5. Liu X et al., “SARS-CoV-2 spike protein-induced cell fusion activates the cGAS-STING pathway and the interferon response,” *Sci Signal.* 2022, 15, 729: eabg8744. doi: 10.1126/scisignal.abg8744

6. Martinez-Marmol R et al., “SARS-CoV-2 infection and viral fusogens cause neuronal and glial fusion that compromises neuronal activity,” *Sci. Adv.* 2023, 9, 23. doi: 10.1126/sciadv.adg2248

7. Rajah MM et al., “SARS-CoV-2 Alpha, Beta, and Delta variants display enhanced spike-mediated syncytia formation,” *EMBO J.* 2021, 40: e108944. doi: https://doi.org/10.15252/embj.2021108944

8. Shirato K and Takako Kizaki, “SARS-CoV-2 Spike Protein S1 Subunit Induces Pro-inflammatory Responses via Toll-Like Receptor 4 Signaling in Murine and Human Macrophages,” *Heliyon* 2021, 7, 2: e06187. doi: 10.1016/j.heliyon.2021.e06187

9. Theuerkauf SA et al., “Quantitative assays reveal cell fusion at minimal levels of SARS-CoV-2 spike protein and fusion from without,” *iScience* 2021, 24, 3: 102170. doi: https://doi.org/10.1016/j.isci.2021.102170

10. Zhang Z et al., “SARS-CoV-2 spike protein dictates syncytium-mediated lymphocyte elimination,” *Cell Death Differ.* 2021, 28: 2765–2777. doi: 10.1038/s41418-021-00782-3

GG. Therapeutics

1. Almehdi AM et al., “SARS-CoV-2 Spike Protein: Pathogenesis, Vaccines, and Potential Therapies,” *Infection* 2021, 49, 5: 855–876. doi: 10.1007/s15010-021-01677-8

2. Boretti A, “PQQ Supplementation and SARS-CoV-2 Spike Protein-Induced Heart Inflammation,” *Nat. Prod. Commun.* 2022, 17, 1934578x221080929. doi: https://doi.org/10.1177/1934578X221080929

3. Boschi C et al., “SARS-CoV-2 Spike Protein Induces Hemagglutination: Implications for COVID-19 Morbidities and Therapeutics and for Vaccine Adverse Effects,” *Int. J. Biol. Macromol.* 2022, 23, 24: 15480. doi: https://doi.org/10.3390/ijms232415480

4. Braga L et al., “Drugs that inhibit TMEM16 proteins block SARS-CoV-2 spike-induced syncytia,” *Nature* 2021, 594: 88–93. doi: 10.1038/s41586-021-03491-6

5. Chang MH et al., “SARS-CoV-2 Spike Protein 1 Causes Aggregation of α-Synuclein via Microglia-Induced Inflammation and Production of Mitochondrial ROS: Potential

Therapeutic Applications of Metformin," *Biomedicines* 2024, 12, 6: 1223. doi: https://doi.org/10.3390/biomedicines12061223

6. Chittasupho C et al., "Inhibition of SARS-CoV-2-Induced NLRP3 Inflammasome-Mediated Lung Cell Inflammation by Triphala-Loaded Nanoparticle Targeting Spike Glycoprotein S1," *Pharmaceutics* 2024, 16, 6: 751. doi: 10.3390/pharmaceutics16060751

7. Chittasupho C et al., "Targeting spike glycoprotein S1 mediated by NLRP3 inflammasome machinery and the cytokine releases in A549 lung epithelial cells by nanocurcumin," *Pharmaceuticals* (Basel) 2023, 16, 6: 862. doi: 10.3390/ph16060862

8. Corpetti C et al., "Cannabidiol inhibits SARS-Cov-2 spike (S) protein-induced cytotoxicity and inflammation through a PPARγ-dependent TLR4/NLRP3/Caspase-1 signaling suppression in Caco-2 cell line," *Phytother. Res.* 2021, 35, 12: 6893-6903. doi: https://doi.org/10.1002/ptr.7302

9. Cory TJ et al., "Metformin Suppresses Monocyte Immunometabolic Activation by SARS-CoV-2 Spike Protein Subunit 1," *Front. Immunol.* 2021, 12 (Sec. Cytokines and Soluble Mediators in Immunity): 733921. doi: 10.3389/fimmu.2021.733921

10. Del Re A et al., "Intranasal delivery of PEA-producing *Lactobacillus paracasei F19* alleviates SARS-CoV-2 spike protein-induced lung injury in mice," *Transl. Med. Commun.* 2024, 9, 9. doi: https://doi.org/10.1186/s41231-024-00167-x

11. Del Re A et al., "Ultramicronized Palmitoylethanolamide Inhibits NLRP3 Inflammasome Expression and Pro-Inflammatory Response Activated by SARS-CoV-2 Spike Protein in Cultured Murine Alveolar Macrophages," *Metabolites* 2021, 11, 9: 592. doi: https://doi.org/10.3390/metabo11090592

12. Dhandapani S et al., "Lipid-encapsulated gold nanoparticles: an advanced strategy for attenuating the inflammatory response in SARS-CoV-2 infection," *J. Nanobiotechnology* 2025, 23, 15. doi: https://doi.org/10.1186/s12951-024-03064-5

13. Ferrer MD et al., "Nitrite Attenuates the In Vitro Inflammatory Response of Immune Cells to the SARS-CoV-2 S Protein without Interfering in the Antioxidant Enzyme Activation," *Int. J. Mol. Sci.* 2024, 25, 5: 3001. https://doi.org/10.3390/ijms25053001

14. Frank MG et al., "SARS-CoV-2 S1 subunit produces a protracted priming of the neuroinflammatory, physiological, and behavioral responses to a remote immune challenge: A role for corticosteroids," *Brain Behav. Immun.* 2024, 121: 87-103. doi: https://doi.org/10.1016/j.bbi.2024.07.034

15. Frühbeck G et al., “FNDC4 and FNDC5 reduce SARS-CoV-2 entry points and spike glycoprotein S1-induced pyroptosis, apoptosis, and necroptosis in human adipocytes,” *Cell Mol Immunol.* 2021, 18, 10: 2457–9. doi: 10.1038/s41423-021-00762-0

16. Gasparello J et al., “Aged Garlic Extract (AGE) and Its Constituent S-Allyl-Cysteine (SAC) Inhibit the Expression of Pro-Inflammatory Genes Induced in Bronchial Epithelial IB3-1 Cells by Exposure to the SARS-CoV-2 Spike Protein and the BNT162b2 Vaccine,” *Molecules* 2024, 29, 24: 5938. doi: 10.3390/molecules29245938

17. Gasparello J et al., “In vitro induction of interleukin-8 by SARS-CoV-2 Spike protein is inhibited in bronchial epithelial IB3-1 cells by a miR-93-5p agomiR,” *Int. Immunopharmacol.* 2021, 101: 108201. doi: 10.1016/j.intimp.2021.108201

18. Gasparello J et al., “Sulforaphane inhibits the expression of interleukin-6 and interleukin-8 induced in bronchial epithelial IB3-1 cells by exposure to the SARS-CoV-2 Spike protein,” *Phytomedicine* 2021, 87: 153583. doi: https://doi.org/10.1016/j.phymed.2021.153583

19. Halma MTJ et al., “Exploring autophagy in treating SARS-CoV-2 spike protein-related pathology,” *Endocrinol Metab (EnM)* 2024, 14: 100163. doi: 10.1016/j.endmts.2024.100163

20. Halma MTJ et al., “Strategies for the Management of Spike Protein-Related Pathology,” *Microorganisms* 2023, 11, 5: 1308. doi: 10.3390/microorganisms11051308

21. Jana S et al., “Cell-free hemoglobin does not attenuate the effects of SARS-CoV-2 spike protein S1 subunit in pulmonary endothelial cells,” *Int. J. Mol. Sci.* 2021, 22, 16: 9041. doi: https://doi.org/10.3390/ijms22169041

22. Jugler C et al., “SARS-CoV-2 Spike Protein-Induced Interleukin 6 Signaling Is Blocked by a Plant-Produced Anti-Interleukin 6 Receptor Monoclonal Antibody,” *Vaccines* 2021, 9, 11: 1365. doi: https://doi.org/10.3390/vaccines9111365

23. Ken W et al., “Low dose radiation therapy attenuates ACE2 depression and inflammatory cytokines induction by COVID-19 viral spike protein in human bronchial epithelial cells,” *Int J Radiat Biol.* 2022, 98, 10:1532-1541. doi: https://doi.org/10.1080/09553002.2022.2055806

24. Kumar N et al., “SARS-CoV-2 spike protein S1-mediated endothelial injury and pro-inflammatory state Is amplified by dihydrotestosterone and prevented by mineralocorticoid antagonism,” *Viruses* 2021, 13, 11: 2209. doi: 10.3390/v13112209

25. Liu T et al., “RS-5645 attenuates inflammatory cytokine storm induced by SARS-CoV-2 spike protein and LPS by modulating pulmonary microbiota,” *Int J Biol Sci*. 2021, 17, 13: 3305–3319. doi: 10.7150/ijbs.63329

26. Loh D, “The potential of melatonin in the prevention and attenuation of oxidative hemolysis and myocardial injury from cd147 SARS-CoV-2 spike protein receptor binding,” *Melatonin Research* 2020, 3, 3: 380-416. doi: 10.32794/mr11250069

27. Loh JT et al., “Dok3 restrains neutrophil production of calprotectin during TLR4 sensing of SARS-CoV-2 spike protein,” *Front. Immunol.* 2022, 13 (Sec. Molecular Innate Immunity). doi: https://doi.org/10.3389/fimmu.2022.996637

28. Marrone L et al., “Tirofiban prevents the effects of SARS-CoV-2 spike protein on macrophage activation and endothelial cell death,” *Heliyon* 2024, 10, 15: e35341. doi: 10.1016/j.heliyon.2024.e35341

29. Norris B et al., “Evaluation of Glutathione in Spike Protein of SARS-CoV-2 Induced Immunothrombosis and Cytokine Dysregulation,” *Antioxidants* 2024, 13, 3: 271. doi: https://doi.org/10.3390/antiox13030271

30. Oka N et al., “SARS-CoV-2 S1 protein causes brain inflammation by reducing intracerebral acetylcholine production,” *iScience* 2023, 26, 6: 106954. doi: 10.1016/j.isci.2023.106954

31. Olajide OA et al., “Induction of Exaggerated Cytokine Production in Human Peripheral Blood Mononuclear Cells by a Recombinant SARS-CoV-2 Spike Glycoprotein S1 and Its Inhibition by Dexamethasone,” *Inflammation* 2021, 44: 1865–1877. doi: https://doi.org/10.1007/s10753-021-01464-5

32. Petrosino S and N Matende, “Elimination/Neutralization of COVID-19 Vaccine-Produced Spike Protein: Scoping Review,” *Mathews Journal of Nutrition & Dietetics* 2024, 7, 2. doi: https://doi.org/10.30654/MJND.10034

33. Roy A et al., “Ultradiluted *Eupatorium perfoliatum* Prevents and Alleviates SARS-CoV-2 Spike Protein-Induced Lung Pathogenesis by Regulating Inflammatory Response and Apoptosis,” *Diseases* 2025, 13, 2: 36. doi: 10.3390/diseases13020036

34. Satta S et al., “An engineered nano-liposome-human ACE2 decoy neutralizes SARS-CoV-2 Spike protein-induced inflammation in both murine and human macrophages,” *Theranostics* 2022, 12, 6: 2639–2657. doi: 10.7150/thno.66831

35. Segura-Villalobos D et al., “Jacareubin inhibits TLR4-induced lung inflammatory response caused by the RBD domain of SARS-CoV-2 Spike protein,” *Pharmacol. Rep.* 2022, 74: 1315–1325. doi: https://doi.org/10.1007/s43440-022-00398-5

36. Semmarath W et al., “Cyanidin-3-O-glucoside and Peonidin-3-O-glucoside-Rich Fraction of Black Rice Germ and Bran Suppresses Inflammatory Responses from SARS-CoV-2 Spike Glycoprotein S1-Induction In Vitro in A549 Lung Cells and THP-1 Macrophages via Inhibition of the NLRP3 Inflammasome Pathway,” *Nutrients* 2022, 14, 13: 2738. doi: https://doi.org/10.3390/nu14132738

37. Solopov PA et al., “KVX-053, a protein tyrosine phosphatase 4A3 inhibitor, ameliorates SARS-CoV-2 spike protein subunit 1–induced acute lung injury in mice,” *J. Pharmacol. Exp. Ther.* 2025, 392, 3: 100022. doi: 10.1124/jpet.124.002154

38. Suprewicz L et al., “Recombinant human plasma gelsolin reverses increased permeability of the blood-brain barrier induced by the spike protein of the SARS-CoV-2 virus,” *J Neuroinflamm*. 2022, 19, 1: 282. doi: 10.1186/s12974-022-02642-4

39. Tsilioni I et al., “Nobiletin and Eriodictyol Suppress Release of IL-1β, CXCL8, IL-6, and MMP-9 from LPS, SARS-CoV-2 Spike Protein, and Ochratoxin A-Stimulated Human Microglia,” *Int. J. Mol. Sci.* 2025, 26, 2: 636. doi: 10.3390/ijms26020636

40. Vargas-Castro R et al., “Calcitriol prevents SARS-CoV spike-induced inflammation in human trophoblasts through downregulating *ACE2* and *TMPRSS2* expression,” *J Steroid Biochem Mol Biol* 2025, 245: 106625. doi: 10.1016/j.jsbmb.2024.106625

41. Visvabharathy L et al., “Case report: Treatment of long COVID with a SARS-CoV-2 antiviral and IL-6 blockade in a patient with rheumatoid arthritis and SARS-CoV-2 antigen persistence,” *Front. Med*. 2022, 9 (Sec. Infectious Diseases – Surveillance). doi: https://doi.org/10.3389/fmed.2022.1003103

42. Yonker LM et al., “Multisystem inflammatory syndrome in children is driven by zonulin-dependent loss of gut mucosal barrier,” *J Clin Invest*. 2021, 131, 14: e149633. doi: https://doi.org/10.1172/JCI149633

43. Youn JY et al., “Therapeutic application of estrogen for COVID-19: Attenuation of SARS-CoV-2 spike protein and IL-6 stimulated, ACE2-dependent NOX2 activation, ROS production and MCP-1 upregulation in endothelial cells,” *Redox Biol.* 2021, 46: 102099. doi: https://doi.org/10.1016/j.redox.2021.102099

44. Yu J et al., “Direct activation of the alternative complement pathway by SARS-CoV-2 spike proteins is blocked by factor D inhibition,” *Blood* 2020, 136, 18: 2080–2089. doi: https://doi.org/10.1182/blood.2020008248

HH. Toll-like receptors (TLRs)

1. Aboudounya MM and RJ Heads, "COVID-19 and Toll-Like Receptor 4 (TLR4): SARS-CoV-2 May Bind and Activate TLR4 to Increase ACE2 Expression, Facilitating Entry and Causing Hyperinflammation," *Mediators Inflamm.* 2021: 8874339. doi: https://doi.org/10.1155/2021/8874339

2. Burnett FN et al., "SARS-CoV-2 Spike Protein Intensifies Cerebrovascular Complications in Diabetic hACE2 Mice through RAAS and TLR Signaling Activation," *Int. J. Mol. Sci.* 2023, 24, 22: 16394. doi: https://doi.org/10.3390/ijms242216394

3. Carnevale R et al., "Toll-Like Receptor 4-Dependent Platelet-Related Thrombosis in SARS-CoV-2 Infection," *Circ. Res.* 2023, 132, 3: 290–305. doi: https://doi.org/10.1161/CIRCRESAHA.122.321541

4. Corpetti C et al., "Cannabidiol inhibits SARS-Cov-2 spike (S) protein-induced cytotoxicity and inflammation through a PPARγ-dependent TLR4/NLRP3/Caspase-1 signaling suppression in Caco-2 cell line," *Phytother. Res.* 2021, 35, 12: 6893–6903. doi: https://doi.org/10.1002/ptr.7302

5. Fontes-Dantas FL, "SARS-CoV-2 Spike Protein Induces TLR4-Mediated Long- Term Cognitive Dysfunction Recapitulating Post-COVID-19 Syndrome in Mice," *Cell Reports* 2023, 42, 3: 112189. doi: https://doi.org/10.1016/j.celrep.2023.112189

6. Khan S et al., "SARS-CoV-2 Spike Protein Induces Inflammation via TLR2-Dependent Activation of the NF-κB Pathway," *eLife* 2021, 10: e68563. doi: 10.7554/elife.68563

7. Kim MJ et al., "The SARS-CoV-2 spike protein induces lung cancer migration and invasion in a TLR2-dependent manner," *Cancer Commun* (London) 2023, 44, 2: 273–277. doi: https://doi.org/10.1002/cac2.12485

8. Kircheis R and O Planz, "Could a Lower Toll-like Receptor (TLR) and NF-κB Activation Due to a Changed Charge Distribution in the Spike Protein Be the Reason for the Lower Pathogenicity of Omicron?" *Int. J. Mol. Sci.* 2022, 23, 11: 5966. doi: https://doi.org/10.3390/ijms23115966

9. Loh JT et al., "Dok3 restrains neutrophil production of calprotectin during TLR4 sensing of SARS-CoV-2 spike protein," *Front. Immunol.* 2022, 13 (Sec. Molecular Innate Immunity). doi: https://doi.org/10.3389/fimmu.2022.996637

10. Segura-Villalobos D et al., "Jacareubin inhibits TLR4-induced lung inflammatory response caused by the RBD domain of SARS-CoV-2 Spike protein," *Pharmacol. Rep.* 2022, 74: 1315–1325. doi: https://doi.org/10.1007/s43440-022-00398-5

11. Sirsendu J et al., “Cell-Free Hemoglobin Does Not Attenuate the Effects of SARS-CoV-2 Spike Protein S1 Subunit in Pulmonary Endothelial Cells,” *Int. J. Mol. Sci.*, 2021, 22, 16: 9041. doi: https://doi.org/10.3390/ijms22169041

12. Sung PS et al., “CLEC5A and TLR2 Are Critical in SARS-CoV-2-Induced NET Formation and Lung Inflammation,” *J. Biomed. Sci.* 2022, 29, 52. doi: https://doi.org/10.1186/s12929-022-00832-z

13. Zaki H and S Khan, “SARS-CoV-2 spike protein induces inflammatory molecules through TLR2 in macrophages and monocytes,” *J. Immunol.* 2021, 206 (1_supplement): 62.07. doi: https://doi.org/10.4049/jimmunol.206.Supp.62.07

14. Zaki H and S Khan, “TLR2 senses spike protein of SARS-CoV-2 to trigger inflammation,” *J. Immunol.* 2022, 208 (1_Supplement): 125.30. doi: https://doi.org/10.4049/jimmunol.208.Supp.125.30

15. Zhao Y et al., “SARS-CoV-2 spike protein interacts with and activates TLR4,” *Cell Res.* 2021, 31: 818–820. doi: https://doi.org/10.1038/s41422-021-00495-9

II. Spike protein and “vaccine” mRNA biodistribution studies

Compiled by Dr. Martin Wucher, MSC Dent Sc (eq DDS), Erik Sass, et al.

Last updated July 1, 2025. Corresponding author: eriksass@gmail.com

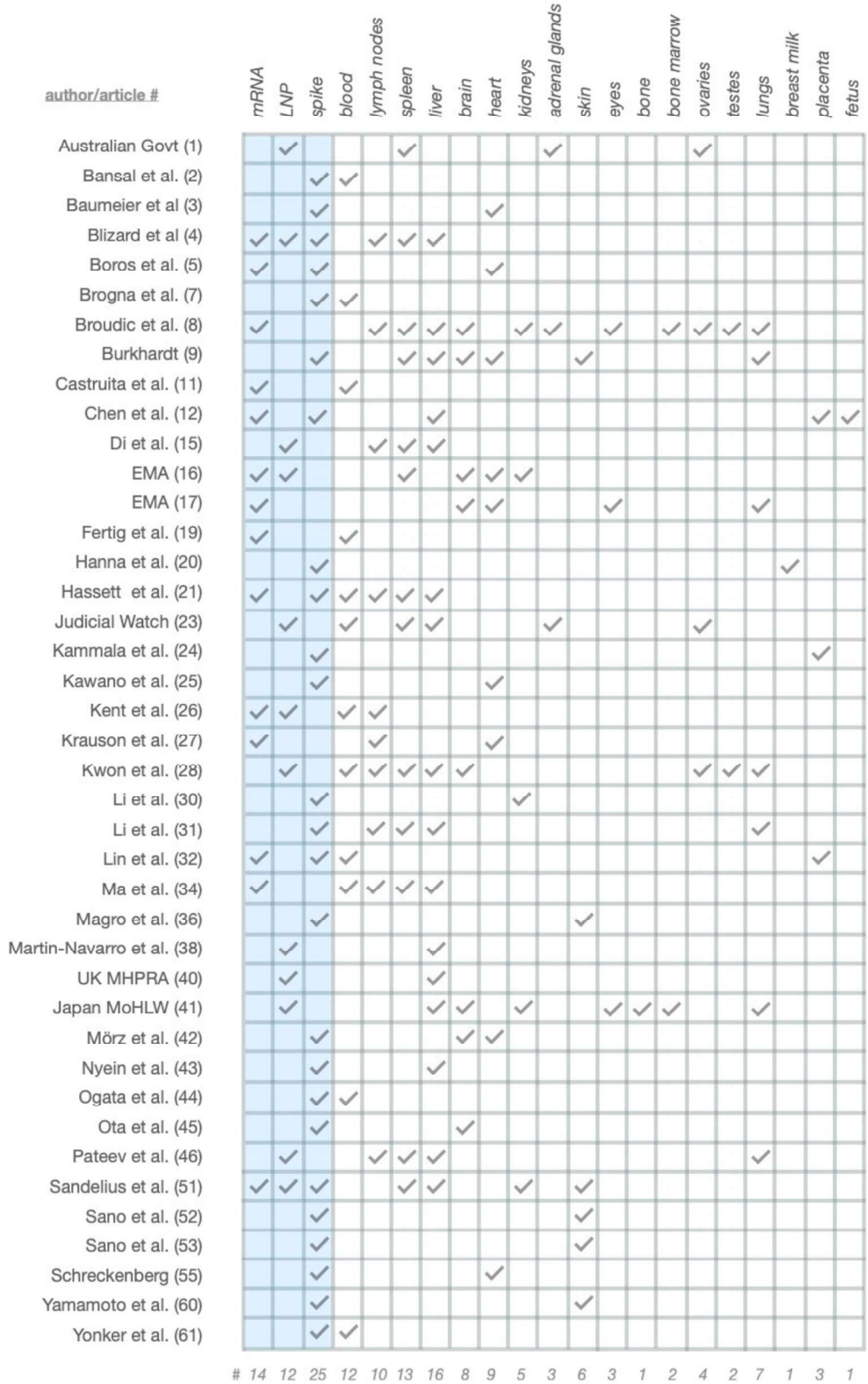
Biodistribution studies show that both the “vaccine” mRNA encoding for the spike protein antigen and the spike protein itself can penetrate distant tissues, causing systemic harms to a variety of organs and organ systems, including the placenta. The following research collection presents over 60 peer-reviewed studies (**n=61**) documenting the wide distribution of “vaccine” mRNA and the associated spike protein throughout human beings and animal test subjects.

These articles confirm that “vaccine” mRNA and spike protein can reach tissues and organs including the heart, liver, brain, lungs, placenta, umbilical cord, breast milk, lymph nodes, thymus, kidneys, spleen, bladder, large intestine, eyes, adrenal glands, ovaries, testes, bone marrow, skin, lacrimal glands, and appendix. Additionally, a small number of studies demonstrate the viral spike protein’s ability to cross important physiological barriers independently of the rest of the virus, suggesting identical “vaccine”-derived spike protein can do the same.

A chart below summarizes the findings of dozens of studies collected in this section II, showing which “vaccine” components and products were examined (mRNA, LNP, and/or spike protein) and key tissues and organs affected. Taken together with evidence of the spike protein’s pathogenicity, these findings suggest that the mRNA “vaccines” can distribute harmful, long-lasting spike protein uncontrollably throughout the body, causing injuries and death by various means.

This compilation originated with Dr. Wucher's contribution to *TOXIC SHOT: Facing the Dangers of the COVID "Vaccines,"* (Chapter 4: The Spike Protein Is Harmful By Itself).

Chart: COVID-19 mRNA “vaccine” biodistribution studies



Original source: “mRNA ‘vaccine’ biodistribution, persistence, and adjuvant toxicity library,” https://zenodo/org/records14559625
Update: mRNA ‘vaccine’ harms research collection v2,” see most recent version: https://zenodo.org/records/14559644

ANNOTATED REFERENCES (n=61)

1. Australian Government Department of Health—Therapeutic Goods Administration, "Nonclinical evaluation of BNT162b2 [mRNA] COVID-19 vaccine (COMIRNATY)," 2021, Available from: https://www.tga.gov.au/sites/default/files/foi-2389-06.pdf
   - "Lipid nanoparticle formulation… and encapsulation efficiency similar to LNP in BNT162b2 vaccine… distribution mainly into liver, adrenal glands, spleen and ovaries over 48 h."

2. Bansal S et al., "Cutting Edge: Circulating Exosomes with COVID Spike Protein Are Induced by BNT162b2 (Pfizer-BioNTech) Vaccination prior to Development of Antibodies: A Novel Mechanism for Immune Activation by mRNA Vaccines," *J. Immunol.* 2021, 207, 10: 2405–2410. doi: 10.4049/jimmunol.2100637
   - plasma

3. Baumeier C et al., "Intramyocardial Inflammation after COVID-19 Vaccination: An Endomyocardial Biopsy-Proven Case Series," *Int. J. Mol. Sci.* 2022, 23, 13: 6940. doi: https://doi.org/10.3390/ijms23136940
   - "The expression of SARS-CoV-2 spike protein within the heart and the dominance of CD4+ lymphocytic infiltrates indicate an autoimmunological response to the vaccination."

4. Blizard GS et al., "Monitoring mRNA vaccine antigen expression in vivo using PET/CT," *Nat. Commun.* 2025, 16: 2234. doi: 10.1038/s41467-025-57446-w
   - lymph nodes, liver, spleen

5. Boros LG et al., "Long-lasting, biochemically modified mRNA, and its frameshifted recombinant spike proteins in human tissues and circulation after COVID-19 vaccination," *Pharmacol Res Perspect* 2024, 12, 3: e1218. doi: 10.1002/prp2.1218
   - "… clinical studies now report that modified SARS-CoV-2 mRNA routinely persist up to a month from injection and can be detected in cardiac and skeletal muscle at sites of inflammation and fibrosis, while the recombinant spike protein may persist a little over half a year in blood."

6. Brady M et al., "Spike protein multiorgan tropism suppressed by antibodies targeting SARS-CoV-2," *Comm. Biol.* 2021, 4, 1318. doi: 10.1038/s42003-021-02856-x
   - After intravenous injection, "SP had a body-wide biodistribution, slow regional elimination, except for the liver, which showed an accumulation, and differential organ uptake. SP uptake was highest for the lungs and this was followed by the kidney, heart, and liver, but lowest in the brain."

7. Brogna C et al., “Detection of recombinant Spike protein in the blood of individuals vaccinated against SARS-CoV-2: Possible molecular mechanisms,” *Proteonomics Clin App.* 2023, 17, 6. doi: 10.1002/prca.202300048
    - plasma

8. Broudic K et al., “Nonclinical safety assessment of an mRNA Covid-19 vaccine candidate following repeated administrations and biodistribution,” *J. Appl. Toxicol.* 2024, 44, 3: 371-390. doi: https://doi.org/10.1002/jat.4548
    - lymph nodes, spleen, liver, lacrimal glands, brain, thymus, lungs, adrenal glands, bone marrow, kidneys, testes, ovaries

9. Burkhardt A, “Pathology Conference: Vaccine-Induced Spike Protein Production in the Brain, Organs etc., now Proven.” Report24.news, 2022, available online: https://report24.news/pathologie-konferenz-impfinduzierte-spike-produktion-in-gehirn-u-a-organen-nun-erwiesen/
    - Heart, brain, liver, appendix, bronchi, skin, spleen

10. Buzhdygan TP et al., “The SARS-CoV-2 spike protein alters barrier function in 2D static and 3D microfluidic in-vitro models of the human blood–brain barrier,” *Neurobiol Dis.* 2020m 146: 105131. doi: 10.1016/j.nbd.2020.105131

11. Castruita JAS et al., “SARS-CoV-2 spike mRNA vaccine sequences circulate in blood up to 28 days after COVID-19 vaccination,” *APMIS* 2023, 131: 128–132. doi: https://doi.org/10.1111/apm.13294
    - plasma

12. Chen JC et al., “mRNA-1273 is placenta-permeable and immunogenic in the fetus,” *Mol. Ther. Nucleic Acids* 2025, 36, 1: 102489. doi: 10.1016/j.omtn.2025.102489
    - placenta, fetus. “Although spike mRNA in fetal circulation faded away within 4–6 h, it could accumulate in fetal tissues, mainly the liver and get translated into spike protein.”

13. Cosentino M and Franca Marino, “Understanding the Pharmacology of COVID- 19 mRNA Vaccines: Playing Dice with the Spike?” *Int. J. Mol. Sci.* 2022, 23, 18: 10881. doi: https://doi.org/10.3390/ijms231810881
    - “Taken as a whole, evidence strongly supports the possible link between inappropriate expression of S protein in sensitive tissues and subsequent tissue damage.”

14. DeOre BJ et al., “SARS-CoV-2 Spike Protein Disrupts Blood-Brain Barrier Integrity via RhoA Activation,” *J Neuroimmune Pharmacol.* 2021, 16, 4:722-728. Doi: https://doi.org/10.1007/s11481-021-10029-0

15. Di J et al., “Biodistribution and Non-linear Gene Expression of mRNA LNPs Affected by Delivery Route and Particle Size,” *Pharm Res* 2022, 39: 105-114. doi: https://doi.org/10.1007/s11095-022-03166-5
    - liver, spleen, muscle, and inguinal lymph nodes

16. European Medicines Agency, *Assessment Report,* available online: https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf
    - “Synthetic mRNAs encapsulated in LNPs can reach many organs, such as the spleen, heart, kidneys, lungs and brain. The mRNAs were found in the ovaries and the testicles in small quantities, during the biodistribution studies of this vaccine after 9 days.”

17. European Medicines Agency, *COVID-19 Vaccine Moderna*, available online: https://www.ema.europa.eu/en/documents/assessment-report/spikevax-previously-covid-19-vaccine-moderna-epar-public-assessment-report_en.pdf
    - Vaccine mRNAs are detectable in brain, heart, lungs, eyes, gonads.

18. Fertig TE et al., “Beyond the injection site: identifying the cellular targets of mRNA vaccines,” J *Cell Ident* 2024, 3, 1. doi: 10.47570/joci.2024.004
    - Overview of studies showing wide distribution throughout the body.

19. Fertig TE et al., “Vaccine mRNA Can Be Detected in Blood at 15 Days Post Vaccination,” *Biomedicines* 2022, 10, 7: 1538. doi: 10.3390/biomedicines10071538
    - plasma

20. Hanna N et al. “Biodistribution of mRNA COVID-19 vaccines in human breast milk,” *eBioMedicine* 2023, 96, 104800. doi: 10.1016/j.ebiom.2023.104800
    - “Of 13 lactating women receiving the vaccine (20 exposures), trace mRNA amounts were detected in 10 exposures up to 45 h post-vaccination. “

21. Hassett KJ et al., “mRNA vaccine trafficking and resulting protein expression after intramuscular administration,” *Mol. Ther. Nucleic Acids* 2024, 35, 1: 102083. doi: 10.1016/j.omtn.2023.102083
    - plasma, lymph nodes, liver, spleen

22. Hulscher N et al., “Autopsy findings in cases of fatal COVID-19 vaccine-induced myocarditis,” *ESC Heart Failure* 2024. doi: https://doi.org/10.1002/ehf2.14680
    - “COVID-19 vaccine Spike protein is produced in the body for an uncontrolled duration and in unknown quantity resulting in deleterious effects, especially on the heart, explaining the cardiovascular deaths seen in our study without evidence of other organ system involvement.”

23. Judicial Watch, “JW v HHS FDA Pfizer BioNTech Vaccine prod 3 02418,” March 21, 2022, https://www.judicialwatch.org/documents/jw-v-hhs-fda-pfizer-biontech-vaccine-prod-3-02418/
    - LNP biodistribution to liver, spleen, adrenal glands, ovaries. “Outside the injection site, low levels of radioactivity were detected in most tissues, with the greatest levels in plasma observed 1-4 hours post-dose.”

24. Kammala AK et al., “In vitro mRNA-S maternal vaccination induced altered immune regulation at the maternal-fetal interface,” *Am. J. Reprod. Immunol.* 2024, 91, 5: e13861. doi: https://doi.org/10.1111/aji.13861
    - “… our study indicates that mRNA-S-based maternal vaccination during pregnancy may influence the maternal-fetal interface's COVID-19 interaction and immune regulation. Further investigation is warranted to assess safety and implications.”

25. Kawano H et al., “Fulminant Myocarditis 24 Days after Coronavirus Disease Messenger Ribonucleic Acid Vaccination,” *Intern. Med.* 2022, 61, 15: 2319-2325. doi: https://doi.org/10.2169/internalmedicine.9800-22
    - “… positive immunostaining for severe acute respiratory syndrome coronavirus 2 spike protein and C4d in the myocardium."

26. Kent SJ et al., “Blood Distribution of SARS-CoV-2 Lipid Nanoparticle mRNA Vaccine in Humans,” *ACS Nano* 2024, 18, 39: 27077-27089. doi: 10.1021/acsnano.4c11652
    - “The similar kinetics of intact mRNA and the ionizable lipid in blood and the slow degradation of the mRNA suggest that mRNA lipid nanoparticles remain intact and travel from injection sites or lymph nodes into the bloodstream within 4 h postvaccination. The rapid dissemination of mRNA lipid nanoparticles in blood found in our study is consistent with the recent findings on the detection of mRNA in breast milk at 3−45 h postvaccination.”

27. Krauson AM et al., “Duration of SARS-CoV-2 mRNA vaccine persistence and factors associated with cardiac involvement in recently vaccinated patients,” *npj Vaccines,* 8, 141. doi: https://doi.org/10.1038/s41541-023-00742-7
    - axillary lymph nodes, myocardium

28. Kwon MH et al., “The Pharmacokinetics of mRNA Vaccine Carrier using Carbon-14,” *J. Radiopharm. Mol. Probes* 2024, 10, 1: 73-81. doi: 10.22643/JRMP.2024.10.1.73
    - serum, lymph nodes, muscle, spleen, liver, testis, ovary, thymus, lung, brain

29. Lehmann KJ, “SARS-CoV-2-Spike Interactions with the Renin-Angiotensin-Aldosterone System – Consequences of Adverse Reactions of Vaccination,” *J Biol Today’s World* 2023, 12/4: 001-013. doi: https://doi.org/10.31219/osf.io/27g5h

- "The presented analysis provides a substantial body of evidence for the causal involvement of Ang II/activated RAAS in eliciting adverse reactions after application of spike-inducing vaccine. As an example, some serious organ disturbances or adverse reactions, in which the connection with an activated RAAS is obvious (cardiovascular and blood coagulation disorders, disorders of the nervous and muscular system, inflammatory reactions, auto-immunological, vascular and renal disorders), are presented and discussed..."

30. Li C. et al., "Intravenous Injection of Coronavirus Disease 2019 (COVID-19) MRNA Vaccine Can Induce Acute Myopericarditis in Mouse Model," *Clin. Infect. Dis.* 2022, 74, 11: 1933-1950. doi: https://doi.org/10.1093/cid/ciab707
    - "Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike antigen expression by immunostaining was occasionally found in infiltrating immune cells of the heart or injection site, in cardiomyocytes and intracardiac vascular endothelial cells, but not skeletal myocytes."

31. Li C et al., "Mechanisms of innate and adaptive immunity to the Pfizer-BioNTech BNT162b2 vaccine," *Nature Immunol.* 2022, 23: 543-555. doi: https://doi.org/10.1038/s41590-022-01163-9
    - spleen, muscle, liver, lung and non-dLNs

32. Lin X et al., "Transplacental transmission of the COVID-19 vaccine messenger RNA: evidence from placental, maternal, and cord blood analyses postvaccination," *Am J Obstet Gynecol* 2024, 92, 4: e13934. doi: https://doi.org/10.1111/aji.13934
    - "The vaccine mRNA was detected in the 2 placentas evaluated using quantitative ddPCR and ISH... Using WES, the spike protein expression was detected in the placenta of patient 2, but not in patient 1... Furthermore, the vaccine mRNA was detected in the umbilical cord and maternal blood of patient 1 using ddPCR."

33. Luo Y et al., "SARS-Cov-2 spike induces intestinal barrier dysfunction through the interaction between CEACAM5 and Galectin-9," *Front. Immunol.* 2024, 15. doi: https://doi.org/10.3389/fimmu.2024.1303356

34. Ma L et al., "6.3. FDA-Approved mRNA Vaccines: Interpretation of Preclinical Pharmacokinetic (PK) Data," in *Drug Metabolism and Pharmacokinetics: Frontiers, Strategies, and Applications*, ed. L Shen et al., Wiley & Sons, Hoboken, 2025. ISBN: 978-1-394-30013-6
    - Plasma, lymph nodes, liver, adrenal glands, spleen, ovaries, brain, lung, eye, testes, kidney

35. Magen E et al., “Clinical and Molecular Characterization of a Rare Case of BNT162b2 mRNA COVID-19 Vaccine-Associated Myositis,” *Vaccines* 2022, *10*, 7: 1135. doi: https://doi.org/10.3390/vaccines10071135
    - “… although the BNT162b2 vaccine mRNA was not properly expressed in blood cells seven days after receipt of the first vaccine dose, it was still expressed in muscle tissue distant from the vaccination site one month after receipt of the first vaccine dose.”

36. Magro C et al., “The histologic and molecular correlates of COVID-19 vaccine-induced changes in the skin,” *Clin. Dermatol.* 2021, 39, 6: 966-984. doi: https://doi.org/10.1016/j.clindermatol.2021.07.011
    - Spike detected in deep dermis or blood vessels serving skin in 10/34 cases.

37. Magro C et al., “Disruption of the blood-brain barrier is correlated with spike endocytosis by ACE2 + endothelia in the CNS microvasculature in fatal COVID-19. Scientific commentary on ‘Detection of blood-brain barrier disruption in brains of patients with COVID-19, but no evidence of brain penetration by SARS-CoV-2’,” *Acta Neuropathol.* 2024, 147, 1: 47. doi: https://doi.org/10.1007/s00401-023-02681-y

38. Martin-Navarro L et al., “In situ detection of vaccine mRNA in the cytoplasm of hepatocytes during COVID-19 vaccine-related hepatitis,” *J. Hepatol.* 2023, 78, 1: e20-e22. doi: 10.1016/j.jhep.2022.08.039
    - “… our results suggest that lipid nanoparticles bearing mRNA molecules encoding SARS-CoV-2 proteins can reach hepatocytes under certain circumstances and deliver mRNA in high quantities that could be used by the translational machinery of the cells to produce spike.”

39. Maugeri M et al.. “Linkage between endosomal escape of LNP-mRNA and loading into EVs for transport to other cells,” *Nat Commun* 2019, 10: 4333. doi: https://doi.org/10.1038/s41467-019-12275-6
    - “The present study shows that LNP components (mRNA and ionizable lipids) are partly incorporated into endo-EVs… these endo-EVs protect exogenous mRNA during in vivo transport to organs…”

40. Medicines & Healthcare Products Regulatory Agency, “Summary of the Public Assessment Report for Pfizer/BioNTech COVID-19 Vaccine,” available online: https://www.gov.uk/government/publications/regulatory-approval-of-pfizer-biontech-vaccine-for-covid-19/summary-public-assessment-report-for-pfizerbiontech-covid-19-vaccine
    - liver

41. Ministry of Health, Labour and Welfare of Japan, “SARS-CoV-2 mRNA Vaccine (BNT162, PF-07302048): Summary Text of the Pharmacokinetic Study,” available online: https://www.docdroid.net/xq0Z8B0/pfizer-report-japanese-government-pdf
    - bladder, bone, bone marrow, brain, eyes, heart, kidneys, large intestine, liver, lung

42. Mörz M, “A Case Report: Multifocal Necrotizing Encephalitis and Myocarditis after BNT162b2 mRNA Vaccination against COVID-19,” *Vaccines* 2022, 10, 10: 1651. doi: https://doi.org/10.3390/vaccines10101651
    - “Only spike protein but no nucleocapsid protein could be detected within the foci of inflammation in both the brain and the heart, particularly in the endothelial cells of small blood vessels.”

43. Nyein CM et al., “Severe *de novo* liver injury after Moderna vaccination – not always autoimmune hepatitis,” *J. Hepatol.* 2022, 77, 2: 556-558. doi: 10.1016/j.jhep.2022.03.041
    - “Unique to this case is the demonstration of anti-SARS-CoV-2 spike protein within the liver parenchyma.”

44. Ogata AF et al., “Circulating Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Vaccine Antigen Detected in the Plasma of mRNA-1273 Vaccine Recipients,” *Clin. Infect. Dis.* 2022, 75, 4: 715–718. doi: 10.1093/cid/ciab465
    - “Here we provide evidence that circulating SARS-CoV-2 proteins are present in the plasma of participants vaccinated with the mRNA-1273 vaccine.”

45. Ota N et al., “Expression of SARS-CoV-2 spike protein in cerebral Arteries: Implications for hemorrhagic stroke Post-mRNA vaccination,” *J. Clin. Neurosci.* 2025, 136: 111223. doi: https://doi.org/10.1016/j.jocn.2025.111223

46. Pateev I et al., “Biodistribution of RNA Vaccines and of Their Products: Evidence from Human and Animal Studies,” *Biomedicines* 2024, 12, 1: 59. doi: https://doi.org/10.3390/biomedicines12010059
    - “Intravenous injection led to the detection of fluorescent proteins in the liver, spleen, lungs, and lymph nodes.”

47. Petrovszki D et al., “Penetration of the SARS-CoV-2 Spike Protein across the Blood-Brain Barrier, as Revealed by a Combination of a Human Cell Culture Model System and Optical Biosensing,” *Biomedicines* 2022, 10, 1: 188. doi: https://doi.org/10.3390/biomedicines10010188

48. Rhea EM et al., “The S1 protein of SARS-CoV-2 crosses the blood-brain barrier in mice,” *Nature Neuroscience* 2021, 24, 3: 368-378. doi: 10.1038/s41593-020-00771-8

49. Röltgen K et al., “Immune imprinting, breadth of variant recognition, and germinal center response in human SARS-CoV-2 infection and vaccination,” *Cell,* 2022, 185, 6: 1025-1040. doi: 10.1016/j.cell.2022.01.018
    - “mRNA vaccination stimulates robust GCs containing vaccine mRNA and spike antigen up to 8 weeks postvaccination in some cases.”

50. Rzymski P and Andrzej Fal, “To aspirate or not to aspirate? Considerations for the COVID-19 vaccines,” *Pharmacol. Rep* 2022, 74: 1223–1227. doi: https://doi.org/10.1007/s43440-022-00361-4
    - “As shown in vivo in mice, intravenous injection of the BNT162b2 vaccine (BioNTech/Pfizer, Germany/USA) resulted in histopathological changes characteristic for myopericarditis… the amount of mRNA encoding SARS-CoV-2 spike protein and its subsequent myocardial expression was significantly higher in heart tissue when compared to the animals receiving the intramuscular injection.”

51. Sandelius A et al., “Biodistribution of lipid nanoparticle, eGFP mRNA and translated protein following subcutaneous administration in mouse,” *Bioanalysis* 2024, 16, 14: 721-733. doi: https://doi.org/10.1080/17576180.2024.2360361
    - skin, spleen, liver, kidney

52. Sano H et al., “A case of persistent, confluent maculopapular erythema following a COVID-19 mRNA vaccination is possibly associated with the intralesional spike protein expressed by vascular endothelial cells and eccrine glands in the deep dermis,” *J Dermatol* 2023, 50, 9: 1208-1212. doi: 10.1111/1346-8138.16816
    - “Surprisingly, immunohistochemical staining of the lesion 100 days after the disease onset revealed the COVID-19 spike protein expressed by vascular endothelial cells and eccrine glands in the deep dermis. As she had no episode of COVID-19 infection, it is highly likely that the spike protein was derived from the mRNA vaccine and it might be the cause of the development and persistence of her skin lesions.”

53. Sano S et al., “SARS-CoV-2 spike protein found in the acrosyringium and eccrine gland of repetitive miliaria-like lesions in a woman following mRNA vaccination,” *J. Dermatol.* 2024, 51, 9: e293-e295. doi: https://doi.org/10.1111/1346-8138.17204
    - cutaneous

54. Sattar S Et al., “Nuclear translocation of spike mRNA and protein is a novel feature of SARS-CoV-2,” 2023 *Front. Microbiol.* 2023, 14 (Sec. Virology). doi: https://doi.org/10.3389/fmicb.2023.1073789
    - “Although the S protein is a surface transmembrane type 1 glycoprotein, it has been predicted to be translocated into the nucleus due to the novel nuclear

localization signal (NLS) 'PRRARSV,' which is absent from the S protein of other coronaviruses. Indeed, S proteins translocate into the nucleus in SARS-CoV-2-infected cells. S mRNAs also translocate into the nucleus. S mRNA colocalizes with S protein, aiding the nuclear translocation of S mRNA."

55. Schreckenberg R et al., "Cardiac side effects of RNA-based SARS-CoV-2 vaccines: Hidden cardiotoxic effects of mRNA-1273 and BNT162b2 on ventricular myocyte function and structure," *Br. J. Pharmacol.* 2024, 181, 3: 345-361. doi: https://doi.org/10.1111/bph.16262
    - "After 48 h, expression of the encoded spike protein was detected in ventricular cardiomyocytes for both mRNAs… mRNA-1273 induced arrhythmic as well as completely irregular contractions associated with irregular as well as localized calcium transients, which provide indications of significant dysfunction of the cardiac ryanodine receptor (RyR2)… BNT162b2 increased cardiomyocyte contraction via significantly increased protein kinase A (PKA) activity…"

56. Stern B et al., "SARS-CoV-2 spike protein induces endothelial dysfunction in 3D engineered vascular networks," *J. Biomed. Mater. Res. A.* 2023, 112, 4: 524-533. doi: https://doi.org/10.1002/jbm.a.37543

57. Suprewicz L et al., "Blood-brain barrier function in response to SARS-CoV-2 and its spike protein," *Neurol. Neurochir Pol.* 2023, 57: 14–25. doi: 10.5603/PJNNS.a2023.0014
    - "… S1, S1RBD, and S2 subunits exhibit pro-inflammatory effects, resulting in increased BBB permeability via damage to tight junctions (TJs)…"

58. Suprewicz L et al., "Recombinant human plasma gelsolin reverses increased permeability of the blood-brain barrier induced by the spike protein of the SARS-CoV-2 virus," *J Neuroinflamm.* 2022, 19, 1: 282. doi: 10.1186/s12974-022-02642-4

59. Takanashi A et al., "Delivery and Expression of mRNA in the Secondary Lymphoid Organs Drive Immune Responses to Lipid Nanoparticle-mRNA Vaccines after Intramuscular Injection," *Mol. Pharmaceutics* 2023, 20, 8: 3876–3885. doi: https://doi.org/10.1021/acs.molpharmaceut.2c01024
    - "Our results suggest that the mRNA delivery and transfection of secondary lymphatic organs, not LNP adjuvancy or RNA expression in muscle, are the main drivers for adaptive immune response in mice."

60. Yamamoto M et al., "Persistent varicella zoster virus infection following mRNA COVID-19 vaccination was associated with the presence of encoded spike protein in the lesion," *J. Cutan. Immunol. Allergy* 2022, 6, 1: 18-23. doi: 10.1002/cia2.12278
    - Spike expressed in vesicular keratinocytes and endothelial cells in the dermis.

61. Yonker LM et al., “Circulating Spike Protein Detected in Post–COVID-19 mRNA Vaccine Myocarditis,” *Circulation* 2023, 147, 11. doi: 10.1161/CIRCULATIONAHA.122.061025
    - Plasma

III. Spike protein and vaccine mRNA persistence studies

Compiled by Dr. Martin Wucher, MSC Dent Sc (eq DDS), Erik Sass, et al.

Last updated July 1, 2025. Corresponding author: eriksass@gmail.com

Dozens of studies collected here (**n=41**) demonstrate that both "vaccine" mRNA, and the spike protein antigen it encodes, persist in the tissues of human vaccine recipients and animal test subjects far longer than claimed by public health officials: up to eight weeks in the case of mRNA (Röltgen K et al.) and up to six months for spike protein (Brogna C et al.). Numerous studies have also shown that viral spike proteins can persist even longer in individuals recovered from SARS CoV2 infection or with "long COVID," with spike protein detected 15 months (Patterson BK et al.) to two years (Fraser ME at al.) after infection. Long-lasting viral spike proteins have frequently been detected in the absence of viable virus, as reflected in negative PCR tests and RNA assays, suggesting identical "vaccine" spike proteins may also persist for a year or more.

This compilation originated with Dr. Wucher's contribution to *TOXIC SHOT: Facing the Dangers of the COVID "Vaccines,"* (Chapter 4: The Spike Protein Is Harmful By Itself).

ANNOTATED REFERENCES (n=41)

1. Alghmadi A et al., "Altered Circulating Cytokine Profile Among mRNA-Vaccinated Young Adults: A Year-Long Follow-Up Study," *Immun. Inflamm. Dis.* 2025, 13, 4: e70194. doi: https://doi.org/10.1002/iid3.70194
    - "The findings of this study indicated that COVID-19 vaccination resulted in an increase in cytokine levels, which signifies the persistence of the humoral immune response to messenger RNA (mRNA) vaccines. This effect may be attributed to the persistent production of spike protein and highly inflammatory nature of mRNA-lipid nanoparticle."

2. Bansal S, et al. "Cutting Edge: Circulating Exosomes with COVID Spike Protein Are Induced by BNT162b2 (Pfizer-BioNTech) Vaccination prior to Development of Antibodies: A Novel Mechanism for Immune Activation by mRNA Vaccines," *J. Immunol.* 2021, 207, 10: 2405–2410. doi: 10.4049/jimmunol.2100637
    - circulating exosomes with spike protein detected four months after vaccination.

3. Boros LG et al., "Long-lasting, biochemically modified mRNA, and its frameshifted recombinant spike proteins in human tissues and circulation after COVID-19 vaccination," *Pharmacol Res Perspect* 2024, 12, 3: e1218. doi: 10.1002/prp2.1218
    - "... clinical studies now report that modified SARS-CoV-2 mRNA routinely persist up to a month from injection and can be detected in cardiac and skeletal muscle

at sites of inflammation and fibrosis, while the recombinant spike protein may persist a little over half a year in blood."

4. Brogna C et al., "Detection of recombinant Spike protein in the blood of individuals vaccinated against SARS-CoV-2: Possible molecular mechanisms," *Proteonomics Clin App.* 2023, 17, 6. doi: 10.1002/prca.202300048
   - "The minimum and maximum time at which PP-Spike was detected after vaccination was 69 and 187 days, respectively."

5. Castruita JAS et al., "SARS-CoV-2 spike mRNA vaccine sequences circulate in blood up to 28 days after COVID-19 vaccination," *APMIS* 2023, 131: 128–132. doi: https://doi.org/10.1111/apm.13294

6. Cheung CCL et al., "Residual SARS-CoV-2 viral antigens detected in GI and hepatic tissues from five recovered patients with COVID-19," *Gut* 2022, 71, 1: 226–9. doi: https://doi.org/10.1136/gutjnl-2021-324280
   - Persistence of residual SARS-CoV-2 antigens up to 180 days in the colon, appendix, ileum, haemorrhoid, liver, gallbladder and lymph nodes; unable to detect viral RNA in many patients' tissues.

7. Colmenero I et al., "SARS-CoV-2 endothelial infection causes COVID-19 chilblains: histopathological, immunohistochemical and ultrastructural study of seven paediatric cases," *Br J Dermatol.* 2020, 183: 729-737. doi: 10.1111/bjd.19327
   - Spike protein detected in lesions up to 30 days after onset of acute infection. SARS-CoV-2 PCR from nasopharyngeal and oropharyngeal swabs was negative in all cases tested (six of six).

8. Craddock V et al., "Persistent circulation of soluble and extracellular vesicle-linked Spike protein in individuals with postacute sequelae of COVID-19," *J Med. Virol.* 2023, 95, 2: e28568. doi: https://doi.org/10.1002/jmv.28568
   - "… our findings suggest that Spike and/or viral RNA fragments persist in the recovered COVID-19 patients with PASC up to 1 year or longer after acute SARS-CoV-2 infection." Further, "this is the first report to show that part of the circulating Spike is linked to extracellular vesicles without any presence of viral RNA in these vesicles."

9. European Medicines Agency, *Assessment Report,* available online: https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf
   - "Synthetic mRNAs encapsulated in LNPs can reach many organs, such as the spleen, heart, kidneys, lungs and brain. The mRNAs were found in the ovaries and the testicles in small quantities, during the biodistribution studies of this vaccine after 9 days…"

10. Fertig TE et al., “Vaccine mRNA Can Be Detected in Blood at 15 Days Post Vaccination,” *Biomedicines* 2022, 10, 7: 1538. doi: 10.3390/biomedicines10071538

11. Fraser ME at al., “SARS-CoV-2 Spike Protein and Viral RNA Persist in the Lung of Patients With Post-COVID Lung Disease (abstract),” *Am J Respir Crit Care Med* 2024, 209: A4193. doi: 10.1164/ajrccm-conference.2024.209.1_MeetingAbstracts.A4193
    - “Spike protein and RNA persists in BAL from patients with post-COVID lung disease up to two years after acute infection.”

12. Gaebler C et al., “Evolution of antibody immunity to SARS-CoV-2,” *Nature* 2021, 591: 639-644. doi: https://doi.org/10.1038/s41586-021-03207-w
    - Gastrointestinal tract biopsies suggest spike antigen persisted in the small bowel in 7 of 14 individuals who were asymptomatic at 4 months after infection… Clinically approved nasopharyngeal-swab PCR assays were negative in all 14 individuals at the time of biopsy. However, biopsy samples from 3 of the 14 participants produced PCR amplicons that were sequence-verified as SARS-CoV-2. In addition, viral RNA was detected by in situ hybridization in biopsy samples from the two participants who were tested.

13. George S et al., “Evidence for SARS-CoV-2 Spike Protein in the Urine of COVID-19 Patients,” *Kidney360* 2021, 2, 6: 924-936. doi: 10.34067/KID.0002172021
    - “The SARS-CoV-2 spike protein could be detected in urine from day 1 to day 44 post–hospital admission… Of the 23 adults who were Ur-S+, only one individual showed detectable viral RNA in urine.”

14. Goh D et al., “Case report: Persistence of residual antigen and RNA of the SARS-CoV-2 virus in tissues of two patients with long COVID,” *Front. Immunol.* 2022, 13 (Sec. Viral Immunology). doi: https://doi.org/10.3389/fimmu.2022.939989
    - Persistence of spike protein 426 days after symptom onset; residual viral RNA also detected.

15. Hano S et al., “A case of persistent, confluent maculopapular erythema following a COVID-19 mRNA vaccination is possibly associated with the intralesional spike protein expressed by vascular endothelial cells and eccrine glands in the deep dermis,” *J Dermatol* 2023, 50, 9: 1208-1212. doi: 10.1111/1346-8138.16816
    - “Surprisingly, immunohistochemical staining of the lesion 100 days after the disease onset revealed the COVID-19 spike protein expressed by vascular endothelial cells and eccrine glands in the deep dermis. As she had no episode of COVID-19 infection, it is highly likely that the spike protein was derived from the mRNA vaccine and it might be the cause of the development and persistence of her skin lesions.”

16. Karaba AH et al., “Detectable plasma severe acute respiratory syndrome coronavirus 2 spike antigen is associated with poor antibody response following third messenger RNA vaccination in kidney transplant recipients,” *Transpl Infect Dis* 2024, 26, 3: e14281. doi: https://doi.org/10.1111/tid.14281
    - Spike protein detectable in 3/16 (19%) participants 14 days after vaccination.

17. Kawano H et al., “Fulminant Myocarditis 24 Days after Coronavirus Disease Messenger Ribonucleic Acid Vaccination,” *Intern. Med.* 2022, 61, 15: 2319-2325. doi: https://doi.org/10.2169/internalmedicine.9800-22
    - “... positive immunostaining for severe acute respiratory syndrome coronavirus 2 spike protein and C4d in the myocardium."

18. Kent SJ et al., “Blood Distribution of SARS-CoV-2 Lipid Nanoparticle mRNA Vaccine in Humans,” *ACS Nano* 2024, 18, 39: 27077-27089. doi: 10.1021/acsnano.4c11652
    - “The vaccine mRNA was detectable and quantifiable up to 14–15 days postvaccination in 37% of subjects.”

19. Krauson AM et al., “Duration of SARS-CoV-2 mRNA vaccine persistence and factors associated with cardiac involvement in recently vaccinated patients,” *npj Vaccines,* 8, 141. doi: https://doi.org/10.1038/s41541-023-00742-7
    - “Vaccine was detected in the axillary lymph nodes in the majority of patients dying within 30 days of vaccination... Vaccine was detected in the myocardium in a subset of patients vaccinated within 30 days of death.”

20. Li C et al., “Mechanisms of innate and adaptive immunity to the Pfizer-BioNTech BNT162b2 vaccine,” *Nature Immunol.* 2022, 23: 543-555. doi: https://doi.org/10.1038/s41590-022-01163-9
    - “mRNA could be detected in the spleen, and the spike protein itself was detectable in the serum, for up to 7 d after immunization.”

21. Ma L et al., “6.3. FDA-Approved mRNA Vaccines: Interpretation of Preclinical Pharmacokinetic (PK) Data,” in *Drug Metabolism and Pharmacokinetics: Frontiers, Strategies, and Applications*, ed. L Shen et al., Wiley & Sons, Hoboken, 2025. ISBN: 978-1-394-30013-6
    - “Notably, mRNA may have a persistent distribution at the injection site, lymph nodes, and spleen for 2–3 weeks, with a slow elimination rate.”

22. Magen E et al., “Clinical and Molecular Characterization of a Rare Case of BNT162b2 mRNA COVID-19 Vaccine-Associated Myositis,” *Vaccines* 2022, 10, 7: 1135. doi: https://doi.org/10.3390/vaccines10071135
    - “... although the BNT162b2 vaccine mRNA was not properly expressed in blood cells seven days after receipt of the first vaccine dose, it was still expressed in

muscle tissue distant from the vaccination site one month after receipt of the first vaccine dose."

23. Mayordomo-Colunga J et al., "SARS-CoV-2 spike protein in intestinal cells of a patient with coronavirus disease 2019 multisystem inflammatory syndrome," *J Pediatr.* 2022, 243: 214-18e215. doi: https://doi.org/10.1016/j.jpeds.2021.11.058
    - Spike protein detected 6 weeks after acute infection. "At presentation, the patient tested negative for SARS-CoV-2 by reverse-transcriptase polymerase chain reaction on nasopharyngeal swab but positive for serum SARS-CoV-2 immunoglobulin G."

24. Mörz M, "A Case Report: Multifocal Necrotizing Encephalitis and Myocarditis after BNT162b2 mRNA Vaccination against COVID-19," *Vaccines* 2022, 10, 10: 1651. doi: https://doi.org/10.3390/vaccines10101651
    - Vaccine-induced spike detected on autopsy three weeks after last injection.

25. Ogata AF et al., "Circulating Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Vaccine Antigen Detected in the Plasma of mRNA-1273 Vaccine Recipients," *Clin. Infect. Dis.* 2022, 74, 4: 715-728. doi: 10.1093/cid/ciab465
    - "Spike protein was detectable in 3 of 13 participants an average of 15 days after the first injection."

26. Parcial ALN et al., "SARS-CoV-2 Is Persistent in Placenta and Causes Macroscopic, Histopathological, and Ultrastructural Changes," *Viruses* 2022, 14, 9: 1885. doi: https://doi.org/10.3390/v14091885
    - "Three of five placentas presented SARS-CoV-2 RNA detected by RT-PCRq at least two to twenty weeks after primary pregnancy infection symptoms, and SARS-CoV-2 spike protein was detected in all placentas by immunoperoxidase assay."

27. Pateev I et al., "Biodistribution of RNA Vaccines and of Their Products: Evidence from Human and Animal Studies," *Biomedicines* 2024, 12, 1: 59. doi: https://doi.org/10.3390/biomedicines12010059
    - (Roltgen K et al) "The amount of the spike antigen declined significantly at 4 months after the double vaccination but was still detectable."
    - "Immunohistochemical staining for the spike antigen in the lymph nodes of vaccinated patients revealed peak amounts of the spike protein in germinal centers 16 days after dose 2, with the spike antigen still detectable on day 60."
    - (Brogna C et al.) "It is noteworthy that in this study, spike protein was still detected in human blood on the 187th day after vaccination."

28. Patterson BK et al., "Detection of S1 spike protein in CD16+ monocytes up to 245 days in SARS-CoV-2-negative post-COVID-19 vaccine syndrome (PCVS) individuals,"

Hum Vaccin Immunother. 2025, 21, 1: 2494934. doi: 10.1080/21645515.2025.2494934

29. Patterson BK et al., "Persistence of SARS CoV-2 S1 Protein in CD16+ Monocytes in Post-Acute Sequelae of COVID-19 (PASC) up to 15 Months Post-Infection," *Front. Immunol.* 2022, 12: 746021. doi: 10.3389/fimmu.2021.746021
    - Intact viral RNA undetectable in monocytes.

30. Peluso MJ et al., "Plasma-based antigen persistence in the post-acute phase of COVID-19," *Lancet* 2024, 24, 6: E345-E347. doi: 10.1016/S1473-3099(24)00211-1
    - "Of 660 pandemic-era specimens tested, 61 (9·2%) specimens from 42 participants (25% of the group), had one or more detectable SARS-CoV-2 antigens. The most commonly detected antigen was spike (n=33, 5·0%), followed by S1 (n=15, 2·3%)..."
    - "... our data provide strong evidence that SARS-CoV-2, in some form or location, persists for up to 14 months following acute SARS-CoV-2 infection."
    - "... our findings provide no direct evidence regarding the persistent presence of replication-competent or even transcriptionally active virus."

31. Peluso MJ et al., "SARS-CoV-2 and mitochondrial proteins in neural-derived exosomes of COVID-19," *Ann Neurol* 2022, 91, 6: 772-781. doi: 10.1002/ana.26350
    - Exosomes containing spike protein were detected in plasma of long COVID patients with neuropsychiatric symptoms at two months.

32. Roden AC et al., "Comparison of In Situ Hybridization, Immunohistochemistry, and Reverse Transcription–Droplet Digital Polymerase Chain Reaction for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Testing in Tissue," *Arch Pathol Lab Med* 2021, 145, 7: 785–796. doi: https://doi.org/10.5858/arpa.2021-0008-SA
    - Detected viral protein 46 days after onset of symptoms.
    - "All patients from our institution had tested positive for COVID-19 by nasopharyngeal swab within a median of 14.5 days (range, 0–67 days) before death. All patients from our institution but one were tested for COVID-19 again at time of autopsy; 10 of 13 (76.9%) tested positive."

33. Röltgen K et al., "Immune imprinting, breadth of variant recognition, and germinal center response in human SARS-CoV-2 infection and vaccination," *Cell,* 2022, 185, 6: 1025-1040. doi: 10.1016/j.cell.2022.01.018
    - "mRNA vaccination stimulates robust GCs containing vaccine mRNA and spike antigen up to 8 weeks postvaccination in some cases."
    - "... with spike antigen still present as late as 60 days post-second dose"

34. Rong Z et al., “Persistence of spike protein at the skull-meninges-brain axis may contribute to the neurological sequelae of COVID-19,” *Cell Host Microbe* 2024, 26: S1931-3128(24)00438-4. doi: 10.1016/j.chom.2024.11.007
    - “In a time course experiment, we found the spike protein in the skull marrow, kidney, liver, and lung 3 days post-injection, remaining detectable in the kidney and liver 14 days post-injection.”

35. Sano H et al., “A case of persistent, confluent maculopapular erythema following a COVID-19 mRNA vaccination is possibly associated with the intralesional spike protein expressed by vascular endothelial cells and eccrine glands in the deep dermis,” *J. Dermatol.* 2023, 50: 1208–1212. doi: 10.1111/1346-8138.16816
    - “Surprisingly, immunohistochemical staining of the lesion 100 days after the disease onset revealed the COVID-19 spike protein expressed by vascular endothelial cells and eccrine glands in the deep dermis. As she had no episode of COVID-19 infection, it is highly likely that the spike protein was derived from the mRNA vaccine and it might be the cause of the development and persistence of her skin lesions.”

36. Schultheiss C et al., “Liquid biomarkers of macrophage dysregulation and circulating spike protein illustrate the biological heterogeneity in patients with post-acute sequelae of COVID-19,” *J Med Virol* 2023, 95, 1: e28364. doi: 10.1002/jmv.28364
    - Detected SARS-CoV-2 S1 protein in the plasma of approximately 64% of PASC study participants recruited at a median of 8 months (range 1–17 months) after acute COVID-19, but only in approximately 35% of convalescent control patients.

37. Swank Z et al., “Persistent circulating SARS-CoV-2 spike is associated with post-acute COVID-19 sequelae,” *Clin. Infect. Dis.* 2022, 76: e487-e490. doi: https://doi.org/10.1093/cid/ciac722
    - “We detect severe acute respiratory syndrome coronavirus 2 spike predominantly in PASC patients up to 12 months after diagnosis… Although the detection of spike in PASC patients months after diagnosis suggests the presence of replicating viral reservoirs, further analyses are needed to confirm this hypothesis.”

38. Visvabharathy L et al., “Case report: Treatment of long COVID with a SARS-CoV-2 antiviral and IL-6 blockade in a patient with rheumatoid arthritis and SARS-CoV-2 antigen persistence,” *Front. Med.* 2022, 9 (Sec. Infectious Diseases – Surveillance). doi: https://doi.org/10.3389/fmed.2022.1003103
    - “The patient tested RT-PCR– for SARS-CoV-2 at 14 days post-infection and multiple times thereafter but continued to test intermittently antigen+ for 14

weeks post-infection despite no overt exposure to SARS-CoV-2 infected individuals."

39. Wu H et al., "Molecular evidence suggesting the persistence of residual SARS-CoV-2 and immune responses in the placentas of pregnant patients recovered from COVID-19," *Cell Prolif.* 2021, 54, 9: e13091. doi: https://doi.org/10.1111/cpr.13091
    - "Our study showed that SARS-CoV-2 nucleic acid (in one patient) and protein (in five patients) were present in the placentas of clinically recovered pregnant patients for more than 3 months after diagnosis."

40. Yamamoto M et al., "Persistent varicella zoster virus infection following mRNA COVID-19 vaccination was associated with the presence of encoded spike protein in the lesion," *J. Cutan. Immunol. Allergy* 2022, 6, 1: 18-23. doi: 10.1002/cia2.12278
    - "multi-dermatomal vesicles, necrotizing vasculitis and superficial thrombophlebitis-like lesions, which lasted as long as 3 months possibly associated with two doses of BNT162b2"

41. Yonker LM et al., "Multisystem inflammatory syndrome in children is driven by zonulin-dependent loss of gut mucosal barrier," *J Clin Invest*. 2021, 131, 14: e149633. doi: https://doi.org/10.1172/JCI149633
    - "...our studies showed that spike antigens rose over the first few days of MIS-C symptoms and persisted for more than 10 days, occasionally through 6 months..."
    - "... we measured SARS-CoV-2 RNA from MIS-C stool samples collected several weeks after the initial SARS-CoV-2 infection or exposure. Indeed, a majority of the patients showed detectable viral loads in the stool ranging from 1.5×10^2 to 2.5×10^7 RNA copies/mL, suggesting an ongoing nidus of infection in MIS-C."

42. Zollner A et al., "Postacute COVID-19 is Characterized by Gut Viral Antigen Persistence in Inflammatory Bowel Diseases," *Gastroenterology* 2022, 163, 2: 495-506.e8. doi: https://doi.org/10.1053/j.gastro.2022.04.037
    - Viral spike protein detected 219 days after original positive endoscopy in gut lining of 15 out of 132 subjects.
    - "We were unable to culture SARS-CoV-2 from gut tissue of patients with viral antigen persistence."

IV. Lipid nanoparticle toxicity and allergenicity studies

Compiled by Dr. Byram Bridle, PhD, Erik Sass, et al.

Last updated July 1, 2025. Corresponding author: eriksass@gmail.com

The anti-SARS CoV2 mRNA injections rely on lipid nanoparticles (LNPs) bonded with polyethylene glycol (PEG) to deliver mRNA coding for the spike protein antigen into human cells. However, a growing body of evidence suggests that the ionizable LNPs used in the experimental mRNA injections are highly inflammatory on their own, while PEG has long been recognized as an allergen with the potential to trigger anaphylaxis (a severe, possibly life-threatening allergic reaction). This annotated research collection presents (**n=80**) scientific papers detailing the potential harms of LNPs, PEG, and other components of the mRNA injections to the human body and setting forth possible or established mechanisms. Some of the research annotated here also suggests a far higher incidence of anaphylaxis due to the mRNA injections than claimed in official estimates, ranging from 1/2,280 doses (Warren CM et al.) to 1/4,049 (Blumenthal KG et al.) and 1/13,882 (Somiya A et al.).

This compilation originated with one of Dr. Bridle's contributions to *TOXIC SHOT: Facing the Dangers of the COVID "Vaccines,"* (Chapter 1: The COVID Shots Are Not Real Vaccines).

ANNOTATED REFERENCES (n=80)

1. Ahn JH et al., "Impact of administration routes and dose frequency on the toxicology of SARS-CoV-2 mRNA vaccines in mice model," *Arch Toxicol.* 2024. doi: https://doi.org/10.1007/s00204-024-03912-1
    - "These results suggest that mRNA vaccines may exhibit various potential toxicities, and the toxicological phenotype may vary depending on the LNP composition."

2. Awaya T et al., "Cytokine Storms and Anaphylaxis Following COVID-19 mRNA-LNP Vaccination: Mechanisms and Therapeutic Approaches," *Diseases* 2024, 12, 10: 231. doi: https://doi.org/10.3390/diseases12100231
    - "...during the process of endosomal escape, ionizable lipids disrupt the endosomal membrane to release mRNA, which can, in some cases, lead to the excessive production of inflammatory cytokines."

3. Bakos T et al., "mRNA-LNP COVID-19 Vaccine Lipids Induce Complement Activation and Production of Proinflammatory Cytokines: Mechanisms, Effects of Complement Inhibitors, and Relevance to Adverse Reactions," *Int. J. Mol. Sci.* 2024, 25, 7: 3595. doi: https://doi.org/10.3390/ijms25073595

- "… the novel findings in the present study include (i) the dominance of alternative pathway activation, (ii) the increased strength of C activation relative to corresponding PEGylated liposomes, and (iii) the absence of C activation by naked mRNAs."

4. Barta BA et al., "The COVID-19 mRNA vaccine Comirnaty induces anaphylactic shock in an anti-PEG hyperimmune large animal model," *Eur. Heart J.* 2023, 44 (supp 2): ehad655.3291. doi: https://doi.org/10.1093/eurheartj/ehad655.3291
   - "Consistent with previous studies, our current data show a causal role of anti-PEG Abs in the anaphylaxis to Comirnaty, which involves complement activation…"

5. Bigini P et al., "The role and impact of polyethylene glycol on anaphylactic reactions to COVID-19 nano-vaccines," *Nat. Nanotechnol.* 2021, 16: 1169–1171. doi: https://doi.org/10.1038/s41565-021-01001-3

6. Bitounis D et al., "Strategies to reduce the risks of mRNA drug and vaccine toxicity," *Nat. Rev. Drug Discov.* 2024, 23: 281-300. doi: 10.1038/s41573-023-00859-3
   - "… cell tropism and tissue distribution of mRNA and lipid nanoparticles can lead to toxicity, and their possible reactogenicity."

7. Blumental KG et al., "Acute Allergic Reactions to mRNA COVID-19 Vaccines," *JAMA* 2021, 325, 15:1562-1565. doi: 10.1001/jama.2021.3976
   - "… severe reactions consistent with anaphylaxis occurred at a rate of 2.47 per 10 000 vaccinations… The incidence rate of confirmed anaphylaxis in this study is larger than that reported by the Centers for Disease Control and Prevention based on passive spontaneous reporting methods (0.025-0.11 per 10 000 vaccinations)."

8. Borgsteede SD et al. "Other excipients than PEG might cause serious hypersensitivity reactions in COVID-19 vaccines," *Allergy* 2021, 76: 1941–2. doi: https://doi.org/10.1111/all.14774

9. Cabanillas B et al., "Allergic reactions to the first COVID-19 vaccine: A potential role of polyethylene glycol?" Allergy 2021, 76, 6: 1617-1618. doi: 10.1111/all.14711
   - "Although the trigger of the adverse allergic reactions suffered by the two NHS workers after receiving the vaccine BNT162b2 against COVID-19 has yet to be determined, the potential role of the excipient ALC-0159 containing PEG as a high-risk hidden trigger of dangerous allergic reactions should be carefully considered before advising the administration of BNT162b2 vaccine."

10. Calogiuri G et al., “Polyethylene glycols and polysorbates: Two still neglected ingredients causing true IgE-mediated reactions,” *J Allergy Clin Immunol Pract* 2019, 7, 7: 2509-2510. doi: 10.1016/j.jaip.2019.05.058
    - “In the light of increased exposure of PEGs and polysorbates in our environment, a greater incidence of PEG hypersensitivity should be expected in the next years.”

11. Calzetta L et al., “The BNT162b2 mRNA COVID-19 Vaccine Increases the Contractile Sensitivity to Histamine and Parasympathetic Activation in a Human Ex Vivo Model of Severe Eosinophilic Asthma,” *Vaccines* 2023, 11, 2: 282. doi: 10.3390/vaccines11020282

12. Camera GL et al., “A Step-by-Step Approach to Improve Clinical Translation of Liposome-Based Nanomaterials, a Focus on Innate Immune and Inflammatory Responses,” *Int. J. Mol. Sci.* 2021, 22, 2: 820. doi: 10.3390/ijms22020820
    - “… a large proportion of the selected, commercially available carriers failed to pass the first homogeneity tests, and further products were found to be cytotoxic or interact with the immune system in an undesired way.”

13. Carreno JM et al., “mRNA-1273 but not BNT162b2 induces antibodies against polyethylene glycol (PEG) contained in mRNA-based vaccine formulations,” *Vaccine* 2022, 40, 42: 6114-6124. doi: https://doi.org/10.1016/j.vaccine.2022.08.024
    - “We detected an increase in the reactivity to mRNA vaccine formulations in mRNA-1273 but not BNT162b2 vaccinees’ sera in a prime-boost dependent manner. Furthermore, we observed the same pattern of reactivity against irrelevant lipid nanoparticles.”

14. Catenacci L et al., “Effect of Lipid Nanoparticle Physico-Chemical Properties and Composition on Their Interaction with the Immune System,” *Pharmaceutics* 2024, 16, 12: 1521. doi: https://doi.org/10.3390/pharmaceutics16121521
    - “COVID-19 mRNA vaccines administered in the deltoid muscle in humans stimulate inflammation and recruitment of neutrophils, monocytes, and dendritic cells…”

15. Chen BM et al., “Polyethylene Glycol Immunogenicity: Theoretical, Clinical, and Practical Aspects of Anti-Polyethylene Glycol Antibodies,” *ACS Nano* 2021, 15, 9: 14022–14048. doi: https://doi.org/10.1021/acsnano.1c05922
    - “Hypersensitivity reactions including anaphylaxis after infusion of pegylated medicines are well documented in both animal and clinical studies… Pegylated liposomes encapsulating oligonucleotides induce anti-PEG IgM antibodies in mice and cause anaphylactic shock upon a second injection of liposomes.”

16. Chen WA et al., “Antibodies against Poly(ethylene glycol) Activate Innate Immune Cells and Induce Hypersensitivity Reactions to PEGylated Nanomedicines,” *ACS Nano* 2023, 17, 6: 5757–5772. doi: https://doi.org/10.1021/acsnano.2c12193
    - “We demonstrate that anti-PEG IgG but not IgM antibodies induce hypersensitivity-like symptoms against PLD and other PEGylated nanoparticles and macromolecules in mice that depend primarily on neutrophils, macrophages, and basophils.”

17. de Vriez J, “Pfizer's vaccine raises allergy concerns. Polymer in mRNA's “packaging” may cause rare anaphylactic reactions,” *Science* 2021, 371, 6524: 10-11. doi: 10.1126/science.371.6524.10
    - “Severe allergy-like reactions in at least 12 people who received the COVID-19 vaccine produced by Pfizer and BioNTech may be due to a compound in the packaging of the messenger RNA (mRNA) that forms the vaccine's main ingredient, scientists say. A similar mRNA vaccine developed by Moderna also contains the compound, polyethylene glycol (PEG).”

18. Dézsi L et al., “A naturally hypersensitive porcine model may help understand the mechanism of COVID-19 mRNA vaccine-induced rare (pseudo) allergic reactions: Complement activation as a possible contributing factor,” *Geroscience* 2022, 44: 597–618. doi: https://doi.org/10.1007/s11357-021-00495-y

19. du Preez HN et al., “COVID-19 vaccine adverse events: Evaluating the pathophysiology with an emphasis on sulfur metabolism and endotheliopathy,” *Eur J Clin Invest*. 2024, 54, 10: e14296. doi: https://doi.org/10.1111/eci.14296
    - “We hypothesize that after COVID-19 vaccination, the combination of the genetic-vaccine-generated (GVG) Sp antigen, the genetic material and LNPs, will ultimately contribute to GL [glycocalyx] degradation; mainly through the generation of chronic, skewed or excessive inflammatory responses, and oxidative stress. Therefore, AEs experienced postvaccination results from compromised barrier functions, circulating pro-inflammatory cytokines, reactive oxygen species (ROS), GL fragments, harmful NPs, and soluble GVG Sp and its fragments, all of which cause various cytotoxic effects.”

20. Eberlein B et al., “Allergy to PEG (polyethylene glycol) – sensitivity of basophil activation test with COVID-19 mRNA-vaccine BNT162B2,” *Hum Vaccin Immunother.* 2024, 20, 1. doi: https://doi.org/10.1080/21645515.2024.2312600

21. Gao Z et al., “Exploring the impact of lipid nanoparticles on protein stability and cellular proteostasis,” *J. Colloid Interface Sci.* 2025, 678(A): 656-665. doi: https://doi.org/10.1016/j.jcis.2024.08.146
    - “… LNPs may induce subtle proteome stress by compromising protein stability and proteostasis even without obvious damage to cell viability.”

22. Garces M et al., “Current understanding of nanoparticle toxicity mechanisms and interactions with biological systems,” *New J. Chem.* 2021, 45: 14328-14344. doi: https://doi.org/10.1039/D1NJ01415C

23. Giavina-Bianchi P and J Kalil, “May polyethylene glycol be the cause of anaphylaxis to mRNA COVID-19 vaccines?” *World Allergy Organ J.* 2021, 14, 4: 100532. doi: https://doi.org/10.1016/j.waojou.2021.100532

24. Guo C et al., “The interplay between PEGylated nanoparticles and blood immune system,” *Adv Drug Deliv Rev.* 2023, 200: 114004. doi: 10.1016/j.addr.2023.115044
    - “Complement activation-related pseudoallergy (CARPA) and accelerated blood clearance (ABC) phenomenon are the most notorious problems. CARPA is a non-IgE-activated hypersensitivity reaction (HSR) that manifests as a hemodynamic disturbance and an inflammatory response that can cause serious consequences or even fatalities.”

25. Haroon HB et al., “Activation of the complement system by nanoparticles and strategies for complement inhibition,” *Eur. J. Pharm. Biopharm.* 2023, 193: 227-240. doi: https://doi.org/10.1016/j.ejpb.2023.11.006

26. Hashimoto T et al., “High anaphylaxis rates following vaccination with the Pfizer BNT162b2 mRNA vaccine against COVID-19 in Japanese healthcare workers: a secondary analysis of initial post-approval safety data,” *J. Travel Med.* 2021, 28, 7: taab090. doi: https://doi.org/10.1093/jtm/taab090

27. Ibrahim M et al., “Polyethylene glycol (PEG): The nature, immunogenicity, and role in the hypersensitivity of PEGylated products,” *J Control Release* 2022, 351: 215-230. doi: https://doi.org/10.1016/j.jconrel.2022.09.031
    - “… the main causes and exact mechanisms of hypersensitivity to mRNA COVID-19 vaccines have not been fully elucidated, but reports of hypersensitivity reactions have focused on the role of the PEG polymer that is used in the preparation of these vaccines… we explain the potential role of PEG in the reports of the immunogenicity and hypersensitivity that has been encountered post-mRNA COVID-19 vaccination.”

28. Igyarto BZ et al., “Future considerations for the mRNA-lipid nanoparticle vaccine platform,” *Curr Opin Virol.* 2021, 48: 65–72. doi: 10.1016/j.coviro.2021.03.008
    - “… some of the immediate allergic responses observed with the first shot of mRNA-LNP vaccines might be related to pre-existing PEG antibodies. Since these vaccines often require a booster shot, anti-PEG antibody formation is expected after the first shot. Thus, the allergic events are likely to increase upon re-vaccination.”
    - “It has been shown that mRNA-LNP vaccines have an altered tissue distribution, dynamics, and uptake in animals that have been pre-exposed to inflammatory

agents. These findings suggest that people with pre-existing inflammatory conditions might show altered immune responses to these vaccines and might present with more severe side-effects."

29. Igyarto BZ and Zhen Qin, "The mRNA-LNP vaccines – the good, the bad and the ugly?" *Front. Immunol.* 2024, 15 (Sec. Vaccines and Molecular Therapeutics). doi: https://doi.org/10.3389/fimmu.2024.1336906
    - "... the LNPs' ionizable lipid component of the mRNA-LNP vaccine is highly inflammatory ... another potential explanation for the distinct lots triggering different levels of adverse events could be that the amounts of mRNA-LNP or the mRNA : LNP ratio differed between lots."

30. Jiang SY et al., "Non–immunoglobulin E-mediated allergy associated with Pfizer-BioNTech coronavirus disease 2019 vaccine excipient polyethylene glycol," *Ann Allergy Asthma Immunol.* 2021, 127, 6: 694-696. doi: 10.1016/j.anai.2021.09.012

31. Jo H et al., "Regulating Immune Responses Induced by PEGylated Messenger RNA–Lipid Nanoparticle Vaccine," *Vaccines* 2025, 13, 1: 14. doi: 10.3390/vaccines13010014

32. Ju Y et al., "Anti-PEG Antibodies Boosted in Humans by SARS-CoV-2 Lipid Nanoparticle mRNA Vaccine," *ACS Nano* 2022, 16, 8: 11769–11780. doi: https://doi.org/10.1021/acsnano.2c04543
    - "We conclude that PEG-specific antibodies can be boosted by LNP mRNA vaccination and that the rise in PEG-specific antibodies is associated with systemic reactogenicity and an increase of PEG particle–leukocyte association in human blood."

33. Ju Y et al., "Impact of anti-PEG antibodies induced by SARS-CoV-2 mRNA vaccines," *Nat. Rev. Immunol*. 2023, 23: 135-135. doi: 10.1038/s41577-022-00825-x

34. Klimek L et al., "Allergenic components of the mRNA-1273 vaccine for COVID-19: Possible involvement of polyethylene glycol and IgG-mediated complement activation," *Allergy* 2021, 76, 11: 3307-3313. doi: https://doi.org/10.1111/all.14794
    - "Allergic reactions to such PEGylated lipids are IgE-mediated. However, non-IgE-mediated reactions should also be considered."

35. Korzun T et al., "From Bench to Bedside: Implications of Lipid Nanoparticle Carrier Reactogenicity for Advancing Nucleic Acid Therapeutics," *Pharmaceuticals* 2023, 16, 8: 1088. doi: https://doi.org/10.3390/ph16081088
    - "... the current data raise important questions revolving around LNP-associated side effects. For instance, the use of a greater mRNA–LNP dose in the mRNA-1273 vaccine and different ionizable lipids used in the formulation are potential explanations for the increased reactogenicity of mRNA-1273 compared with

BNT162b formulations in the Moderna and Pfizer-BioNTech COVID-19 vaccines, respectively."

36. Korzun T et al., "Lipid Nanoparticles Elicit Reactogenicity and Sickness Behavior in Mice Via Toll-Like Receptor 4 and Myeloid Differentiation Protein 88 Axis," *ACS Nano* 2024, 18, 36: 24842–24859. doi: https://doi.org/10.1021/acsnano.4c05088
    - "Our comprehensive investigation utilizing gene ablation studies and pharmacological receptor manipulation proves that TLR4 activation by LNPs triggers distinct physiologically meaningful responses in mice. We show that TLR4 and MyD88 are essential for reactogenic signal initiation, pro-inflammatory gene expression, and physiological outcomes like food intake and body weight – robust metrics of sickness behavior in mice."

37. Kozma GT et al., "Anti-PEG antibodies: Properties, formation, testing and role in adverse immune reactions to PEGylated nano-biopharmaceuticals," Adv. Drug Deliv. Rev. 2020, 154-155: 163-175. doi: https://doi.org/10.1016/j.addr.2020.07.024
    - "Considering the known causal relationships among C [complement] activation, ABC [accelerated blood clearance], HSRs [hypersensitivity reactions], opsonization and immunogenicity, we proposed the possible rise of an immune stimulatory vicious cycle among these effects…"

38. Kozma GT et al., "Role of anti-polyethylene glycol (PEG) antibodies in the allergic reactions to PEG-containing Covid-19 vaccines: Evidence for immunogenicity of PEG," *Vaccine* 2023, 41, 31: 4561-4570. doi: 10.1016/j.vaccine.2023.06.009
    - "The anti-PEG IgG and/or IgM levels in the 15 vaccine reactors (3 anaphylaxis) were significantly higher compared to nonreactors. Serial testing of plasma showed significant correlation between the booster injection-induced rises of anti-S and anti-PEG IgGs, suggesting coupled anti-S and anti-PEG immunogenicity."

39. Laisuan W, "COVID-19 vaccine anaphylaxis: current evidence and future approaches," *Front Allergy.* 2021, 2: 801322. doi:10.3389/falgy.2021.801322

40. Li Y et al., "Nanoparticle-Binding Immunoglobulins Predict Variable Complement Responses in Healthy and Diseased Cohorts," *ACS Nano* 2024, 18, 42: 28649–28658. doi: https://doi.org/10.1021/acsnano.4c05087

41. Lim XR et al., "Anaphylatoxin Complement 5a in Pfizer BNT162b2-Induced Immediate-Type Vaccine Hypersensitivity Reactions," *Vaccines* 2023, 11, 6: 1020. doi: https://doi.org/10.3390/vaccines11061020

42. Luxi N et al., "Allergic Reactions to COVID-19 Vaccines: Risk Factors, Frequency, Mechanisms and Management," *BioDrugs* 2022, 36: 443-458. doi: https://doi.org/10.1007/s40259-022-00536-8

- "PEG is the only excipient in COVID-19 vaccines that has been clearly demonstrated to cause mainly immediate HRs, while the role of trometamol and PS80 as relevant allergens in these vaccines remains more questionable."

43. Maltezou HC et al., "Anaphylaxis rates following mRNA COVID-19 vaccination in children and adolescents: Analysis of data reported to EudraVigilance," *Vaccine* 2023, 41, 14: 2382-2386. doi: https://doi.org/10.1016/j.vaccine.2023.02.067
    - "The overall mean anaphylaxis rate was 12.81 [95% confidence interval (CI): 11.49–14.12] per 10^6 mRNA vaccine doses [12.14 (95% CI: 6.37–17.91) per 10^6 doses for mRNA-1273 and 12.84 (95% CI: 11.49–14.19) per 10^6 doses for BNT162b2]."

44. Maugeri M et al.. "Linkage between endosomal escape of LNP-mRNA and loading into EVs for transport to other cells," *Nat Commun* 2019, 10: 4333. doi: https://doi.org/10.1038/s41467-019-12275-6
    - "... the systemic delivery of both EVs and LNPs cause the expression of proinflammatory cytokines in mice..."

45. Moghimi SM, "Allergic reactions and anaphylaxis to LNP-based COVID-19 vaccines," *Mol. Ther.* 2021, 29, 3: 898-900. doi: 10.1016/j.ymthe.2021.01.030
    - "Limited information is available on LNP size distribution, polydispersity index, particle number, and presence of likely co-existing vesicles and micelles in the Pfizer-BioNTech and Moderna vaccines. Batch-to-batch variations in these parameters could further play a modulatory role in allergic reactions, and these possibilities were previously suggested for liposomes."

46. Moghimi SM et al., "Perspectives on complement and phagocytic cell responses to nanoparticles: from fundamentals to adverse reactions," *J Control Rel.* 2023, 356: 115–129. doi: https://doi.org/10.1016/j.jconrel.2023.02.022

47. Moghimi SM and Dmitri Simberg, "Pro-inflammatory concerns with lipid nanoparticles," *Mo. Ther.* 2022, 30, 6: 2109-2110. doi: 10.1016/j.ymthe.2022.04.011
    - "Considering the pro-inflammatory nature of the currently available ionizable cationic lipids, notably their undesirable immune cascade initiated through the IL-1β release, and of other cationic lipids, the potential application of LNPs for systemic administration must be viewed cautiously."

48. Mouri M et al., "Serum polyethylene glycol-specific IgE and IgG in patients with hypersensitivity to COVID-19 mRNA vaccines," *Allergol Int.* 2022, 71, 4: 512-519. doi: https://doi.org/10.1016/j.alit.2022.05.007
    - "The results suggest that PEG is one of the antigens in the allergy to COVID-19 mRNA vaccines. Cross-reactivity between PEG and PS might be crucial for allergy to the vaccines."

49. Muhaimin M et al., “The Toxicological Profile of Active Pharmaceutical Ingredients–Containing Nanoparticles: Classification, Mechanistic Pathways, and Health Implications,” *Pharmaceuticals* 2025, 18, 5: 703. doi: 10.3390/ph18050703

50. Nakayama T et al., “Comparison of cytokine production in mice inoculated with messenger RNA vaccines BNT162b2 and mRNA-1273,” *Microbiol Immunol* 2022, 67, 3: 120-128. doi: https://doi.org/10.1111/1348-0421.13043
    - “The induction of inflammatory cytokines in the mouse model is related to the cause of adverse events in humans, with a higher incidence of adverse events after the second dose.”

51. Ndeupen S et al., “The mRNA-LNP platform's lipid nanoparticle component used in preclinical vaccine studies is highly inflammatory,” *iScience* 2021, 24, 12: 103479. doi: 10.1016/j.isci.2021.103479
    - “Intradermal injection of these LNPs alone or in combination with non-coding poly-cytosine mRNA led to rapid and robust innate inflammatory responses, characterized by neutrophil infiltration, activation of diverse inflammatory pathways, and production of various inflammatory cytokines and chemokines. The same dose of LNP delivered intranasally led to similar inflammatory responses in the lung and resulted in a high mortality rate.”

52. Nguyen HM et al., “mRNA-LNPs induce immune activation and cytokine release in human whole blood assays across diverse health conditions,” *Mol. Ther.* 2025, 33, 6: 2872-2885. doi: 10.1016/j.ymthe.2024.12.019

53. Omo-Lamai S et al., “Physicochemical Targeting of Lipid Nanoparticles to the Lungs Induces Clotting: Mechanisms and Solutions,” *Adv. Mater.* 2024, 36, 26: 2312026. doi: https://doi.org/10.1002/adma.202312026

54. Parhiz H et al., “Added to pre-existing inflammation, mRNA-lipid nanoparticles induce inflammation exacerbation (IE),” *J Control Release* 2022, 344: 50-61. doi: https://doi.org/10.1016/j.jconrel.2021.12.027
    - “Although fairly benign in the healthy state, LNP potentiated existing inflammation in mice that had received the bacterial cell wall component LPS intratracheally (IT) or intravenously (IV).”

55. Qin Z et al., “Pre-exposure to mRNA-LNP inhibits adaptive immune responses and alters innate immune fitness in an inheritable fashion,” *PLoS Pathog.* 2022, doi: https://doi.org/10.1371/journal.ppat.1010830
    - “The mRNA-LNP-based SARS-CoV-2 vaccine is highly inflammatory, and its synthetic ionizable lipid component responsible for the induction of inflammation has a long in vivo half-life… We found that pre-exposure to mRNA-LNPs or LNP alone led to long-term inhibition of the adaptive immune response.”

56. Radice A et al., “Potential culprits for immediate hypersensitivity reactions to BNT162b2 mRNA COVID-19 vaccine: not just PEG,” *Eur Ann Allergy Clin Immunol 2021*, 53, 5: 240-242. doi: https://doi.org/10.23822/eurannaci.1764-1489.214
    - “Apart from PEG, another component of the LNP, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), should also be considered a potential culprit as it contains a quaternary ammonium (QA) ion.”

57. Rama TA et al., “Hypersensitivity to the Moderna COVID-19 vaccine caused by tromethamine: PEG is not always the culprit excipient,” *J Investig Allergol Clin Immunol.* 2022, 32, 5: 414-415. doi: 10.18176/jiaci.0773
    - “… this case provides further evidence that the excipient, and specifically IgE-mediated hypersensitivity to tromethamine, may be an underlying mechanism for immediate hypersensitivity to mRNA COVID-19 vaccines.”

58. Sampath V et al., “Vaccines and allergic reactions: The past, the current COVID-19 pandemic, and future perspectives,” *Allergy* 2021, 76, 6: 1640-1660. doi: https://doi.org/10.1111/all.14840
    - “This suggests that the incidence of anaphylaxis in the mRNA BNT162b2 (11.1 cases per million doses) and mRNA-1273 COVID-19 vaccines (2.5 cases per million doses) may be about 2 to 8.5 times as high as the incidence reported in the 2016 VSD study for all vaccines (1.31 per million doses).”

59. Sellaturay P et al., “Polyethylene glycol (PEG) is a cause of anaphylaxis to the Pfizer/BioNTech mRNA COVID-19 vaccine,” *Clin Exp Allergy* 2021, 51, 6: 861-863. doi: https://doi.org/10.1111/cea.13874
    - “Here, we show polyethylene glycol allergy caused one of the first cases of anaphylaxis to the Pfizer/BioNTech COVID-19 vaccine. Allergy skin prick testing with polyethylene glycol triggered anaphylaxis, highlighting the importance of safety procedures during investigation.”

60. Shah MM et al., “Elucidating allergic reaction mechanisms in response to SARS-CoV-2 mRNA vaccination in adults,” *Allergy* 2024 79, 9: 2502-2523. doi: https://doi.org/10.1111/all.16231

61. Sharma N et al., “Nanoparticles toxicity: an overview of its mechanism and plausible mitigation strategies,” *J. Drug. Target.* 2024, 32, 5: 457-469. doi: https://doi.org/10.1080/1061186X.2024.2316785

62. Shi D et al., “To PEGylate or not to PEGylate: Immunological properties of nanomedicine’s most popular component, polyethylene glycol and its alternatives,” *Adv. Drug Deliv. Rev.* 2022, 180: 114079. doi: 10.1016/j.addr.2021.114079
    - “First, phagocytic cells of the immune system are at the forefront of clearance of PEG and PEGylated materials; therefore, toxicity to these cells may influence

body's general defense against infections and damaged or transformed host's cells. Second, generation of the specific immune response to PEG in the form of antibodies contributes to hypersensitivity reactions (HSRs) to PEG and PEGylated products. Such HSRs include true allergy (IgE mediated, type I hypersensitivity), anaphylactoid reactions (complement-mediated immediate type hypersensitivity or complement-mediated pseudoallergy, CARPA), type II and type III hypersensitivity (IgM and IgG-mediated) reactions. Third, neutralization and cross-reactivity of such antibodies may contribute to HSRs and altered PK of other products containing PEG or other structures similar to PEG."

63. Simberg D et al., "PEGylation technology: addressing concerns, moving forward," *Drug Deliv.* 2025, 32, 1: 2494775. doi: 10.1080/10717544.2025.2494775

64. Somiya M et al., "Sex differences in the incidence of anaphylaxis to LNP-mRNA COVID-19 vaccines," *Vaccine* 2021, 39, 25): 3313–3314. doi: 10.1016/j.vaccine.2021.04.066
    - "On February 17, 2021, Japan started vaccinating healthcare workers with the Pfizer-BioNTech lipid nanoparticle (LNP)-mRNA COVID-19 vaccine. Among total 79 anaphylaxis cases, 70 cases have been reported in women (89.9%) after 1,096,698 doses of the vaccine until April 4, 2021… Another report confirmed the female predominance of anaphylaxis cases in over 60,000 doses of LNP-mRNA vaccinations; 15 (94%) of the 16 confirmed cases were women… One possible explanation for the sex imbalance is that sensitization to PEG is more common in women due to the relatively frequent exposure to PEG-containing products, such as cutaneous exposure to cosmetics or the use of medications such as contraceptive injections."

65. Stone CA, Jr., et al., "Immediate Hypersensitivity to Polyethylene Glycols and Polysorbates: More Common Than We Have Recognized," *J Allergy Clin Immunol Pract* 2019, 7, 5: 1533-1540.e8. doi: https://doi.org/10.1016/j.jaip.2018.12.003
    - "Immediate hypersensitivity to PEG 3350 with cross-reactive polysorbate 80 hypersensitivity may be underrecognized in clinical practice."

66. Szebeni J et al., "Applying lessons learned from nanomedicines to understand rare hypersensitivity reactions to mRNA-based SARS-CoV-2 vaccines," *Nat. Nanotechnol.* 2022, 17: 337–346. doi: https://doi.org/10.1038/s41565-022-01071-x
    - "In summary, all the components of LNP–mRNA vaccines… have various immunostimulatory effects… collectively required for vaccine efficacy. The same components, however, also contribute to HSR and other IMAEs…"

67. Tahtinen S and Ira Mellman, "IL-1-mediated inflammation induced by different RNA vaccines is context-specific," *Nature Immunol.* 2022, 23, 4: 485-486. doi: https://doi.org/10.1038/s41590-022-01177-3

- "Systemic inflammatory responses generated by lipid-formulated RNA vaccines are driven by differential induction of pro- and anti-inflammatory interleukin-1 (IL-1) family members in mice and humans… We discovered that the RNA-LPX vaccine induces the release of the cytokine IL-1. IL-1 initiates an innate immune cascade that results in systemic cytokine release and the adverse events that limit vaccine dosing in humans."

68. Tahtinen S et al., "IL-1 and IL-1ra are key regulators of the inflammatory response to RNA vaccines," *Nat. Immunol.* 2022, 23: 532-542. doi: 10.1038/s41590-022-01160-y
    - "In human immune cells, RNA vaccines induce production of IL-1 cytokines, predominantly IL-1β, which is dependent on both the RNA and lipid formulation. IL-1 in turn triggers the induction of the broad spectrum of pro-inflammatory cytokines (including IL-6)."

69. Tenchov R et al, "PEGylated Lipid Nanoparticle Formulations: Immunological Safety and Efficiency Perspective," *Bioconjug. Chem.* 2023, 34, 6: 941-960. doi: https://doi.org/10.1021/acs.bioconjchem.3c00174
    - "A search in the CAS Content Collection identified nearly 900 documents, including ∼150 patents, related to the PEG–lipids immunologically induced adverse effects such as anti-PEG antibodies generation, accelerated blood clearance, and complement activation-related pseudoallergies."

70. Tinari S, "The EMA covid-19 data leak, and what it tells us about mRNA instability," *BMJ* 2021, 372: n672. doi: https://doi.org/10.1136/bmj.n627
    - "JW Ulm, a gene therapy specialist who has published on tissue targeting of therapeutic vectors, raised concerns about the biodistribution of LNPs: 'At present, relatively little has been reported on the tissue localisation of the LNPs used to encase the SARS-CoV-2 spike protein-encoding messenger RNA, and it is vital to have more specific information on precisely where the liposomal nanoparticles are going after injection.' It is an unknown that Ulm worries could have implications for vaccine safety."

71. Tran TT and SR Roffler, "Interactions between nanoparticle corona proteins and the immune system," *Curr Opin Biotechnol.* 2023, 84: 103010. doi: 10.1016/j.copbio.2023.103010
    - "Intravenous administration of pegylated liposomal formulations containing Toll-like receptor agonists to mice on days 0, 4, and 8 resulted in hypersensitivity reaction symptoms…"

72. Troelnikov A et al., "Basophil reactivity to BNT162b2 is mediated by PEGylated lipid nanoparticles in patients with PEG allergy," *J Allergy Clin Immunol.* 2021, 148, 1: 91-95. doi: https://doi.org/10.1016/j.jaci.2021.04.032

- "Our findings implicate PEG, as covalently modified and arranged on the vaccine lipid nanoparticle, as a potential trigger of anaphylaxis in response to BNT162b2, and highlight shortcomings of current skin testing protocols for allergy to PEGylated liposomal drugs."

73. Tsilingiris D et al., "Potential implications of lipid nanoparticles in the pathogenesis of myocarditis associated with the use of mRNA vaccines against SARS-CoV-2," *Metabol. Open* 2022, 13: 100159. doi: https://doi.org/10.1016/j.metop.2021.100159
    - "The recent observation of a similar adverse event [myocarditis] in a recipient of the non-mRNA, peptide-based NVX-CoV2373 in the frame of a phase III clinical trial with 7020 participants in the active treatment arm raises the question whether the lipid nanoparticle sheath, which is a common structural component of these platforms could be implicated in the pathogenesis of vaccine-induced myocarditis."

74. Wang H et al., "Polyethylene glycol (PEG)-associated immune responses triggered by clinically relevant lipid nanoparticles in rats," *npj Vaccines* 2023, 8: 169. doi: https://doi.org/10.1038/s41541-023-00766-z
    - "... 'antigen-antibody' complexes may induce severe side effects including hypersensitivity reactions, although the underlying mechanisms have not been fully clarified... Overall, these data provided strong evidence for the dose- and time-dependent induction of anti-PEG IgM."

75. Wang J et al., "Recent Advances in Lipid Nanoparticles and Their Safety Concerns for mRNA Delivery," *Vaccines* 2024, 12, 10: 1148. doi: 10.3390/vaccines12101148
    - "... as the immunological activation in response to mRNA-LNP treatment increases, the body's defense capability may also rise, but there is a high possibility of the mRNA-LNP complexes causing adverse effects, including allergies and autoimmune diseases."

76. Warren CM et al. "Assessment of Allergic and Anaphylactic Reactions to mRNA COVID-19 Vaccines With Confirmatory Testing in a US Regional Health System," *JAMA Netw. Open*. 2021, 4, 9: e2125524. doi: 10.1001/jamanetworkopen.2021.25524
    - "These findings suggest that non–IgE-mediated allergic reactions to PEG may be responsible for many documented cases of allergy to mRNA vaccines."

77. Xuan L et al., "Nanoparticles-Induced Potential Toxicity on Human Health: Applications, Toxicity Mechanisms, and Evaluation Models," *MedComm* 2023, 4, 4: e327. doi: https://doi.org/10.1002/mco2.327

78. Yang M et al., "Effects of PEG antibodies on in vivo performance of LNP-mRNA vaccines," *Int J Pharm*. 2024, 650: 123695. doi: 10.1016/j.ijpharm.2023.123695

- “PEG antibodies binding on the LNP vaccine increased probability of complement activation in animal as well as in human serum and led to lethal side effect in large dosage via intravenous injection of mice. Our data suggested that PEG antibodies in human was a risky factor of LNP-based vaccines for biosafety concerns but not efficacy.”

79. Yuan Z et al., “Impact of physicochemical properties on biological effects of lipid nanoparticles: Are they completely safe,” *Sci Total Environ.* 2024, 927: 172240. doi: https://doi.org/10.1016/j.scitotenv.2024.172240
    - “The physicochemical properties of LNPs, like size, surface hydrophobicity, surface charge, surface modification and lipid composition, determine the interaction of LNPs with macromolecules and organelles to a large extent, resulting in negative effects on cells, especially cytotoxicity and genotoxicity, and cell death.”

80. Zhou ZH et al ., “Anti-PEG IgE in anaphylaxis associated with polyethylene glycol,” *J Allergy Clin Immunol Pract* 2021, 9, 4: 1731-1733.e3. doi: 10.1016/j.jaip.2020.11.011
    - “… all the anaphylaxis case samples and none of the control samples were clearly positive for anti-PEG IgE.”

V. COVID "vaccine" immune imprinting library

Compiled by Dr. Steven Hatfill, MD, MMed, Erik Sass, et al.

Last updated July 1, 2025. Corresponding author: eriksass@gmail.com

Immune imprinting, dubbed "original antigenic sin" by Thomas Francis Jr., occurs when memory B lymphocytes produced in response to an initial viral infection dominate subsequent responses to related viruses, producing antibodies geared to the original exposure. Long-term immune memory has many advantages, but immune imprinting can be harmful if it interferes with immune response to later infections.

The following collection of peer-reviewed papers (**n=140**) suggests that COVID "vaccines" imprinted the immune systems of recipients through exposure to the "wild type" spike protein from the original Wuhan strain, shaping their response to subsequent variants in potentially harmful ways. Immune imprinting impaired responses to new variants by skewing B cell production of antibodies toward the "ancestral" spike protein at the expense of new antibodies specifically tailored to the variants' heavily mutated spike. Additionally, by imprinting a single antigen – the spike protein – on recipients' immune systems, the "vaccines" prevented them from forming antibodies to other, less mutation-prone parts of the virus, such as proteins from the virus nucleocapsid (Ahmed MIM et al., Delgado JF et al., Paula NM et al., Smith CP et al., Yao D et al). Further findings point to "deep immunological imprinting" or "hybrid immune damping," in which "vaccination" combined with infection alters later immune response unpredictably (Aguilar-Bretones M et al., Gao B et al., Hornsby H et al., Ju B et al., Reynolds CJ et al., Wang Q et al.).

This collection originated with Dr. Steven Hatfill's contribution to *TOXIC SHOT: Facing the Dangers of the COVID "Vaccines"* (Chapter 5: Debunking CDC's Bad Science).

ANNOTATED REFERENCES (N=140)

1. Addetia A et al., "Neutralization, effector function and immune imprinting of Omicron variants," *Nature* 2023, 621: 592-601. doi: 10.1038/s41586-023-06487-6
   - "Omicron breakthrough infections of Wu-vaccinated subjects primarily recall cross-reactive MBCs specific for epitopes shared by multiple SARS-CoV-2 variants rather than priming naive B cells that recognize Omicron RBD-specific epitopes. We observed an unexpectedly small number of MBCs specific for Omicron RBDs (and not cross-reacting with the Wu RBD) even after two exposures to Omicron S antigens, including after Wu/BA.5 or Wu/BA.1 bivalent mRNA vaccination."

2. Aguilar-Bretones M et al., "Impact of antigenic evolution and original antigenic sin on SARS-CoV-2 immunity," *J Clin Invest*. 2023, 133, 1: e162192. doi: 10.1172/JCI162192
   - "… vaccinated individuals infected with the Alpha or Delta variant have a relatively decreased response to variant-specific epitopes compared with unvaccinated individuals, which is indicative of OAS… In addition, more traits of immune imprinting have recently been identified in hybrid-immune individuals who were infected with Wuhan-1 strain before vaccination, in whom enhancement of VOC cross-reactive antibody titers and T cells by Omicron infection was nullified, a phenomenon termed hybrid immune damping."

3. Ahmed MIM et al., "Enhanced Spike-specific, but attenuated Nucleocapsid-specific T cell responses upon SARS-CoV-2 breakthrough versus non-breakthrough infections," *Front. Immunol*. 2022, 13 (Sec. Vaccines and Molecular Therapeutics). doi: https://doi.org/10.3389/fimmu.2022.1026473
   - "Subjects with vaccine breakthrough infection had significantly higher CD4 and CD8 T cell responses targeting the vaccine-encoded Spike during the first and third/fourth week after PCR diagnosis compared to non-vaccinated controls, respectively. In contrast, CD4 T cells targeting the non-vaccine encoded Nucleocapsid antigen were of significantly lower magnitude in BTI as compared to non-BTI. Hence, previous vaccination was linked to enhanced T cell responses targeting the vaccine-encoded Spike antigen, while responses against the non-vaccine encoded Nucleocapsid antigen were significantly attenuated."

4. Alsoussi WB et al., "SARS-CoV-2 Omicron boosting induces de novo B cell response in humans," *Nature* 2023, 617, 7961: 592-598. doi: https://doi.org/10.1038/s41586-023-06025-4
   - "mRNA-1273 and mRNA-1273.213 both elicited robust germinal centre responses and maturation of the MBC and BMPC responses, but we did not isolate any antibodies specifically targeting S proteins from the variant strains encoded by the mRNA-1273.213 vaccine that did not cross-react to the original WA1/2020 S protein. Thus, the B cell response after boosting with the mRNA-1273.213 vaccine was imprinted by the primary vaccination series with mRNA-1273, which encodes the ancestral S protein."

5. Altmann DM et al., "COVID-19 vaccination: The road ahead," *Science* 2022, 375, 6586: 1127-1132. doi: 10.1126/science.abn1755
   - "In terms of immune imprinting ('original antigenic sin'), the data show that different repertoires emerge, with associated implications for variable quality and quantity of neutralization of current or future VOC. For example, our comparative analysis of differential VOC neutralization patterns in vaccinees shows the development of imprinted differences between those who had a prior infection with either the ancestral or Alpha virus. Faced with these diverse

scenarios, the question is whether to keep developing boosters carrying prototypic Wuhan Hu-1 spike sequence or focus on being reactive to regionally predominant VOCs. The iteration of this that pools VOC sequences into multivalent vaccines has appeal, although the immune imprinting data argue the potential for unforeseen, differential response patterns dependent on prior history and subsequent SARS-CoV-2 exposure. There is a danger that, even with 'plug and play' platforms and rapid pipelines, this entails a future of playing catchup against oncoming VOCs for diminishing and unpredictable returns in protective immunity."

6. Amano M et al., "Restoration of Neutralization Activity Against Omicron BA.2 and BA.5 in Older Adults and Individuals With Risk Factors Following the Fourth Dose of Severe Acute Respiratory Syndrome Coronavirus 2 BNT162b2 Vaccine," *J. Infect. Dis.* 2023, 227, 1: 161-163. doi: https://doi.org/10.1093/infdis/jiac393
   - "The present data, that a fourth vaccine dose restores protection but does not further enhance the humoral response, may be related to 'original antigenic sin,' wherein high-affinity memory B cells inhibit the recruitment of naive B cells against subsequent antigenic stimuli, in particular, against new stimuli. Thus, it is likely that despite the fourth dose, breakthrough infections continue to occur."

7. Arunachalam PS et al. "Systems vaccinology of the BNT162b2 mRNA vaccine in humans," *Nature* 2021, 596: 410-416. doi: 10.1038/s41586-021-03791-x
   - "BNT162b2 vaccination also induced a neutralizing antibody response against the B.1.351 variant of concern, albeit at a tenfold-lower magnitude than against the wild-type WA1/2020 (WA1) strain."

8. Atari N et al., "Omicron BA.2.75 variant is efficiently neutralised following BA.1 and BA.5 breakthrough infection in vaccinated individuals, Israel, June to September 2022," *Eurosurveillance* 2022, 27, 44: 2200785. doi: https://doi.org/10.2807/1560-7917.ES.2022.27.44.2200785
   - "The neutralisation efficiency in HCW who were infected with BA.1/BA.5 and had previously been vaccinated with three doses of Comirnaty vaccine was significantly higher for all of Omicron variants (unpaired T-test, p value > 0.0008) than in vaccinated but SARS-CoV-2-naïve HCW."

9. Aydillo T et al., "Immunological imprinting of the antibody response in COVID-19 patients," *Nat. Commun.* 2021, 12: 3781. doi: 10.1038/s41467-021-23977-1
   - "Our findings thus provide evidence of immunological imprinting by previous seasonal coronavirus infections that can potentially modulate the antibody profile to SARS-CoV-2 infection… A similar scenario to our studies in infected people could be proposed for the vaccines, with some differences due to the nature of the stimulus itself. Back-boost of cross-reactive antibody responses might lead to less protective antibodies directed against non-neutralizing

conserved epitopes between the S antigen of the vaccine and the S proteins of seasonal human betacoronaviruses."

10. Baerends EAM et al., "Omicron Variant-Specific Serological Imprinting Following BA.1 or BA.4/5 Bivalent Vaccination and Previous SARS-CoV-2 Infection: A Cohort Study," *Clin. Infect. Dis.* 2023, 77, 11: 1511-1520. doi: 10.1093/cid/ciad402
    - "Vaccination and previous infection leave a clear serological imprint that is focused on the variant-specific antigen."

11. Bayarri-Olmos R et al., "Unraveling the impact of SARS-CoV-2 mutations on immunity: insights from innate immune recognition to antibody and T cell responses," *Front Immunol.* 2024, 15 (Sec. Viral Immunology). doi: https://doi.org/10.3389/fimmu.2024.1412873
    - "Of note, we observed no significant difference in T cell reactivity against the Delta and Omicron spike MP in those with an Omicron infection, nor in T cell reactivity against the Omicron spike in the different donor groups, suggesting that immune imprinting from vaccination may had dampened the induction of Omicron-specific T cells after infection… Taken together, these findings suggest that deployment of Omicron-based vaccines, or other highly divergent SARS-CoV-2 strains, in immune-naïve individuals may induce poorly cross-reactive antibody responses, while Omicron boosters in vaccinees may be of limited use due to the imprinted responses from the ancestral strain-based vaccines."

12. Belik M et al., "Long-term COVID-19 vaccine- and Omicron infection-induced humoral and cell-mediated immunity," *Front. Immunol.* 2024, 15 (Sec. Viral Immunology). doi: https://doi.org/10.3389/fimmu.2024.1494432
    - "Interestingly, the bivalent vaccine induced equally high neutralizing antibodies against D614G as the monovalent vaccine, and repeated vaccinations with the original Wuhan-type monovalent vaccine or booster vaccination with a bivalent BA.1 or BA.4/5 vaccine did not broaden the specificity of neutralizing antibodies against XBB.1.5. These results indicate that the vaccines elicit antibody responses based on immune imprinting and the repeated Omicron exposure does not override ancestral SARS-CoV-2 immune imprinting."

13. Blanco J et al., "Rethinking Optimal Immunogens to Face SARS-CoV-2 Evolution Through Vaccination," *Influenza Other Respir Viruses* 2025, 19, 1: e70076. doi: https://doi.org/10.1111/irv.70076
    - "In this repeated vaccination context, antibody repertoire diversification was evidenced, although immune imprinting after booster doses or reinfection was also demonstrated and identified as a major determinant of immunological responses to repeated antigen exposures."

14. Blankson JN, "Bivalent COVID-19 Vaccines: Can the Original Antigenic Sin Be Forgiven?" *J. Infect. Dis.* 2023, 11, 1: 1221-1223. doi: 10.1093/infdis/jiad073

- "… the lack of a more potent response to BA.5 following bivalent vaccination in some cases may reflect the fact that we are looking at a primary immune response. If that is the case, then there is a chance that subsequent exposure to BA.5 spike protein, either by vaccination or natural infection, will lead to an improved response. Unfortunately, by the time 2 bivalent booster shots are given to a significant part of the population—an unlikely prospect given the limited uptake of the bivalent vaccine and the vaccine weariness of the US population—the variant in question will probably no longer be the dominant variant in circulation."

15. Boynton JR and DM Altmann, "Imprinted hybrid immunity against XBB reinfection," *Lancet Infect Dis*. 2023, 23, 7: 764-765. doi: 10.1016/S1473-3099(23)00138-X
    - "If we now appreciate that even hybrid immunity to SARS-CoV-2 infection is (differentially, depending on previous immune experience) poorly durable and annual debates on booster strategy are required, how should we move forward? The dataset from Singapore reminds us that suggesting the booster strategy will simply involve tweaking vaccines annually, as for influenza, seriously underestimates the complexity of the current challenge. The long-term strategy will require considerable effort towards the development of both next-generation vaccines (targeting neutralising epitopes that are truly conserved and disadvantageous for viral mutations) and vaccine platforms that provide durable, local protection in the nasal mucosa, thereby blocking viral transmission."

16. Brown E and HT Essigmann, "Original Antigenic Sin: the Downside of Immunological Memory and Implications for COVID-19," *mSphere* 2021, 6, 2. doi: https://doi.org/10.1128/msphere.00056-21
    - "The impact of OAS on the elicitation of protective immunity should not be ignored in vaccine development. Selection of a vaccine candidate or candidates that are too similar to antigens already 'seen' by the population at large could result in three distinct outcomes: (i) a "back-boost" or enhanced protective immunity resulting from a second round of GCRs in response to shared antigens between primary and secondary exposures, (ii) boosting of a nonprotective antibody response, or (iii) in the context of a multicomponent vaccine formulation, the masking of a protective response against some vaccine components if other antigens in the formulation have been previously "seen" by the population as observed with Gardasil 9."

17. Cao Y et al., "BA.2.12.1, BA.4 and BA.5 escape antibodies elicited by Omicron infection," *Nature* 2022, 608, 593-602. doi: 10.1038/s41586-022-04980-y
    - "Of note, BA.2.12.1 and BA.4/BA.5 display increased evasion of neutralizing antibodies compared with BA.2 against plasma from triple-vaccinated individuals or from individuals who developed a BA.1 infection after vaccination… BA.1 infection after vaccination predominantly recalls humoral immune memory directed against ancestral… SARS-CoV-2 spike protein."

18. Cao Y et al., "Imprinted SARS-CoV-2 humoral immunity induces convergent Omicron RBD evolution," *Nature* 2023, 614: 521–529. doi: https://doi.org/10.1038/s41586-022-05644-7
    - "In this work, we showed that due to immune imprinting, our humoral immune repertoire is not effectively diversified by infection with new Omicron variants. The immune pressure on the RBD becomes increasingly concentrated and promotes convergent evolution, explaining the observed sudden acceleration of SARS-CoV-2 RBD evolution and the convergence pattern. Although this study only examines inactivated vaccines, immune imprinting is also observed in those receiving mRNA vaccines."

19. Carreño JM et al., "Bivalent COVID-19 booster vaccines and the absence of BA.5-specific antibodies," *Lancet Microbe* 2023, 4, 8: E569. doi: 10.1016/S2666-5247(23)00118-0
    - "Pre-booster and post-booster RBD antibody avidity was lower against BA.5 RBD than wild-type RBD, which prompted us to look for BA.5 specific antibodies. Wild-type RBD depleted serum samples had undetectable reactivity to wild-type RBD—as expected—and to BA.5 RBD, suggesting that a single exposure to BA.5 antigens by the administration of bivalent vaccine boosters does not elicit robust concentrations of BA.5 specific serum antibodies."

20. Cerqueira-Silva T et al., "Effectiveness of monovalent and bivalent COVID-19 vaccines," *Lancet Infect Dis.* 2023, 23, 11: 1208-1209. doi: 10.1016/S1473-3099(23)00379-1
    - "A possible explanation for the lack of increased protection against infection with bivalent vaccines is immune imprinting against the wild-type variant of SARS-CoV-2. This could impair the production of neutralising antibodies against omicron variants after immunological stimulation with a mix of wild-type and omicron antigens (ie, bivalent vaccines) because production of antibodies against antigens that the immune system had previously been exposed to would be prioritized."

21. Chalkias S et al., "A Bivalent Omicron-Containing Booster Vaccine against Covid-19," *N Eng J Med* 2022, 387: 1279-1291. doi: 10.1056/NEJMoa2208343
    - "In the primary analysis set of participants without evidence of previous SARS-CoV-2 infection, the observed geometric mean titers of neutralizing antibodies against ancestral SARS-CoV-2 (D614G) were 5977.3 (95% confidence interval [CI], 5321.9 to 6713.3) and 5649.3 (95% CI, 5056.8 to 6311.2) and against omicron were 2372.4 (95% CI, 2070.6 to 2718.2) and 1473.5 (95% CI, 1270.8 to 1708.4) 28 days after the mRNA-1273.214 and mRNA-1273 boosters, respectively."

22. Chemaitelly H et al., “2332. COVID-19 Primary Series and Booster Vaccination and Potential for Immune Imprinting,” *Open Forum Infect. Dis.* 2023, 10 (Issue Supplement_2): ofad500.1954. doi: https://doi.org/10.1093/ofid/ofad500.1954
- “History of primary-series vaccination enhanced immune protection against omicron reinfection, but history of booster vaccination compromised protection against omicron reinfection.”

23. Chemaitelly H et al., “Long-term COVID-19 booster effectiveness by infection history and clinical vulnerability and immune imprinting: a retrospective population-based cohort study,” *Lancet Infect Dis.* 2023, 23, 7: 816-827. doi: 10.1016/S1473-3099(23)00058-0
- “Protection against omicron infection waned after the booster, and eventually suggested a possibility for negative immune imprinting.”

24. Chen JJ et al., “Neutralization against XBB.1 and XBB.1.5 after omicron subvariants breakthrough infection or reinfection,” *Lancet Reg Health West Pac.* 2023, 33: 100759. doi: 10.1016/j.lanwpc.2023.100759
- “In all six groups, neutralization titers were lower against all omicron subvariants than against the D614G strain; the level of neutralizing antibodies was lowest against the XBB.1, followed by XBB.1.5… In addition, significantly enhanced neutralizing activity against all omicron subvariants was observed after BA.5.2 reinfection.”

25. Chen SY et al., “The Effectiveness of Bivalent COVID-19 Vaccination: A Preliminary Report,” *Life* 2023, 13, 10: 2094. doi: https://doi.org/10.3390/life13102094
- “Therefore, the human immune system elicits more robust immunity against the initial strain following a booster with an MV or BV. This ‘first love phenomenon’ may explain why the induced immunogenicity against BA.5 is not promising in people who receive a BA.5-containing booster. Our study also demonstrates much higher levels of immunogenicity against ancestral strains than new additional variant strains across enrolled studies.”

26. Cho A et al., “Anti-SARS-CoV-2 receptor-binding domain antibody evolution after mRNA vaccination,” *Nature* 2021, 600: 517-522. doi: 10.1038/s41586-021-04060-7
- “Between prime and boost, memory B cells produce antibodies that evolve increased neutralizing activity, but there is no further increase in potency or breadth thereafter. Instead, memory B cells that emerge five months after vaccination of naive individuals express antibodies that are similar to those that dominate the initial response.”

27. Collier ARY et al. “Immunogenicity of BA.5 Bivalent mRNA Vaccine Boosters,” *N Engl J Med* 2023, 388, 6: 565-567. doi: 10.1056/NEJMc2213948

- "Our data indicate that both monovalent and bivalent mRNA boosters markedly increased antibody responses but did not substantially augment T-cell responses. Neutralizing antibody titers against the ancestral strain of SARS-CoV-2 were higher than titers against BA.5 after both monovalent and bivalent boosting… Our findings suggest that immune imprinting by previous antigenic exposure may pose a greater challenge than is currently appreciated for inducing robust immunity against SARS-CoV-2 variants."

28. Corbett KS et al., "Protection against SARS-CoV-2 Beta variant in mRNA-1273 vaccine–boosted nonhuman primates," *Science* 2021, 374, 6573: 1343-1353. doi: 10.1126/science.abl8912
    - "The relative frequency of B cells specific for WA-1, β, or both did not change after boost with either the homologous or heterologous mRNA, suggesting that priming with mRNA-1273 imprinted the B cell repertoire."

29. Cui T et al., "Dynamic immune landscape in vaccinated-BA.5-XBB.1.9.1 reinfections revealed a 5-month protection-duration against XBB infection and a shift in immune imprinting," *eBioMedicine* 2024, 99: 104903. doi: 10.1016/j.ebiom.2023.104903
    - "… XBB.1.9.1 reinfection results in immune imprinting shifting from WT antigen induced by previous vaccination to the new XBB.1.9.1 antigen."

30. da Silva ES et al., "Vaccine- and Breakthrough Infection-Elicited Pre-Omicron Immunity More Effectively Neutralizes Omicron BA.1, BA.2, BA.4 and BA.5 Than Pre-Omicron Infection Alone," *Curr Issues Mol Biol.* 2023, 45, 2: 1741-1761. doi: 10.3390/cimb45020112
    - "… immune imprinting by first generation vaccines may restrict, but not abolish, cross-neutralization."

31. Davis-Gardner ME et al., "Neutralization against BA.2.75.2, BQ.1.1, and XBB from mRNA Bivalent Booster," *N Engl J Med* 2022, 388, 2: 183-185. doi: 10.1056/NEJMc2214293
    - "The results in both of these cohorts correspond with neutralization titers against BA.1 and BA.5 that were 5 to 9 times as low as that against WA1/2020 and neutralization titers against BA.2.75.2, BQ.1.1, and XBB that were 23 to 63 times as low as that against WA1/2020."

32. Degryse J et al., "Antigenic Imprinting Dominates Humoral Responses to New Variants of SARS-CoV-2 in a Hamster Model of COVID-19," *Microorganisms* 2024, 12, 12: 2591. doi: 10.3390/microorganisms12122591
    - "Our results show that both Comirnaty® XBB.1.5 and YF-S0* induce robust, however, poorly cross-reactive, neutralizing antibody (nAb) responses. In either case, total antibody and nAb levels increased following infection. Intriguingly,

the specificity of these boosted nAbs did not match the respective challenge virus, but was skewed towards the primary antigen used for immunization, suggesting a marked impact of antigenic imprinting, confirmed by antigenic cartography… our findings strongly suggest that antigenic imprinting by previous encounter (in this case, by vaccination) dominates the subsequent humoral response to new SARS-CoV-2 variants."

33. Delgado JF et al., "SARS-CoV-2 Spike Protein Vaccine-Induced Immune Imprinting Reduces Nucleocapsid Protein Antibody Response in SARS-CoV-2 Infection," *J. Immunol. Res.* 2022. doi: https://doi.org/10.1155/2022/8287087
    - "SARS-CoV-2 primary infection in vaccinated healthcare workers (HCWs) produced significantly lower titers of anti-N antibodies than that in nonvaccinated HCWs: 5.7 (IQR 2.3-15.2) versus 12.2 (IQR 4.2-32.0), respectively ($p = 0.005$). Therefore, spike protein vaccine-induced immune imprinting (original antigenic sin) reduces N protein antibody response."

34. Dowell AC et al., "Immunological imprinting of humoral immunity to SARS-CoV-2 in children," *Nat. Commun.* 2023, 14: 3845. doi: 10.1038/s41467-023-39575-2
    - "Prior pre-Omicron SARS-CoV-2 virus infection or vaccination primes for robust antibody responses following Omicron infection but these remain primarily focussed against ancestral variants."

35. Edara VV et al., "mRNA-1273 and BNT162b2 mRNA vaccines have reduced neutralizing activity against the SARS-CoV-2 omicron variant," *Cell Rep Med.* 2022, 3, 2: 100529. doi: 10.1016/j.xcrm.2022.100529
    - "Six months after the initial two-vaccine doses, sera from naive vaccinated subjects show no neutralizing activity against omicron. In contrast, COVID-19-recovered individuals 6 months after receiving the primary series of vaccinations show a 22-fold reduction, with the majority of the subjects retaining neutralizing antibody responses."

36. Einhauser S et al., "Longitudinal effects of SARS-CoV-2 breakthrough infection on imprinting of neutralizing antibody responses," *eBioMedicine* 2024, 110: 105438. doi: 10.1016/j.ebiom.2024.105438
    - "Notably, the longitudinal analysis reveals an initial augmentation of the vaccine-primed nAb response upon infection, followed by a progressive expansion of neutralization capacity towards the infecting SARS-CoV-2 variant. Long-term observation reveals a subsequent contraction and inclination towards dominant wild-type (WT) immunity post-breakthrough infection."

37. Emmelot ME et al., "SARS-CoV-2 Omicron BA.4/BA.5 Mutations in Spike Leading to T Cell Escape in Recently Vaccinated Individuals," *Viruses* 2023, 15, 1: 101. doi: https://doi.org/10.3390/v15010101

- "In summary, our study shows that several BA.4/BA.5 mutations in the spike protein lead to a reduced responsiveness of epitope-specific T cells in subjects that received two doses of a mRNA vaccine based on the ancestral WT spike sequence. Other currently circulating Omicron sublineages, such as BA.2.75, BA.4.6, BQ.1.1 and XBB, share many of these spike mutations, making our findings also relevant for the impact of the T cell response on these emerging Omicron variants."

38. Erice A et al., "Immune Imprinting, Non-Durable Hybrid Immunity, and Hybrid Immune Damping Following SARS-CoV-2 Primary Vaccination with BNT162b2 and Boosting with mRNA-1273," *Vaccines* 2025, 13, 3: 310. doi: 10.3390/vaccines13030310
    - "These findings suggest a modulating effect of previous SARS-CoV-2 infection on the humoral immune response to mRNA vaccination, a non-durable hybrid immunity following mRNA vaccination in previously infected subjects, and attenuation of the humoral immune response (immune damping) after repeated exposure to SARS-CoV-2 antigens through mRNA vaccination and/or infection."

39. Erice A et al., "Long-Term Analyses of SARS-CoV-2 Humoral and T Cell Responses and Breakthrough SARS-CoV-2 Infections after Two Doses of BNT162b2 Followed by mRNA-1273 and Bivalent Omicron-Adapted BNT162b2 Vaccines: A Prospective Study over 2 Years in Non-Immunocompromised Individuals," *Vaccines* 2023, 11, 12: 1835. doi: https://doi.org/10.3390/vaccines11121835
    - "In healthy adults who received two doses of BNT162b2 followed by a booster of mRNA-273 and the bivalent Omicron-adapted BNT162b2 over a 26-month period, the evolution of anti-RBD antibodies suggests modulation of the immune response through immune imprinting."

40. Faraone JN and SL Liu, "Immune imprinting as a barrier to effective COVID-19 vaccines," *Cell Rep Med*. 2023, 4, 11: 101291. doi: 10.1016/j.xcrm.2023.101291
    - "Imprinting from three doses of monovalent vaccine can be alleviated by BA.5 or BQ-lineage breakthrough infection but not by a bivalent booster."

41. Faraone JN et al., "Immune evasion and membrane fusion of SARS-CoV-2 XBB subvariants EG.5.1 and XBB.2.3," *Emerg Microbes Infect* 2023, 12, 2: 2270069. doi: https://doi.org/10.1080/22221751.2023.2270069
    - "Bivalent vaccination-induced antibodies neutralized ancestral D614G efficiently, but to a much less extent, two new EG.5.1 and XBB.2.3 variants. In fact, the enhanced neutralization escape of EG.5.1 appeared to be driven by its key defining mutation XBB.1.5-F456L."

42. Fujita S et al., "Impact of Imprinted Immunity Induced by mRNA Vaccination in an Experimental Animal Model," *J Infect Dis*. 2023, 228, 8: 1060-1065. doi: https://doi.org/10.1093/infdis/jiad230
    - "The concept of 'imprinted immunity' suggests that individuals vaccinated with ancestral virus-based vaccines may not develop effective immunity against newly emerging Omicron subvariants, such as BQ.1.1 and XBB.1. In this study, we investigated this possibility using hamsters. Although natural infection induced effective antiviral immunity, breakthrough infections in hamsters with BQ.1.1 and XBB.1 Omicron subvariants after receiving the 3-dose mRNA-lipid nanoparticle vaccine resulted in only faintly induced humoral immunity, supporting the possibility of imprinted immunity."

43. Gagne M et al., "mRNA-1273 or mRNA-Omicron boost in vaccinated macaques elicits similar B cell expansion, neutralizing responses, and protection from Omicron," *Cell* 2022, 185, 9: P1556-1571.E18. doi: 10.1016/j.cell.2022.03.038
    - "The observation that boosting with either mRNA-1273 or mRNA-Omicron resulted in the expansion of a similarly high frequency of cross-reactive B cells likely stems from the recall of prior immune memory after a related antigenic encounter. This principle has been termed original antigenic sin, imprinting, and back boosting… As we have now shown in two different NHP studies, boosting animals with either mRNA-Beta or mRNA-Omicron has not yet been shown to provide any significant advantage over mRNA-1273 in recalling high titer neutralizing antibodies across all variants tested in the short term and protecting from virus replication after challenge. These considerations may apply to the large numbers of individuals with prior immunity from vaccination or infection with current and previous variants."

44. Gao B et al., "Repeated vaccination of inactivated SARS-CoV-2 vaccine dampens neutralizing antibodies against Omicron variants in breakthrough infection," *Cell Res*. 2023, 33: 258-261. doi: https://doi.org/10.1038/s41422-023-00781-8
    - "Strikingly, we found that although nAb titers against SARS-CoV-2 were comparable between the 2-dose and the 3-dose groups of patients with BA.2 breakthrough infection, nAb titers against the Omicron BA.2, BA.4 and BA.5 variants were significantly lower in the 3-dose group. Our data suggest that repeated vaccination with inactivated virus vaccine back-boosts previous memory and dampens the immune response to a new antigenically related but distinct viral strain. Such vaccination-induced immune imprint could reflect the 'original antigenic sin' doctrine…"

45. Garcia-Beltran WF et al., "Multiple SARS-CoV-2 variants escape neutralization by vaccine-induced humoral immunity," *Cell* 2021, 184, 9: P2372-2383.E9. doi: 10.1016/j.cell.2021.03.013
    - "Strikingly, neutralization of all three South African B.1.351 strains was substantially decreased for both two-dose vaccines (v1: 34.5-fold for BNT162b2

and 27.7-fold for mRNA-1273; v2: 41.2-fold for BNT162b2 and 20.8-fold for mRNA-1273; v3: 42.4-fold for BNT162b2 and 19.2-fold for mRNA-1273; $p < 0.0001$ for all comparisons). These strains contain the same three RBD mutations as P.1 except for an asparagine versus threonine substitution at K417 (K417N) and several additional mutations in non-RBD regions… Notably, 36.7% (11/30) recipients of two-dose BNT162b2 and 42.9% (15/35) recipients of two-dose mRNA-1273 vaccines did not have detectable neutralization of at least one of the B.1.351 variants."

46. Germanio CD et al., "Spike and nucleocapsid antibody dynamics following SARS-CoV-2 infection and vaccination: Implications for sourcing COVID-19 convalescent plasma from routinely collected blood donations," *Transfusion* 2024, 64, 11: 2063-2074. doi: https://doi.org/10.1111/trf.18017
    - "In our study, seroreactivity for variant-specific bAb (MSD) and nAb (RVPN) assays to omicron variant S proteins was lower than the other variants in all the donor groups, including among VI cases during the omicron wave. Since all these donors were vaccinated during 2020–2021 when Moderna, Pfizer-BioNTech, or Janssen monovalent vaccines based on the ancestral virus S RNA/protein were administered, this phenomenon may be a result of 'immune imprinting'. Studies have shown that the first encounter with SARS-CoV-2 S protein, by either vaccination or infection, establishes immunologic memory to the corresponding S antigenic determinants, which impacts capacity for responses to SARS-CoV-2 variant S antigens during subsequent infections."

47. Gong X et al., "Repeated Omicron infection dampens immune imprinting from previous vaccination and induces broad neutralizing antibodies against Omicron sub-variants," *J. Infect.* 2024, 89, 2: 106208. doi: 10.1016/j.jinf.2024.106208
    - "Neutralizing potency against the corresponding infected variant is significantly hampered along with the doses of vaccination during first infection… Breakthrough infection with BA.1 predominantly recalls humoral immune memory against the WT SARS-CoV-2 spike protein and elicited non-neutralizing antibodies, and repeated vaccination of inactivated SARS-CoV-2 vaccine dampens neutralizing antibodies against Omicron variants in breakthrough infection."

48. Gruell H et al., "mRNA booster immunization elicits potent neutralizing serum activity against the SARS-CoV-2 Omicron variant," *Nat. Med.* 2022, 28: 477-480. doi: https://doi.org/10.1038/s41591-021-01676-0
    - "We report a near-complete lack of neutralizing activity against Omicron in polyclonal sera from individuals vaccinated with two doses of the BNT162b2 COVID-19 vaccine and from convalescent individuals, as well as resistance to different monoclonal antibodies in clinical use. However, mRNA booster immunizations in vaccinated and convalescent individuals resulted in a significant increase of serum neutralizing activity against Omicron."

49. Haralambieva IH et al., “Restricted Omicron-specific cross-variant memory B-cell immunity after a 3rd dose/booster of monovalent Wuhan-Hu-1-containing COVID-19 mRNA vaccine,” *Vaccine* 2024, 42, 4: 912-917. doi: 10.1016/j.vaccine.2024.01.032
    - “… we observed significantly lower frequencies of MBCs reactive to the receptor-binding domain/RBD, the N-terminal domain/NTD, and the S1 of Omicron/BA.1, compared to Wuhan and Delta, even after a 3rd vaccine dose/booster. Our study is a proof of concept that MBC cross-reactivity to variants with greater sequence divergence from the vaccine strain may be overestimated and suggests that these variants may exhibit immune escape with reduced recognition by circulating pre-existing MBCs upon infection.”

50. Hoffman M et al., “Effect of hybrid immunity and bivalent booster vaccination on omicron sublineage neutralization,” *Lancet Infect Dis.* 2023, 23, 1: 25-28. doi: 10.1016/S1473-3099(22)00792-7
    - “Collectively, our results show that the emerging omicron sublineages BQ.1.1 and particularly BA.2.75.2 efficiently evade neutralisation independent of the immunisation history. Although monovalent and bivalent vaccine boosters both induce high neutralising activity and increase neutralisation breadth, BA.2.75.2-specific and BQ.1.1-specific neutralisation activity remained relatively low. This finding is in keeping with the concept of immune imprinting by initial immunisation with vaccines targeting the ancestral SARS-CoV-2 B.1 lineage. Furthermore, the observation that neutralisation of BA.2.75.2pp and BQ.1.1pp was most efficient in the cohort that had a breakthrough infection during the BA.1 and BA.2 wave and later received a bivalent booster vaccination, but was still less efficient than neutralisation of B.1pp, implies that affinity maturation of antibodies and two-time stimulation with different omicron antigens might still not be sufficient to overcome immune imprinting.”

51. Hoffman M et al., “Profound neutralization evasion and augmented host cell entry are hallmarks of the fast-spreading SARS-CoV-2 lineage XBB.1.5,” *Cel Mol Immunol* 2023, 20, 419-422. doi: https://doi.org/10.1038/s41423-023-00988-0
    - “Finally, we investigated the neutralization sensitivity of XBB.1.5pp to antibodies induced by vaccination with or without breakthrough infection (BTI). For this, we utilized plasma from triple-vaccinated individuals that experienced a BTI during the BA.5 wave in Germany, and plasma from quadruple-vaccinated individuals that received a monovalent or bivalent mRNA-vaccine booster as fourth vaccination. All tested plasma showed high neutralizing activity against B.1pp, while neutralizing activity against BA.4-5pp and BQ.1.1pp was moderately (BA.4-5pp: 2.3–7.2-fold reduced compared to B.1pp) or strongly (BQ.1.1pp: 6.4–19.9-fold reduced compared to B.1pp) reduced, as expected. In line with published results, neutralizing activity against XBB.1pp was even further reduced compared to BA.4-5pp and BQ.1.1pp (XBB.1pp: 22.5–38.2-fold reduced

compared to B.1pp), and neutralizing activity against XBB.1.5pp was comparable to that of XBB.1pp (XBB.1pp: 23.7–35.9-fold reduced compared to B.1pp)."

52. Hornsby H et al., "Omicron infection following vaccination enhances a broad spectrum of immune responses dependent on infection history," *Nat. Commun.* 2023, 14: 56. doi: https://doi.org/10.1038/s41467-023-40592-4
    - "These 'previously-infected' individuals have higher spike-specific serum antibody and T-cell responses after each vaccine dose compared to infection-naive vaccinees. Hybrid immunity generated by post-vaccination infections may be quantitatively and qualitatively different from responses seen in individuals who experienced SARS-CoV-2 infection before receiving a vaccination course. This may be due to differences in the priming SARS-CoV-2 exposure or lower antigenic exposure during the attenuated disease course of omicron viruses; although it is difficult to tease apart the contributions of viral phenotype change from those of pre-existing immunity."

53. Jia T et al., "Expanded immune imprinting and neutralization spectrum by hybrid immunization following breakthrough infections with SARS-CoV-2 variants after three-dose vaccination," *J. Infect.* 2024, 89, 6: 106362. doi: 10.1016/j.jinf.2024.106362
    - "Following Omicron breakthrough infections, the levels of nAbs against WT and pre-Omicron VOCs were higher due to immune imprinting established by WT-based vaccination, in comparison to nAbs against Omicron variants."

54. Johnston TS et al., "Immunological imprinting shapes the specificity of human antibody responses against SARS-CoV-2 variants," *Immunity* 2024, 57, 4: P912-925.E4. doi: 10.1016/j.immuni.2024.02.017
    - "We determined the specificity and functionality of antibody and B cell responses following exposure to BA.5 and XBB variants in individuals who received ancestral SARS-CoV-2 mRNA vaccines. BA.5 exposures elicited antibody responses that targeted epitopes conserved between the BA.5 and ancestral spike. XBB exposures also elicited antibody responses that primarily targeted epitopes conserved between the XBB.1.5 and ancestral spike."

55. Ju B et al., "Antigenic sin of wild-type SARS-CoV-2 vaccine shapes poor cross-neutralization of BA.4/5/2.75 subvariants in BA.2 breakthrough infections," *Nat. Commun.* 2022, 13: 7120. https://doi.org/10.1038/s41467-022-34400-8
    - "Compared with the neutralizing antibody titers against BA.2, marked reductions are observed against BA.2.75 in both 2-dose and 3-dose vaccine groups. In addition, although BA.2 breakthrough infections induce a certain cross-neutralization capacity against later Omicron subvariants, the original antigenic sin phenomenon largely limits the improvement of variant-specific antibody response. These findings suggest that BA.2 breakthrough infections seem

unable to provide sufficient antibody protection against later subvariants such as BA.2.75 in the current immunization background with wild-type vaccines."

56. Ju B et al., "Striking antibody evasion of SARS-CoV-2 Omicron sub-lineages BQ.1.1, XBB.1 and CH.1.1," *Natl. Sci. Rev.* 2023, 10, 8: nwad148. doi: 10.1093/nsr/nwad148
    - "Overall, due to the original antigenic sin (or so-called immune imprinting) of the initial WT vaccination, these plasma samples from BA.4 or BA.5 breakthrough infected individuals acquired weaker neutralization against subsequent Omicron sub-lineages, such as BQ.1.1, XBB.1 and CH.1.1."

57. Kaku CI et al., "Evolution of antibody immunity following Omicron BA.1 breakthrough infection," *Nat. Commun.* 2023, 14: 2751. doi: https://doi.org/10.1038/s41467-023-38345-4
    - "While the acute B cell response following BA.1 breakthrough infection was dominated by vaccine-induced cross-reactive clones that exhibited preferential WT binding and neutralization, antibodies isolated from the same donors 5 to 6 months post-infection accumulated additional somatic mutations and displayed enhanced BA.1 recognition at the expense of WT binding… De novo BA.1-specific B cell responses only comprised a small fraction of the total RBD-directed response at both time points studied."

58. Kaku CI et al., "Recall of preexisting cross-reactive B cell memory after Omicron BA.1 breakthrough infection," *Sci. Immunol.* 2022, 7, 73. doi: 10.1126/sciimmunol.abq3511
    - "BA.1 breakthrough infection donors exhibited similar (within twofold) serum IgG binding titers to BA.1 and WT S and RBD. In contrast, uninfected/mRNA-vaccinated donors displayed a two- to fourfold and four- to ninefold reduced serum IgG binding to full-length BA.1 S and BA.1 RBD, respectively, relative to WT."

59. Kaplonek P et al., "Hybrid immunity expands the functional humoral footprint of both mRNA and vector-based SARS-CoV-2 vaccines," *Cell Rep Med.* 2023, 4, 5: 101048. doi: 10.1016/j.xcrm.2023.101048
    - "However, hybrid immunity shows a unique augmentation of S2-domain-specific functional immunity that was poorly induced for the vaccination only. These data highlight the importance of natural infection in breaking the immunodominance away from the evolutionarily unstable S1 domain and potentially affording enhanced cross-variant protection by targeting the more highly conserved S2 domain of SARS-CoV-2."

60. Kim W, "Germinal Center Response to mRNA Vaccination and Impact of Immunological Imprinting on Subsequent Vaccination," *Immune Netw.* 2024, 24, 4: e28. doi: https://doi.org/10.4110/in.2024.24.e28

- "The immunological imprinting induced by ancestral spike-based vaccination was also reflected in serological responses, which are outcomes of B cell responses to subsequent exposures. Individuals who have received two doses of primary vaccination and encountered omicron infection still exhibit low levels of omicron-specific Ab responses."

61. King SM et al., "First Impressions Matter: Immune Imprinting and Antibody Cross-Reactivity in Influenza and SARS-CoV-2," *Pathogens* 2023, 12, 2: 169. doi: https://doi.org/10.3390/pathogens12020169
    - "This issue may already be playing out with the SARS-CoV-2 bivalent vaccines produced by Pfizer-BNT and Moderna. The first bivalent boosters contained mRNA designed to elicit immunity against the original WA1/2020 SARS-CoV-2 strain, present in the previous monovalent boosters, as well as the then newly emergent BA.1 strain. The results of these were disappointing, with only modest increases in anti-BA.1 neutralizing antibodies. As BA.1 was no longer circulating in the United States, the United States Food and Drug Administration approved new bivalent boosters directed against the now dominant circulating variants BA.4 and BA.5. Results emerging from very recent studies suggest limited boosts in antibody levels with modest protection against target strains, with minimal increases in BA.4 and BA.5 protection from the WA1/2020 and BA.1 boosters. These results are thought to be due to immune imprinting from multiple rounds of the prior WU1/2020 monovalent vaccine series."

62. Koutsakos M and AH Ellebedy, "Immunological imprinting: Understanding COVID-19," *Immunity* 2023, 56, 5: 909-913. doi: 10.1016/j.immuni.2023.04.012
    - "… individuals primed with Hu-1-like spike and then infected with a variant like Delta or Omicron maintain higher antibodies against the Hu-1-like antigen than the infecting variant (antigenic seniority)."

63. Kurhade C et al., "Low neutralization of SARS-CoV-2 Omicron BA.2.75.2, BQ.1.1 and XBB.1 by parental mRNA vaccine or a BA.5 bivalent booster," *Nat. Med.* 2023, 29: 344-347. doi: https://doi.org/10.1038/s41591-022-02162-x
    - "The results showed that a BA.5 bivalent booster elicited a high neutralizing titer against BA.4/5 measured at 14–32 days after boost; however, the BA.5 bivalent booster did not produce robust neutralization against the newly emerged BA.2.75.2, BQ.1.1 or XBB.1. Previous infection substantially enhanced the magnitude and breadth of BA.5 bivalent booster-elicited neutralization."

64. Lasrado N et al., "Waning immunity and IgG4 responses following bivalent mRNA boosting," *Sci. Adv.* 2024, 10, 8. doi: 10.1126/sciadv.adj9945
    - "Here, we show limited durability of neutralizing antibody (NAb) responses against XBB variants and isotype switching to immunoglobulin G4 (IgG4) responses following bivalent mRNA boosting. Bivalent mRNA boosting elicited

modest XBB.1-, XBB.1.5-, and XBB.1.16-specific NAbs that waned rapidly within 3 months. In contrast, bivalent mRNA boosting induced more robust and sustained NAbs against the ancestral WA1/2020 strain, suggesting immune imprinting."

65. Lee WS et al., "Durable reprogramming of neutralizing antibody responses following Omicron breakthrough infection," *Sci. Adv.* 2023, 9, 29. doi: 10.1126/sciadv.adg5301
    - "We show that only cross-reactive memory B cells were expanded by breakthrough infection, and the resulting antibody response was dominated by antibodies cross-reactive with ancestral spike, indicating that limited de novo responses were generated against neo-epitopes within BA.1 or BA.2 spike. In line with recent studies, our results are suggestive of immune imprinting, with no evident increase in BA.1 or BA.2 monospecific B cells even up to 4 to 7 months after infection… While the isolation of receptor binding domain (RBD)–specific monoclonal antibodies (mAbs) specific for the BA.1 RBD that do not cross-react with ancestral RBD has been reported, these comprised only a small fraction (median, 4%) of the response to RBD, confirming that neo-epitopes are poorly recognized during breakthrough infection. Immune imprinting is not constrained to breakthrough infections, as monovalent Omicron BA.1 or bivalent Beta/Delta mRNA vaccines also predominantly boost preexisting cross-reactive responses."

66. Li Y et al., "Repeated Omicron Infections Overcome T Cell Immune Imprinting to Original SARS-CoV-2," *J. Med. Virol.* 2025, 97, 2: e70264. doi: 10.1002/jmv.70264
    - "Therefore, similar to humoral immunity vaccination with the original SARS-CoV-2 strain-derived vaccines induces T cell immune imprinting when undergoing Omicron subvariants breakthrough infection."

67. Liang CY et al., "Imprinting of serum neutralizing antibodies by Wuhan-1 mRNA vaccines," *Nature* 2024, 630: 950-960. doi: 10.1038/s41586-024-07539-1
    - "Because serum neutralizing responses against Omicron strains and other sabercoronaviruses were abrogated after pre-clearing with Wuhan-1 spike protein, antibodies induced by XBB.1.5 boosting in humans focus on conserved epitopes targeted by the antecedent mRNA-1273 primary series."

68. Liu S et al., "Sera from breakthrough infections with SARS-CoV-2 BA.5 or BF.7 showed lower neutralization activity against XBB.1.5 and CH.1.1," *Emerg Microb Infect* 2023, 12, 2: 2225638. doi: https://doi.org/10.1080/22221751.2023.2225638
    - "The level of neutralizing antibody against the wild strain is the highest which may be attributed to the imprinted original immune responses against the prototype vaccine strain. "

69. Madhi SA et al., "Efficacy of the ChAdOx1 nCoV-19 Covid-19 Vaccine against the B.1.351 Variant," *N Engl J Med* 2021, 384, 20: 1885-1898. doi: 10.1056/NEJMoa2102214
- "Six of 13 vaccine recipients (46%) without evidence of previous SARS-CoV-2 infection showed no neutralization activity against an RBD triple-mutant pseudovirus (containing K417N, E484K, and N501Y variants), and 11 of the 13 (85%) had no neutralization activity against B.1.351 pseudovirus. Geometric mean titers dropped from 297 against the original virus to 85 against the RBD-only mutant and 74 against the B.1.351 variant."

70. Maltseva M et al., "Immune imprinting: The persisting influence of the first antigenic encounter with rapidly evolving viruses," *Hum Vaccin Immunother* 2024, 20, 1: 2384192. doi: https://doi.org/10.1080/21645515.2024.2384192
- "Breakthrough infections with the Alpha or Delta variants resulted in a greater increase in antibody titers against the ancestral strain compared to the VOC strain in individuals vaccinated with three doses of ancestral mRNA-LNP, highlighting the effects of immune imprinting... Efforts to enhance vaccine efficacy by updating vaccines have led to improved VOC neutralization. However, individuals previously vaccinated with the ancestral mRNA vaccines showed dominant recall antibody responses following monovalent Beta or Delta boosters, or bivalent ancestral and Beta/Delta boosters... Omicron breakthrough infections pre-dominantly promoted recall responses, leading to reduced neutralization of Omicron variants."

71. Marcotte H et al., "Limited cross-variant neutralization after primary Omicron infection: consideration for a variant-containing booster," *Signal Transduct Target Ther* 2022, 7: 294. doi: https://doi.org/10.1038/s41392-022-01146-0
- "The plasma of individuals receiving three doses of mRNA vaccines or a combination of inactivated and mRNA vaccines were shown to neutralize BA.1 but with titers 32-fold lower compared to the wild-type strain. Furthermore, two recent studies showed that sera from individuals who received three doses of vaccines (Pfizer, AstraZeneca, or CoronaVac) and from vaccinated individuals with BA.1 breakthrough infection have a reduced ability to neutralize BA.4, BA.5, and BA.2.12.1 compared with BA.1 and BA.2 due to RBD mutations involving L452R and F486V (BA.4/5) and L452Q (BA.2.12.1). They found that BA.1 Omicron breakthrough infections mainly reactivate WT-induced memory B cells, reducing the diversity of antibodies, and possibly facilitating the emergence of new mutants."

72. Marzi R et al., "Maturation of SARS-CoV-2 Spike-specific memory B cells drives resilience to viral escape," *iScience* 2023, 26, 1: 105726. doi: 10.1016/j.isci.2022.105726

- "Whereas MBCs of infected individuals targeted both prefusion and postfusion Spike (S), most vaccine-elicited MBCs were specific for prefusion S, consistent with the use of prefusion-stabilized S in mRNA vaccines."

73. Medits I et al., "Different Neutralization Profiles After Primary SARS-CoV-2 Omicron BA.1 and BA.2 Infections," *Front. Immunol.* 2022, 13 (Sec. Vaccines and Molecular Therapeutics). doi: https://doi.org/10.3389/fimmu.2022.946318
    - "Serum neutralization of Omicron BA.1 and BA.2 variants was detectable after three-dose mRNA vaccinations, but with reduced titers. Vaccination-breakthrough infections with either Omicron BA.1 or BA.2, however, generated equal cross-neutralizing antibody levels against all SARS-CoV-2 variants tested."

74. Milne G et al., "Does infection with or vaccination against SARS-CoV-2 lead to lasting immunity?" *Lancet Respir Med* 2021, 9, 12: 1450-1466. doi: 10.1016/S2213-2600(21)00407-0
    - "Upon natural infection, the T-cell-mediated response appears to be targeted across a larger variety of epitopes than the humoral response, and hence might be more durable to genetic changes in key immunogenic viral epitopes. Nonetheless, the neutralising antibody response also comprises a key aspect of protection against reinfection... Compared with the immune response to natural infection, vaccination elicits a response of greater magnitude and higher specificity, largely focused on the RBD. Increasing evidence of reduced neutralisation and vaccine effectiveness against emerging variants, alongside emerging data on breakthrough infections, suggests that vaccines will need to be updated in the short-to-medium term."

75. Montiel-Ruiz M et al., "Immune imprinting and antibody profiles to SARS-CoV-2 in urban and rural Ghana," *Cell* 2025, 28, 5: 112511. doi: 10.1016/j.isci.2025.112511
    - "Vaccinated and urban individuals exhibited significantly greater Spike-pseudotyped virus neutralization than nonvaccinated and rural individuals. Notably, plasma antibodies preferentially bound Wuhan-Hu-1 over Omicron Spike variants. Our findings indicate significant prior and ongoing SARS-CoV-2 transmission as well as immunological imprinting by Wuhan-Hu-1-like SARS-CoV-2 in Ghana."

76. Moreno A et al., "Divergence of variant antibodies following SARS-CoV-2 booster vaccines in myeloma and impact of hybrid immunity," *npj Vaccines* 2024, 9: 201. doi: https://doi.org/10.1038/s41541-024-00999-6
    - "It has been suggested that immune imprinting provided by prior infection or SARS-CoV-2 vaccination negatively impacts vaccine immunogenicity of booster immunizations. Consistent with this, we observed preferential boosting of nAb against the ancestral WA1 strain following booster immunization."

77. Mueksche F et al., "Increased memory B cell potency and breadth after a SARS-CoV-2 mRNA boost," *Nature* 2022, 607: 128-134. doi: 10.1038/s41586-022-04778-y
    - "Consistent with prior reports, the third vaccine dose significantly boosted geometric mean NT50 values by 16-fold, 12-fold and 37-fold for the Beta, Delta and Omicron BA.1 variants, respectively. The level of activity against the Beta and Delta variants was not significantly different from that against Wuhan-Hu-1, whereas the activity against Omicron BA.1 was 16-fold lower than that against Wuhan-Hu-1 (P = 0.58, P = 0.24 and P = 0.0013, respectively)... given the correlation between neutralizing antibody levels and protection from Wuhan-Hu-1 infection, the reduced activity against Omicron BA.1 in recipients of a third dose of vaccine probably explains why vaccinated individuals remained particularly susceptible to infection by this variant."

78. Muik A et al., "Immunity against conserved epitopes dominates after two consecutive exposures to SARS-CoV-2 Omicron BA.1," *Cell Rep.* 2024, 43, 8: 114567. doi: 10.1016/j.celrep.2024.114567
    - "Upon exposure to the highly altered Omicron spike glycoprotein, pre-immunized individuals predominantly mount recall responses of Wuhan-Hu-1 (wild-type)-imprinted memory B (B_{MEM}) cells mostly targeting conserved non-neutralizing epitopes, leading to diminished Omicron neutralization. We investigated the impact of imprinting in individuals double/triple vaccinated with a wild-type-strain-based mRNA vaccine who, thereafter, had two consecutive exposures to Omicron BA.1 spike (breakthrough infection followed by BA.1-adapted vaccine). We found that depletion of conserved epitope-recognizing antibodies using a wild-type spike bait results in strongly diminished BA.1 neutralization. Furthermore, spike-specific B_{MEM} cells recognizing conserved epitopes are much more prevalent than BA.1-specific B_{MEM} cells. Our observations suggest that imprinted B_{MEM} cell recall responses limit the induction of strain-specific responses even after two consecutive BA.1 spike exposures. Vaccine adaptation strategies need to consider that prior SARS-CoV-2 infections and vaccinations may cause persistent immune imprinting."

79. Muik A et al., "Progressive loss of conserved spike protein neutralizing antibody sites in Omicron sublineages is balanced by preserved T cell immunity," *Cell Rep.* 2023, 42, 8: 112888. doi: 10.1016/j.celrep.2023.112888
    - "We report that Omicron BA.4/BA.5 breakthrough infection of individuals immunized with SARS-CoV-2 wild-type-strain-based mRNA vaccines results in a boost of Omicron BA.4.6, BF.7, BQ.1.1, and BA.2.75 neutralization but does not efficiently boost BA.2.75.2, XBB, or XBB.1.5 neutralization. In silico analyses showed that the Omicron spike glycoprotein lost most neutralizing B cell epitopes, especially in sublineages BA.2.75.2, XBB, and XBB.1.5."

80. Mykytyn AZ et al., “Antigenic mapping of emerging SARS-CoV-2 omicron variants BM.1.1.1, BQ.1.1, and XBB.1,” *Lancet Microbe* 2023, 4, 5: E294-295. doi: 10.1016/S2666-5247(22)00384-6
- “Our data reveal substantial cross-neutralisation of BA.5 antiserum samples against BQ.1.1 but little cross-neutralisation against XBB.1 and BM.1.1.1. Despite the antigenic similarities between BA.5 and BQ.1.1, thus far there is little evidence for increased neutralisation of BQ.1.1 by BA.5 bivalent vaccines, potentially due to immunological imprinting.”

81. Norton NJ et al., “Characteristics of Vaccine- and Infection-Induced Systemic IgA Anti-SARS-CoV-2 Spike Responses,” *Vaccines* 2023, 11, 9: 1462. doi: https://doi.org/10.3390/vaccines11091462
- “As with circulating IgG responses, vaccination with an ancestral SARS-CoV-2 S antigen imposed immunological imprinting on IgA responses with preferred recognition of ancestral SARS-CoV-2 S protein over Omicron SARS-CoV-2 S protein persisting following Omicron breakthrough infection.”

82. Paciello I et al., “Antigenic sin and multiple breakthrough infections drive converging evolution of COVID-19 neutralizing responses,” *Cell Rep.* 2024, 43, 9: 114645. doi: 10.1016/j.celrep.2024.114645
- “In line with recent studies, our data revealed that while the initial antibody response was different in vaccinated or infected people, breakthrough infections by a distantly related virus such as Omicron induced the expansion of previously unseen germ lines and, most important, rescued the B cell primed by the original antigenic sin.”

83. Pape KA et al., “High-affinity memory B cells induced by SARS-CoV-2 infection produce more plasmablasts and atypical memory B cells than those primed by mRNA vaccines,” *Cell Rep.* 2021, 37, 2: 109823. doi: 10.1016/j.celrep.2021.109823
- “However, infection-induced primary MBCs have better antigen-binding capacity and generate more plasmablasts and secondary MBCs of the classical and atypical subsets than do vaccine-induced primary MBCs. Our results suggest that infection-induced primary MBCs have undergone more affinity maturation than vaccine-induced primary MBCs and produce more robust secondary responses.”

84. Park YJ et al., “Imprinted antibody responses against SARS-CoV-2 Omicron sublineages,” *Science* 2022, 387, 6620: 619-627. doi: 10.1126/science.adc9127
- “Park et al. found that either a vaccination booster or a breakthrough infection elicits neutralization activity against the Omicron variants, but only a breakthrough infection induces an antibody response in the nasal mucosa, which might give better protection against transmission.”

85. Paula NM et al., "Symptomatology and IgG Levels before and after SARS-CoV-2 Omicron Breakthrough Infections in Vaccinated Individuals," *Vaccines* 2024, 12, 10: 1149. doi: https://doi.org/10.3390/vaccines12101149
    - "The anti-N and anti-S IgG titers followed the expected pattern, with anti-S titers raised after a vaccination event, whereas both anti-S and anti-N levels increased after an infection event… [P]reexisting anti-S IgG levels correlate poorly with symptomatology during infections caused by Omicron variants. There was also no correlation between the COVID-19 symptoms and anti-S IgG titers after the infections. Quite surprisingly, COVID-19 symptoms correlated with anti-N IgG levels detected after the infection (Spearman r −0.55, p = 0.03). Thus, individuals with lower anti-N IgG levels after infection were the ones who experienced the most intense COVID-19 symptoms. This observation suggests that human anti-N IgG antibodies may play an important role in resolution of the disease."

86. Pepkowitz SH et al., "Prior vaccination has changed the composition of the COVID-19 convalescent plasma inventory," *Transfusion* 2022, 62, 10: 2153-2154. doi: https://doi.org/10.1111/trf.17089
    - "The lower IgG anti-nucleocapsid antibody, lower IgM anti-spike antibody, and higher IgG anti- RBD antibody present in post-breakthrough COVID-19 CCP are likely due to extensive affinity maturation and a decreased presence of IgM memory-cells post-vaccination and to a component of 'original antigenic sin' in which the immune system is focused on producing those anti-spike antibodies previously developed in response to prior vaccination, while relatively ignoring additional newly presented viral immunogens."

87. Pérez-Alós L et al., "Previous immunity shapes immune responses to SARS-CoV-2 booster vaccination and Omicron breakthrough infection risk," *Nat. Commun.* 2023, 14: 5624. doi: https://doi.org/10.1038/s41467-023-41342-2
    - "Our study shows that both humoral and cellular responses following vaccination were generally higher after SARS-CoV-2 infection compared to infection-naive. Notably, viral exposure before vaccination was crucial to achieving a robust IgA response. Individuals with lower IgG, IgA, and neutralizing antibody responses postvaccination had a significantly higher risk of reinfection and future Omicron infections."

88. Petras M and IV Lesna, "SARS-CoV-2 vaccination in the context of original antigenic sin," *Hum Vaccin Immunother*. 2022, 18, 1: 1949953. doi: https://doi.org/10.1080/21645515.2021.1949953
    - "Given the above, it is most appropriate – when scheduling booster vaccination or even re-vaccination – to carefully monitor the seroresponse of those vaccinated since a reduced immune response to new SARS-CoV-2 variants at the expense of an enhanced response to original variants could in fact result in

inadequate protection of those vaccinated against the current virus variants. Hence, the extremely high levels of specific anti-SARS-CoV-2 antibodies achieved by vaccination, which – as indicated by the most recent data – tend to persist for months post-vaccination, should serve as a warning sign. In addition, it is not yet obvious if the robust vaccination-induced response of T cells can compensate for original antigenic sin to afford a sufficient level of protection against the new SARS-CoV-2 variants."

89. Piubelli C et al., "Subjects who developed SARS-CoV-2 specific IgM after vaccination show a longer humoral immunity and a lower frequency of infection," *eBioMedicine* 2023, 89: 104471. doi: 10.1016/j.ebiom.2023.104471
    - "Taken together these data, including ours, draw attention on the so-called 'original immunological sin', whereby an immune response conditioned by prior immunity against other hCoVs could result in a non-specific SARS-CoV-2 humoral immunity after vaccination, impairing the immune protection."

90. Planas D et al., "Distinct evolution of SARS-CoV-2 Omicron XBB and BA.2.86/JN.1 lineages combining increased fitness and antibody evasion," *Nat. Commun.* 2024, 15: 2254. doi: https://doi.org/10.1038/s41467-024-46490-7
    - "Neutralizing antibody (NAb) responses from vaccinees and BA.1/BA.2-infected individuals are markedly lower compared to BA.1, without major differences between variants."

91. Powers JP et al., "Divergent pathogenetic outcomes in BALB/c mice following Omicron subvariant infection," *Virus Res.* 2024, 341: 199319. doi: https://doi.org/10.1016/j.virusres.2024.199319
    - "Using a live-virus nLuc neutralization assays and sera from mice vaccinated with an alum adjuvanted Wuhan S2P protein vaccine, we observed a significance decrease in neutralizing antibody titer against the three Omicron nLuc viruses as compared to SARS-CoV-2 D614G. Antibodies retained the most activity against BQ.1.1 nLuc, reflecting the reduced numbers of amino acid changes as compared with XBB.1 and XBB.1.5. Further reductions were observed with XBB.1 and XBB.1.5 with only 3 and 2 serum samples neutralizing above the limit of detection, respectively."

92. Pušnik J et al., "Effect of XBB.1.5-adapted booster vaccination on the imprinting of SARS-CoV-2 immunity," *npj Vaccines* 2024, 9: 231. doi: 10.1038/s41541-024-01023-7
    - "Taken together our data support the previously observed imprinting by the original wild-type-based SARS-CoV-2 vaccines but also suggest that vaccination with XBB.1.5-adapted vaccine might help to withdraw antigenic imprinting in some individuals."

93. Pušnik J et al., “Vaccination impairs de novo immune response to omicron breakthrough infection, a precondition for the original antigenic sin,” *Nat. Commun.* 2024, 15: 3102. doi: https://doi.org/10.1038/s41467-024-47451-w
- “Our data demonstrate a robust humoral response in thrice-vaccinated individuals following omicron breakthrough which is a recall of vaccine-induced memory. The humoral and memory B cell responses against the altered regions of the omicron surface proteins are impaired.”

94. Qu P et al., “Enhanced neutralization resistance of SARS-CoV-2 Omicron subvariants BQ.1, BQ.1.1, BA.4.6, BF.7, and BA.2.75.2,” *Cell Host Microb.* 2023, 31, 1: P9-17.E3. doi: 10.1016/j.chom.2022.11.012
- “We also found that BA.4/5-wave patient sera exhibited weaker neutralization of BA.4/5 than of BA.2, which could be related to prior exposure to SARS-CoV-2 variant antigen biasing patient neutralizing antibody response to BA.4/5 infection.”

95. Quandt J et al., “Omicron BA.1 breakthrough infection drives cross-variant neutralization and memory B cell formation against conserved epitopes,” *Sci. Immunol.* 2022, 7, 75. doi: 10.1126/sciimmunol.abq2427
- “We report that Omicron BA.1 breakthrough infection in BNT162b2-vaccinated individuals resulted in strong neutralizing activity against Omicron BA.1, BA.2, and previous SARS-CoV-2 VOCs but not against the Omicron sublineages BA.4 and BA.5. BA.1 breakthrough infection induced a robust recall response, primarily expanding memory B (BMEM) cells against epitopes shared broadly among variants, rather than inducing BA.1-specific B cells… our data also suggest that the immunity in the early stage of Omicron BA.1 infection in vaccinated individuals is based on recognition of conserved epitopes and is narrowly focused on a small number of neutralizing sites that are not altered in Omicron BA.1 and BA.2. Such a narrow immune response bears a high risk that those few epitopes may be lost by acquisition of further alterations in the course of the ongoing evolution of Omicron and may result in immune escape, as is being experienced with sublineages BA.2.12.1, BA.4, and BA.5.”

96. Regev-Yochay G et al., “Efficacy of a Fourth Dose of Covid-19 mRNA Vaccine against Omicron,” *N Eng J Med* 2022, 386, 14: 1377-1380. doi: 10.1056/NEJMc2202542
- “Furthermore, we observed low vaccine efficacy against infections in health care workers, as well as relatively high viral loads suggesting that those who were infected were infectious. Thus, a fourth vaccination of healthy young health care workers may have only marginal benefits.”

97. Reynolds CJ et al., “Heterologous infection and vaccination shapes immunity against SARS-CoV-2 variants,” *Science* 2021, 375, 6577: 183-192. doi: 10.1126/science.abm0811

- "Vaccine responses after infection were found to be less effective if the infection involved heterologous spike from a variant virus. Unfortunately, the N501Y spike mutation, found in many variants, seems to induce the regulatory T cell transcription factor FOXP3, indicating that the virus could subvert effective T cell function. Changes to antibody binding between variants also means that serology data using the Wuhan Hu-1 S1 receptor-binding domain sequence may not be a reliable measure of protection."

98. Reynolds CJ et al., "Immune boosting by B.1.1.529 (Omicron) depends on previous SARS-CoV-2 exposure," *Science* 2022, 377, 6603. doi: 10.1126/science.abq1841
    - "…imprinted patterns such as the specific combination of vaccination with infection during the first ancestral Wuhan Hu-1 wave followed by the B.1.1.529 (Omicron) wave require an additional term: 'hybrid immune damping'… Notably, although B1.1.529 (Omicron) infection in triple-vaccinated previously uninfected individuals could indeed boost antibody, T cell, and MBC responses against other VOCs, responses to Omicron itself were reduced. This relatively poor immunogenicity against itself may help to explain why frequent B.1.1.529 (Omicron) reinfections with short time intervals between infections are proving a novel feature in this wave. It also concurs with observations that mRNA vaccination carrying the B.1.1.529 (Omicron) spike sequence (Omicron third-dose after ancestral sequence prime and boost) offers no protective advantage."

99. Reynolds CJ et al., "Prior SARS-CoV-2 infection rescues B and T cell responses to variants after first vaccine dose," *Science* 2021, 372, 6549: 1418-1423. doi: 10.1126/science.abh1282
    - "Genotyping indicated that a genetic component underlies heterogeneity in immune responses to vaccine and to natural infection. After vaccination, naïve individuals developed antibody responses similar to those seen in naturally infected persons, but T cell responses were more limited and sometimes absent."

100. Rodda LB et al., "Imprinted SARS-CoV-2-specific memory lymphocytes define hybrid immunity," *Cell* 2022, 185, 9: P1588-1601.E14. doi: 10.1016/j.cell.2022.03.018
    - "SARS-CoV-2 infection prior to vaccination elicits a robust CD4+ T Th1/IFN-ɣ response. Infection-induced Th1/IFN-ɣ signature is not reproduced by three vaccinations."

101. Röltgen K et al., "Immune imprinting, breadth of variant recognition, and germinal center response in human SARS-CoV-2 infection and vaccination," *Cell* 2022, 185, 6: P1025-1040.E14. doi: 10.1016/j.cell.2022.01.018

- "Viral variant infection elicits variant-specific antibodies, but prior mRNA vaccination imprints serological responses toward Wuhan-Hu-1 rather than variant antigens."

102. Rössler A et al., "Neutralization Profile after Recovery from SARS-CoV-2 Omicron Infection," *N Engl J Med* 2022, 386, 18: 1764-1766. doi: 10.1056/NEJMc2201607
    - "We found that neutralizing antibody titers against all the variants were high among vaccinated persons after omicron BA.1 breakthrough infection and among vaccinated or unvaccinated persons who had had previous infection with the wild-type, alpha, or delta variant before infection with the omicron BA.1 variant. Mean neutralizing antibody titers against the omicron BA.1 variant were lower than those against the other variants among previously vaccinated persons but were similar to those against the other variants among unvaccinated persons who had had infection with the wild-type, alpha, or delta variant before infection with the omicron BA.1 variant."

103. Selva KJ et al., "Preexisting immunity restricts mucosal antibody recognition of SARS-CoV-2 and Fc profiles during breakthrough infections," *JCI Insight* 2023, 8, 18: e172470. doi: 10.1172/jci.insight.172470
    - "IgG and FcγR engagement, but not IgA, responses to breakthrough COVID-19 variants were dampened and narrowed by increased preexisting vaccine-induced immunity against the ancestral strain."

104. Servellita V et al., "Neutralizing immunity in vaccine breakthrough infections from the SARS-CoV-2 Omicron and Delta variants," *Cell* 2022, 185, 9: P1539-1548.E5. doi: 10.1016/j.cell.2022.03.019
    - "Among immunocompetent, unboosted patients, Delta breakthrough infections induced 10.8-fold higher titers against WT compared with Omicron ($p = 0.037$)... Following either Delta or Omicron breakthrough infection, limited variant-specific cross-neutralizing immunity was observed. These results suggest that Omicron breakthrough infections are less immunogenic than Delta, thus providing reduced protection against reinfection or infection from future variants."

105. Shen X et al., "SARS-CoV-2 variant B.1.1.7 is susceptible to neutralizing antibodies elicited by ancestral spike vaccines," *Cell Host Microbe* 2021, 29, 4: P529-539.E3. doi: 10.1016/j.chom.2021.03.00
    - "The B.1.1.7 variant was neutralized by all vaccine sera, although with modestly diminished susceptibility compared to the D614G variant. A modest decrease in neutralization susceptibility was also seen with convalescent sera, although not to the same extent seen with vaccine sera."

106. Shrestha NK et al., “Effectiveness of the 2023–2024 Formulation of the COVID-19 Messenger RNA Vaccine,” *Clin. Infect. Dis.* 2024, 79, 2: 405-411. doi: https://doi.org/10.1093/cid/ciae132
    - “Risk of COVID-19 was lower among those previously infected with an XBB or more recent lineage and increased with the number of vaccine doses previously received.”

107. Smith CP et al., “The Trajectory of Antibody Responses One Year Following SARS-CoV-2 Infection among Indigenous Individuals in the Southwest United States,” *Viruses* 2024, 16, 10: 1573. doi: https://doi.org/10.3390/v16101573
    - “The peak antibody concentrations and resulting time to seroreversion were the highest for those with a prior history of vaccination and infection and the lowest for those with a prior history of vaccination but not infection. This is consistent with prior findings showing a blunted anti-N response to infection in people who have been vaccinated and a faster time to seroreversion for anti-N compared to anti-S antibodies, likely resulting from vaccine-induced immune imprinting against the S protein, leading to decreased dissemination of the virus and partial inhibition of the immune response to the N protein.”

108. Sokol A et al., “SARS-CoV-2 Omicron BA.1 breakthrough infection drives late remodeling of the memory B cell repertoire in vaccinated individuals,” *Immunity* 2023, 56, 9: P2137-2151.E7. doi: 10.1016/j.immuni.2023.07.007
    - “Here, we show that this imprinting was not limited to the early extrafollicular response but persisted over time, with very few BA.1-restricted naive B cell clones recruited in de novo GCs. High-affinity serum antibodies elicited during the primary response have recently been demonstrated to reduce the recruitment of naive B cells to GCs during secondary responses.”

109. Solforosi L et al., “Booster with Ad26.COV2.S or Omicron-adapted vaccine enhanced immunity and efficacy against SARS-CoV-2 Omicron in macaques,” *Nat. Commun.* 2023, 14, 1944. doi: https://doi.org/10.1038/s41467-023-37715-2
    - “Based on the observation that the booster immunization mostly recalled cross-reactive S WA1/2020 and S Omicron BA.1 B cells, we speculate that de novo induction of neutralizing antibodies targeting key new epitopes in Omicron S is impaired in boosted animals, at least shortly after vaccination, likely mediated by an imprinting effect of the primary Ad26.COV2.S vaccination.”

110. Stamatos L et al., “mRNA vaccination boosts cross-variant neutralizing antibodies elicited by SARS-CoV-2 infection,” *Science* 2021, 372, 6549: 1413-1418. doi: 10.1126/science.abg9175
    - “Vaccination elevated postinfection serum-neutralizing capacity approximately 1000-fold against Wuhan-Hu-1 and other strains, and serum neutralization

against the variant B.1.351 was enhanced. Although responses were relatively muted against the variant, they still showed characteristic memory responses."

111. Stankov MV et al., "Humoral and cellular immune responses following BNT162b2 XBB.1.5 vaccination," *Lancet Infect Dis.* 2024, 24, 1: E1-E3. doi: 10.1016/S1473-3099(23)00690-4
    - "... these data suggest cross-reactive MBC dominance even after multiple exposures to omicron spikes and underscore persistent immune imprinting."

112. Szekely J et al., "Breakthrough SARS-CoV-2 Omicron Variant in Individuals Primed with Heterologous Vaccines Enhances Inhibition Performance of Neutralizing Antibody to BA.2 Parental Lineage," *Vaccines* 2023, 11, 7: 1230. doi: https://doi.org/10.3390/vaccines11071230
    - "Negative results for neutralizing antibody against both Omicron variants were observed in persons with antibody levels to wild-type ranging from 12.78–4679.94 BAU/mL. This observation indicates that the level of IgG antibody to wild-type does not correlate with the presence of effective neutralizing antibodies to Omicron variants."

113. Tan CW et al., "Comparative neutralisation profile of SARS-CoV-2 omicron subvariants BA.2.75 and BA.5," *Lancet Microbe* 2022, 3, 13: E898. doi: 10.1016/S2666-5247(22)00220-8
    - "Despite an overall improvement in neutralising antibody titres following mRNA booster vaccination or an omicron breakthrough infection, there was a significant loss of neutralising antibody potency against omicron subvariants compared with ancestral SARS-CoV-2, with BA.5 being the most effective subvariant at escaping neutralising antibodies. Relative to geometric mean pVNT50s against BA.2, titres against BA.2.75 were 1·1 to 1·4 times lower and those against BA.5 were 2·2 to 3·8 times lower in individuals who had received three doses of mRNA vaccine or recovered from an omicron breakthrough infection."

114. Tan CW et al., "Distinctive serotypes of SARS-related coronaviruses defined by convalescent sera from unvaccinated individuals," *hLife* 2023, 1, 1: 26-34. doi: https://doi.org/10.1016/j.hlife.2023.07.002
    - "Unlike viruses such as measles and polioviruses that have little to no change in their sensitivity to vaccine-induced immunity for decades, the high structure plasticity of the coronavirus spike protein and the vast diversity of animal coronaviruses make the complete eradication an impossible task with current vaccines. Antigenic maps of vaccinated sera showed a greater extent of antigenic differences between the circulating Omicron variants and SARS-CoV-2, implying that pre-existing SARS-CoV-2 immunity is insufficient to prevent current and future infections. In addition, because of the original antigenic

sin, breakthrough infections do not increase NAb epitope diversity but instead further promote the RBD to evolve convergently."

115. Tarke A et al., "SARS-CoV-2 breakthrough infections enhance T cell response magnitude, breadth, and epitope repertoire," *Cell Rep Med.* 2024, 5, 6: 101583. doi: 10.1016/j.xcrm.2024.101583
    - "In conclusion, BMem responses after a variant BTI showed considerable imprinting by the ancestral sequence in the vaccines, consistent with other reports."

116. Tavasolian F et al., "HLA, Immune Response, and Susceptibility to COVID-19," *Front. Immunol.* 2021, 11 (Sec. Viral Immunology). doi: 10.3389/fimmu.2020.601886
    - "Thus, an inadequate immune response to the mutated virus due to the OAS may generate a significant number of sub-neutralizing cross-reactive antibodies that enhance inflammation and may paradoxically promote virus entry into host cells. The intracellular presence of the pathogen activates a pyroptosis mechanism with the subsequent release of danger-associated molecular patterns (DAMPs) to trigger additional inflammatory cells, which in response release a great number of cytokines; which may be the basis of the 'cytokine storm' identified in severe cases of COVID-19."

117. Tian S et al., "Neutralization against emerging Omicron subvariants after SARS-CoV-2 reinfection," *J. Infect.* 2023, 87, 6: 598-601. doi: 10.1016/j.jinf.2023.09.013
    - "XBB subvariants escape the immunity induced by primary infection or reinfection. SARS-CoV-2 reinfection can alleviate WT-vaccination-induced immune imprinting. G339H, G446S, N460K, and F486S/P mutations are essential for immune escape."

118. Torresi J and MA Edeling, "Immune imprinting of SARS-CoV-2 responses: changing first immune impressions," *mSphere* 2024. doi: 10.1128/msphere.00758-23
    - "Although infection with viral variants produces variant-specific antibody responses, prior vaccination with WuH-1 S containing COVID-19 mRNA vaccines has been shown to imprint antibody responses toward the ancestral virus rather than to variant antigens. So prior mRNA vaccination with a WuH-1 vaccine followed by Alpha or Delta infection results in stronger antibody response toward WuH-1 virus and decreased antibody responses to viral variant epitopes compared to unvaccinated individuals infected with these variant viruses. In contrast, individuals infected with Alpha or Delta variants and with no history of vaccination develop antibodies with stronger binding to Alpha or Delta variant receptor binding domain (RBDs) compared to WuH-1 RBD."

119. Tortorici MA et al., “Persistent immune imprinting occurs after vaccination with the COVID-19 XBB.1.5 mRNA booster in humans,” *Immunity* 2024, 57, 4: P904-911.E4. doi: 10.1016/j.immuni.2024.02.016
- “Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) Omicron breakthrough infections and bivalent COVID-19 vaccination primarily recall cross-reactive memory B cells induced by prior Wuhan-Hu-1 spike mRNA vaccination rather than priming Omicron-specific naive B cells… The finding that administration of an XBB.1.5 S booster elicited higher plasma neutralizing activity against Wuhan-Hu-1/D614G S VSV (vaccine mismatched) relative to XBB.1.5 S VSV (vaccine matched) at both time points examined is a serological indication of immune imprinting… These data suggest that XBB.1.5 S vaccination boosts cross-reactive plasma antibody titers previously elicited by Wuhan-Hu-1 S exposure, which are also binding to and neutralizing XBB.1.5 and other variants instead of inducing *de novo* antibody responses against XBB.1.5 S.”

120. Tseng HF et al., “Effectiveness of mRNA-1273 vaccination against SARS-CoV-2 omicron subvariants BA.1, BA.2, BA.2.12.1, BA.4, and BA.5,” *Nat. Commun.* 2023, 14, 189. doi: https://doi.org/10.1038/s41467-023-35815-7
- “Similarly, four-dose VE against infection with BA.2, BA.2.12.1, BA.4, and BA.5 was moderate, and was only approximately 35% against BA.5. The four-dose VE against these subvariants was short-lived, disappearing beyond 90 days after the fourth dose… Taken together, these findings appear to be consistent with those of a recent study that found that the primary benefit of booster vaccines is augmentation of neutralizing antibodies without a strong effect on cellular immunity beyond that already induced by the primary vaccination series.”

121. Uraki R et al., “Humoral immune evasion of the omicron subvariants BQ.1.1 and XBB,” *Lancet Infect Dis.* 2023, 23, 1: 30-32. doi: 10.1016/S1473-3099(22)00816-7
- “The FRNT50 geometric mean titres against BQ.1.1 and XBB were 21·1-fold and 21·6-fold lower, respectively, than those against the ancestral strain (SARS-CoV-2/UT-NC002-1T/Human/2020/Tokyo). In addition, the geometric mean titres against BQ.1.1 and XBB were 1·7-fold and 2·6-fold lower, respectively, than those against BA.5 and BA.2. Similar results were obtained with samples from individuals who received four doses of mRNA vaccine; the FRNT50 geometric mean titres against BQ.1.1 and XBB were 43·3-fold and 51·6-fold lower, respectively, than those against the ancestral strain, and were 3·7-fold and 6·2-fold lower than those against BA.5 and BA.2, respectively. In contrast, most of the samples from vaccinees with BA.2 breakthrough infection neutralised BQ.1.1 and XBB; however, the FRNT50 geometric mean titres against BQ.1.1 and XBB were 35·2-fold and 61·7-fold lower, respectively, than those against the ancestral strain, and were 4·9-fold and 15·1-fold lower than those against BA.5 and BA.2, respectively.”

122. Voss WN et al., “Hybrid immunity to SARS-CoV-2 arises from serological recall of IgG antibodies distinctly imprinted by infection or vaccination,” *Cell Rep Med*. 2024, 5, 8: 101668. doi: 10.1016/j.xcrm.2024.101668
- “Infection primarily triggers S2/N-terminal domain (NTD)-reactive antibodies, whereas vaccination mainly induces anti-receptor-binding domain (RBD) antibodies. This imprint persists after secondary exposures wherein >60% of ensuing hybrid immunity derives from the original IgG pool.”

123. Walls AC et al., “SARS-CoV-2 breakthrough infections elicit potent, broad, and durable neutralizing antibody responses,” *Cell* 2022, 185, 5: P872-880.E3. doi: 10.1016/j.cell.2022.01.011
- “Here, we demonstrate that breakthrough infections induce serum-binding and -neutralizing antibody responses that are markedly more potent, durable, and resilient to spike mutations observed in variants than those in subjects who received only 2 doses of vaccine.”

124. Wang K et al., “Memory B cell repertoire from triple vaccinees against diverse SARS-CoV-2 variants,” *Nature* 2022, 603: 919-925. doi: 10.1038/s41586-022-04466-x
- “Here we examined whether sera from individuals who received two or three doses of inactivated SARS-CoV-2 vaccine could neutralize authentic Omicron. The seroconversion rates of neutralizing antibodies were 3.3% (2 out of 60) and 95% (57 out of 60) for individuals who had received 2 and 3 doses of vaccine, respectively. For recipients of three vaccine doses, the geometric mean neutralization antibody titre for Omicron was 16.5-fold lower than for the ancestral virus (254).”

125. Wang M et al., “Original Antigenic Sin on Antibody Response in SARS-CoV-2 Infection,” *Infect. Dis. Immun.* 2024, 4, 3: 132-137. doi: 10.1097/ID9.0000000000000125
- “OAS is a barrier to the generation of variant-specific antibodies against the current vaccines against rapidly evolving SARS-CoV-2. New vaccine strategies that promote nAb responses to mutated RBD epitopes and avoid boosting imprinted B cell immune responses are required in the future.”

126. Wang Q et al., “Deep immunological imprinting due to the ancestral spike in the current bivalent COVID-19 vaccine,” *Cell Rep Med*. 2023, 4, 11: 101258. doi: 10.1016/j.xcrm.2023.101258
- “Monovalent and BA.5 bivalent mRNA vaccine boosters induced similar antibody responses. BA.5 breakthrough infections yielded higher neutralizing activity than vaccine boosters. The ancestral spike in BA.5 bivalent vaccines caused deep immunological imprinting. Bivalent boosters did not yield superior antibody responses due to immune imprinting.”

127. Wang Z et al., “Ancestral SARS-CoV-2 immune imprinting persists on RBD but not NTD after sequential Omicron infections,” *iScience*, 2025, 28, 1: 111557. doi: 10.1016/j.isci.2024.111557
- “Plasma neutralizing antibody titers against ancestral SARS-CoV-2 and variants indicate that immune imprinting is not consistently induced by inactivated or recombinant protein vaccines. However, once robustly induced, immune imprinting is not countered by successive Omicron challenges.”

128. Weber T et al., “Enhanced SARS-CoV-2 humoral immunity following breakthrough infection builds upon the preexisting memory B cell pool,” *Sci. Immunol.* 2023, 8, 89. doi: 10.1126/sciimmunol.adk5845
- “However, the SARS-CoV-2–specific memory B cell pool was significantly expanded only in individuals with a breakthrough infection after third dose. This was due to selection of pre-existing Omicron-neutralizing memory B cells that potently neutralized a broad range of variants that arose after initial vaccination. These findings demonstrate that SARS-CoV-2 immunity is imprinted during early antigen exposure and adapts to new variants.”

129. Wei D et al., “Sequential reinfection with Omicron variants elicits broader neutralizing antibody profiles in booster vaccinees and reduces the duration of viral shedding,” *J Med Virol* 2023, 95, 10: e29151. doi: 10.1002/jmv.29151
- “Sequential reinfection with Omicron variants elicits broader and high-titer variant-specific neutralizing antibody profiles against Omicron variants. It could also dampen the hyperactivation of WT-specific neutralization induced by previous WT-based vaccination.”

130. Wheatley AK et al., “Immune imprinting and SARS-CoV-2 vaccine design,” *Trend Immunol.* 2021, 42, 11: 956-959. doi: 10.1016/j.it.2021.09.001
- “We hypothesize that updated vaccines against SARS-CoV-2 variants might primarily boost ‘imprinted’ immune responses to conserved regions of the Spike protein to the detriment of new neutralizing responses to antigenically altered sites within new variants.”

131. Wrynla XH et al., “Immune imprinting and vaccine interval determine antibody responses to monovalent XBB.1.5 COVID-19 vaccination,” *Commun. Med.* 2025, 5: 182. doi: https://doi.org/10.1038/s43856-025-00898-4
- “Our findings indicate that immune imprinting continues to affect humoral immunity elicited by the XBB.1.5 vaccine.”

132. Yamamoto S et al., “Omicron BA.1 neutralizing antibody response following Delta breakthrough infection compared with booster vaccination of BNT162b2,” *BMC Infect. Dis.* 2023, 23, 282. doi: https://doi.org/10.1186/s12879-023-08272-2

- "Breakthrough infection cases showed marked increases in NAb titers against Wild-type (4.1-fold) and Delta (5.5-fold), and 64% had detectable NAb against Omicron BA.1 at follow-up, although the NAb against Omicron after breakthrough infection was 6.7- and 5.2-fold lower than Wild-type and Delta, respectively. The increase was apparent only in symptomatic cases and as high as in the third vaccine recipients…"

133. Yang Y et al., "Comparative neutralization profiles of naive and breakthrough infections with Delta, Omicron BA.1 and BA.2 variants of SARS-CoV-2," *Signal Transduct Target Ther* 2022, 7: 316. doi: 10.1038/s41392-022-01166-w
    - "Our results for the naive and breakthrough infections with Delta and BA.1 variants showed that limited cross-neutralizing responses were induced, especially for the currently dominant BA.4/5 variant. This is consistent with previous findings that vaccination with BA.1 specific mRNA vaccine alone or infection with BA.1 provided poor cross-protection, and that BA.4/5 variant could significantly escape the immune response induced by BA.1 breakthrough infection. These observations might result from that BA.1 breakthrough infection predominantly recalls humoral immune memory against the WT SARS-CoV-2 spike protein…"

134. Yao D et al., "Antibody Responses in SARS-CoV-2-Exposed and/or Vaccinated Individuals Target Conserved Epitopes from Multiple CoV-2 Antigens," *Int. J. Mol. Sci.* 2024, 25, 18: 9814. doi: https://doi.org/10.3390/ijms25189814
    - "The majority of the current vaccine efforts against SARS-CoV-2 are limited by targeting the S-protein; however, it is important to consider N and M proteins as potential targets that will allow us to establish cross-reactive responses. Our results demonstrate that mRNA-vaccinated, AstraZeneca-vaccinated, and unvaccinated donors generate N- and M-specific IgG antibody titers. However, within the vaccinated groups, those with known COVID-19 infections showed significantly higher N-specific IgG titer."

135. Yisimayi A et al. "Repeated Omicron exposures override ancestral SARS-CoV-2 immune imprinting," *Nature* 2024, 625: 148-156. doi: 10.1038/s41586-023-06753-7
    - "… immune imprinting induced by vaccination based on the ancestral (hereafter referred to as WT) strain would compromise the antibody response to Omicron-based boosters… in humans, repeated Omicron infections could alleviate WT vaccination-induced immune imprinting and generate broad neutralization responses in both plasma and nasal mucosa."

136. Zelm MCV, "Immune memory to SARS-CoV-2 Omicron BA.1 breakthrough infections: To change the vaccine or not?" *Sci. Immunol.* 2022, 7, 74. doi: 10.1126/sciimmunol.abq5901

- "Analysis of memory B cell responses to Spike antigen after Omicron BA.1 breakthrough infections suggests that 'original antigenic sin' is in play."

137. Zhang L et al., "Neutralisation sensitivity of SARS-CoV-2 lineages EG.5.1 and XBB.2.3," *Lancet Infect Dis.* 2023, 23, 10: e391 - e392. doi: 10.1016/S1473-3099(23)00547-9
    - "Finally, we investigated neutralisation by plasma from quadruple vaccinated people collected 2 months (cohort one) or 4–8 (cohort two) months after vaccination, or from people who were vaccinated three to four times with breakthrough infection (cohort three). Particles bearing XBB S proteins were generally less well neutralised as compared with B.1pp (15–194-fold reduction). No major differences were observed between neutralisation of XBB.1.5pp, XBB.1.16pp, and XBB.2.3pp. However, it is noteworthy that EG.5.1pp evaded neutralisation by plasma collected for cohorts one and three with higher efficiency than XBB.2.3pp, XBB.1.5pp, and XBB.1.16pp."

138. Zhou Z et al., "Immune Imprinting and Implications for COVID-19," *Vaccines* 2023, 11, 4: 875. doi: https://doi.org/10.3390/vaccines11040875
    - "It is plausible that imprinted memory B cells induced by the original mRNA vaccine dominate the response to the booster vaccine. Thus, based on the small-scale preclinical study, at least in the short term, boosting with Omicron-mRNA vaccine has not yet presented big advantage over the original mRNA vaccine regarding the induction of protective NAbs against variant as well as control of viral replication after challenge, and immune imprinting seemingly involved in damping the B cell response to variant epitopes."

139. Zhu A et al., "Antigenic characterization of SARS-CoV-2 Omicron subvariants XBB.1.5, BQ.1, BQ.1.1, BF.7 and BA.2.75.2," *Signal Transduct Target Ther* 2023 8: 125. doi: https://doi.org/10.1038/s41392-023-01391-x
    - "Similar trends were observed for both vaccine- and infection-induced plasma, regardless of the vaccination status, enhanced neutralization resistance of SARS-CoV-2 Omicron subvariants BF.7, BQ.1, BQ.1.1, BA.2.75.2, XBB and XBB.1.5 was observed when compared with their parent BA.2 and BA.4/5. Multiple vaccination strategies... failed to elicit high neutralizing antibody titer against the newly emerged Omicron subvariant..."

140. Zuo F et al., "Heterologous inactivated virus/mRNA vaccination response to BF.7, BQ.1.1, and XBB.1," *Lancet Reg Health West Pac.* 2023, 33: 100762. doi: 10.1016/j.lanwpc.2023.100762
    - "Due to humoral immune imprinting... the bivalent vaccine booster and hybrid immunity may not provide sufficient protection against emerging Omicron subvariants."

VI. SARS-CoV2 vaccine and viral variant research library

Compiled by Dr. Steven Hatfill, MD, MMed, Erik Sass, et al.

Last updated July 1, 2025. Corresponding author: eriksass@gmail.com

In addition to the pathogenicity, distribution, and long persistence of the "vaccine"-produced spike protein, a growing body of research links COVID "vaccination" to the evolution of vaccine-resistant viral variants. The following collection of (**n=70**) peer-reviewed papers suggests the "vaccines" applied strong selective pressure to the fast-mutating SARS-CoV2 virus, quickly giving rise to "vaccine"-resistant variants. It is noteworthy that variants emerged in temporal and geographic proximity to "vaccine" clinical trials or mass "vaccination":

1. The Alpha variant was first identified in the county of Kent in southeast England in November 2020. Phase I/II clinical trials for AstraZeneca's AZD1222 (ChAdOx1 nCoV-19) adenovector "vaccine" enrolled over 1,000 subjects in southern England in April 2020, and thousands more in the phase III trial, May-December 2020.

2. The Delta variant was first identified in Maharashtra state, India, in October 2020. Phase II/III clinical trials for the Covidshield adenovector "vaccine" based on AstraZeneca's AZD1222 enrolled 1,600 subjects at 14 hospital centers, including eight in Maharashtra state, from July-October 2020.

3. The Omicron variant was first identified in Gauteng, South Africa, in November 2021, following an intense provincial "vaccination" campaign from August-October.

On this note, public health officials have warned that "chasing variants" is likely futile:

- In January 2023, Dr. Peter Marks, director of FDA's Center for Biologics Evaluation and Research, wrote: "Continuing along the current path of… variant-specific vaccine boosters is inadequate as a long-term strategy for addressing COVID-19… Simply updating the existing vaccine constructs with new variant sequences or even making trivalent or quadrivalent vaccines… is not likely to provide the depth and breadth of protection needed to interrupt viral transmission…"

- FDA Vaccines and Related Biological Products Advisory Committee (VRBPAC) member Dr. Paul Offit told *Time*: "The experience of the past year has taught us that chasing these Omicron variants with a bivalent vaccine is a losing game."

This compilation originated with Dr. Hatfill's contribution to *TOXIC SHOT: Facing the Dangers of the COVID "Vaccines"* (Chapter 5: Debunking CDC's Bad Science)

ANNOTATED REFERENCES (n=70)

1. Ahmed MN et al., "The impact of pre-existing immunity on the emergence of within-host immune-escape mutations in Omicron lineages," *J. Gen. Virol.* 2025, 106, 5. doi: https://doi.org/10.1099/jgv.0.002108
   - "Non-lineage mutations (39, 33 and 25 in BA.2*, BA.4* and BA.5* lineages, respectively) were detected, some showing higher incidence in vaccinated individuals. Six mutations detected at sub-consensus levels at antigenic sites suggest increased immune pressure on the spike protein in vaccinated individuals. Four high-prevalence antigenic mutations, absent from global GISAID sequences, were identified. Although within-host diversity did not significantly differ between vaccination statuses, detected mutations suggest that vaccine-induced immunity may influence within-host mutation patterns."

2. Al-Khatib HA et al., "Comparative analysis of within-host diversity among vaccinated COVID-19 patients infected with different SARS-CoV-2 variants," *iScience,* 2022, 25, 11: 105438. doi: https://doi.org/10.1016/j.isci.2022.105438
   - "Overall, the relatively higher intra-host diversity among vaccinated individuals and the detection of immune-escape mutations, despite being rare, suggest a potential vaccine-induced immune pressure in vaccinated individuals."

3. Atlani-Duault L et al., "Immune evasion means we need a new COVID-19 social contract," *Lancet Public Health* 2021, 6, 4: E199-E200. doi: 10.1016/S2468-2667(21)00036-0
   - "... the dynamics of natural or vaccinal collective immunity in the regions where these variants emerged might have placed substantial pressure on the viral ecosystem, facilitating the emergence of a variant with enhanced transmissibility... This virological game changer has numerous consequences, not only for vaccines and treatment, but also for prevention and control strategies. The fervently awaited end of this global health crisis might be continually postponed, as new variants emerge and immune evasion reduces vaccination effectiveness in the short and medium term. Hence, it is time to abandon fear-based approaches based on seemingly haphazard stop-start generalised confinement as the main response to the pandemic; approaches which expect citizens to wait patiently until intensive care units are re-enforced, full vaccination is achieved, and herd immunity is reached."

4. Berkhout B and E Herrera-Carrillo, "SARS-CoV-2 Evolution: On the Sudden Appearance of the Omicron Variant," *J. Virol.* 2022, 96, 7. doi: https://doi.org/10.1128/jvi.00090-22
   - "The most compelling evidence for this scenario of regular Darwinian evolution actually comes from inspection of the genetic changes, which reveals a profound preference for mutations that change the amino acid composition of the spike protein: 30 nonsilent changes versus 1 silent mutation."

5. Brand M and Can Kesmir, “Evolution of SARS-CoV-2-specific CD4+ T cell epitopes,” *Immunogent.* 2023, 75: 283-293. doi: https://doi.org/10.1007/s00251-023-01295-8
   - “In this study, we aim to study spike (CD4+) T cell epitopes in silico and investigate the effect of vaccine selection pressure on epitope conservation and mutations in VOCs… we demonstrated in silico that selection induced by vaccination worldwide has marginal effects on SARS-CoV-2 spike-specific CD4 T cell responses, while this might be not at all the case for B cell responses. Therefore, it might be worthwhile to consider inclusion of other less mutating SARS-CoV-2 proteins such as ORF3, NSP3, and the N protein in a future vaccine.”

6. Brandolini M et al., “Omicron Sub-Lineage BA.5 and Recombinant XBB Evasion from Antibody Neutralisation in BNT162b2 Vaccine Recipients,” *Microorganisms* 2023, 11, 1: 191. doi: https://doi.org/10.3390/microorganisms11010191
   - “These evolutionary characteristics have prompted intensively debated questions and speculations, primarily regarding how vaccines will contribute to the emergence of new variants. Moreover, as many vaccines are based on the ancestral Spike protein gene sequence, they elicit a relatively ‘narrow-spectrum’ immune response, which can be easily and rapidly eroded by viral evolution. In fact, there is emerging evidence that the high mutation rate of the S gene constitutes a breeding ground for immune escape mechanisms, reducing the neutralising potential of antibodies produced in vaccinated subjects.”

7. Bushman M et al., “Population impact of SARS-CoV-2 variants with enhanced transmissibility and/or partial immune escape,” *Cell* 2021, 184, 26: P6229-6242.E18. doi: 10.1016/j.cell.2021.11.026
   - “Here, we use a mathematical model to simulate the dynamics of wild-type and variant strains of SARS-CoV-2 in the context of vaccine rollout and nonpharmaceutical interventions. We show that variants with enhanced transmissibility frequently increase epidemic severity, whereas those with partial immune escape either fail to spread widely or primarily cause reinfections and breakthrough infections. However, when these phenotypes are combined, a variant can continue spreading even as immunity builds up in the population, limiting the impact of vaccination and exacerbating the epidemic.”

8. Cao Y et al., “Imprinted SARS-CoV-2 humoral immunity induces convergent Omicron RBD evolution,” *Nature* 2023, 614: 521–529. doi: https://doi.org/10.1038/s41586-022-05644-7
   - “In this work, we showed that due to immune imprinting, our humoral immune repertoire is not effectively diversified by infection with new Omicron variants. The immune pressure on the RBD becomes increasingly concentrated and promotes convergent evolution, explaining the observed sudden acceleration of SARS-CoV-2 RBD evolution and the convergence pattern. Although this study

only examines inactivated vaccines, immune imprinting is also observed in those receiving mRNA vaccines."

9. Carabelli AM et al., "SARS-CoV-2 variant biology: immune escape, transmission and fitness," *Nat Rev Microbiol* 2023, 21, 162–177. doi: 10.1038/s41579-022-00841-7
   - "The increased virus fitness associated with VOCs is the result of a complex interplay of virus biology in the context of changing human immunity due to both vaccination and prior infection."

10. Chaguza C et al., "Rapid emergence of SARS-CoV-2 Omicron variant is associated with an infection advantage over Delta in vaccinated persons," *Clin. Transl. Rep.* 2022, 3, 5: P325-334.E4. doi: 10.1016/j.medj.2022.03.010
    - "As population immunity to SARS-CoV-2 increases through infections and vaccination, selection for variants that are partially resistant to the immune response, in particular neutralizing antibodies, should also increase... We hypothesized that the rapid emergence and spread of the SARS-CoV-2 Omicron variant was partly due to its increased ability to evade immunity from prior infection and/or vaccination. Using a study population seeking outpatient testing when Omicron and Delta were overall relatively equal among infections, we found that Omicron has a relatively higher propensity to cause infections in COVID-19-vaccinated persons."

11. Chang MR et al., "Analysis of a SARS-CoV-2 convalescent cohort identified a common strategy for escape of vaccine-induced anti-RBD antibodies by Beta and Omicron variants," *eBioMedicine* 2022, 80: 104025. doi: 10.1016/j.ebiom.2022.104025
    - "Structural analysis of the Beta and Omicron RBDs reveal a shared immune escape strategy involving residues K417-E484-N501 that is exploited by these variants of concern... Through mutations of the K417-E484-N501 triad, SARS-CoV-2 has evolved to evade neutralization by the class I/II anti-RBD antibody fraction of hybrid immunity plasma as the polyclonal antibody response post-vaccination shows limitations in the ability to solve the structural requirements to bind the mutant RBDs."

12. Cocherie T et al., "Epidemiology and Characteristics of SARS-CoV-2 Variants of Concern: The Impacts of the Spike Mutations," *Microorganisms* 2023, 11, 1: 30. doi: https://doi.org/10.3390/microorganisms11010030
    - "Following the spread of lineage B.1, new lineages emerged in a context of selection pressure related to the extension of vaccination and post-infectious immunization. These lineages have each selected specific sets of mutations, in an asynchronous and geographically isolated manner, which supports the hypothesis of a convergent antigenic evolution, reinforced by the discovery of some of their mutations in independent lineages."

13. Collier DA et al., “Sensitivity of SARS-CoV-2 B.1.1.7 to MRNA vaccine-elicited antibodies,” *Nature* 2021, 593: 136–141. doi: 10.1038/s41586-021-03412-7
    - “Taken together, the presence of multiple escape mutations in the NTD is supportive of the hypothesis that this region of the spike, in addition to the RBM, is also under immune pressure… Our data suggest that vaccine escape by the virus of current spike-directed vaccines designed against the Wuhan-1 strain will be inevitable…”

14. Day T et al., “Pathogen evolution during vaccination campaigns,” *PLoS Biol* 2022, 20, 9: e3001804. doi: https://doi.org/10.1371/journal.pbio.3001804
    - “…vaccine-driven evolution has tended to occur in other pathogens when either the benefits of prophylaxis are small (e.g., the vaccine does not sufficiently suppress pathogen replication below transmissible levels) or when they target a small number of pathogen epitopes. Data increasingly suggest that at least the first of these is true for SARS-CoV-2 and currently deployed vaccines.”

15. Dijokaite-Guraliuc A et al., “Rapid escape of new SARS-CoV-2 Omicron variants from BA.2-directed antibody responses,” *Cell Rep.* 2023, 42, 2: 112271. doi: 10.1016/j.celrep.2023.112271
    - “Overall, in line with the observations on the set of mAbs described above, there were large reductions in neutralization titers against most BA.2 sub-lineages, particularly BA.2.75.2, BA.2.3.20, BQ.1, and XBB, suggesting that they have been selected to escape pre-existing immunity to vaccines or earlier waves of SARS-CoV-2 infection… It is likely that evolution of SARS-CoV-2 Omicron is now primarily driven by extreme pressure to escape antibody responses in vaccinated and/or naturally infected individuals, with compensatory mutations to maintain or increase ACE2 affinity.”

16. Duerr R et al., “Dominance of Alpha and Iota variants in SARS-CoV-2 vaccine breakthrough infections in New York City,” *J Clin Invest* 2021, 131, 18: e152702. doi: 10.1172/JCI152702
    - “Despite the overall effectiveness of vaccination, our full spike mutation analysis revealed a broad set of spike mutations (n = 23) to be elevated in the vaccine breakthrough group. The analysis indicates that adaptive selection is in progress that may subsequently come into full effect.”

17. Duerr R et al., “Selective adaptation of SARS-CoV-2 Omicron under booster vaccine pressure: a multicentre observational study,” *eBioMedicine* 2023, 97: 104843. doi: 10.1016/j.ebiom.2023.104843
    - “Booster shots are required to cope with gaps in immunity. Their discriminative immune pressure contributes to their effectiveness but also requires monitoring of selective viral adaptation processes. Omicron BA.2 and BA.5 had a selective advantage under booster vaccination pressure, contributing to the evolution of BA.2 and BA.5 sublineages and recombinant forms that predominate in 2023.”

18. Fang FF and Pei-Yong Shi, "Omicron: a drug developer's perspective," *Emerg. Microbes & Infect.* 2022, 11, 1. doi: 10.1080/22221751.2021.2023330
    - "Omicron has revealed to us that SARS-CoV-2 has the potential to go beyond the protective threshold provided by vaccines and antibodies. Playing catchup to SARS-CoV-2 selects for more resistant and transmissible variants and may not be successful in the long run."

19. Focosi D et al., "Convergent Evolution in SARS-CoV-2 Spike Creates a Variant Soup from Which New COVID-19 Waves Emerge," *Int. J. Mol. Sci.* 2023, 24, 3: 2264. doi: https://doi.org/10.3390/ijms24032264
    - "The most likely reason for this convergence is the selective pressure exerted by previous infection- or vaccine-elicited immunity… The combined action of increasing cumulative viral loads in the 'human culture medium' and such selective pressures has led to an unprecedented increase in viral diversification in 2022."

20. Garcia-Beltran WF et al., "Multiple SARS-CoV-2 variants escape neutralization by vaccine-induced humoral immunity," *Cell* 2021, 184, 9: p2372-2383.e9. doi: 10.1016/j.cell.2021.03.013
    - "… we found that B.1.351 variants exhibited remarkable resistance to neutralization, largely due to three mutations in RBD but with measurable contribution from non-RBD mutations. The magnitude of the effect is such that B.1.351 strains escaped neutralizing vaccine responses as effectively as distantly related coronaviruses."

21. Gayvert K et al., "Evolutionary trajectory of SARS-CoV-2 genome shifts during widespread vaccination and emergence of Omicron variant," *npj Viruses* 2023, 1: 5. doi: https://doi.org/10.1038/s44298-023-00007-z
    - "Our analysis revealed that during the first year of the pandemic (2020 to 2021), the SARS-CoV-2 genome was subject to strong conservation… However, we observed a sharp increase in the diversification of the RBD during 2021 (8.1% of sites under diversifying pressure up to 2022), indicating selective pressures that promote the accumulation of mutations. This period coincided with broad viral infection and adoption of vaccination worldwide, and we observed the acquisition of mutations that later defined the Omicron lineages in independent SARS-CoV-2 strains…"

22. Ghmire D et al., "Structural Plasticity and Immune Evasion of SARS-CoV-2 Spike Variants," *Viruses* 2022, 14, 6: 1255. doi: https://doi.org/10.3390/v14061255
    - "SARS-CoV-2 viruses are under increased selection pressure from the vaccines, therapeutic approaches, and the host immune system. Whole-genome sequencing technology has allowed identifying the emergence of different SARS-CoV-2 variants… These variants are more transmissible and possibly more

pathogenic and immune–evasive. They carry accumulated mutations in the S protein. The resulting amino acid substitutions in S can impact the binding capacity to hACE2 and antibody recognition, therefore imposing constant challenges in current vaccine and therapeutic regimes."

23. Gobeil SMC et al., "Effect of natural mutations of SARS-CoV-2 on spike structure, conformation, and antigenicity," *Science* 2021, 373, 6555. doi: 10.1126/science.abi6226
    - "Although many of the currently circulating variants of interest/concern likely arose from some combination of genetic drift, host adaptation, and immune evasion, the virus will increasingly experience pressure from vaccine-elicited antibody responses."

24. Habib MT et al., "Natural selection shapes the evolution of SARS-CoV-2 Omicron in Bangladesh," *Front. Genet.* 2023, 14 (Sec. Computational Genomics). doi: https://doi.org/10.3389/fgene.2023.1220906
    - "We found evidence of adaptive evolution within the spike (S) gene of SARS-CoV-2 Omicron isolated from Bangladesh. In total, 22 codon sites of the S gene displayed a signature of positive selection… Moreover, the lack of selection pressure on the S gene representing SARS-CoV-2 Delta from Bangladesh indicates a possible correlation between vaccination and adaptive evolution."

25. Hamburg M and GA Poland, "The time is now for committed and comprehensive action to attain more broadly protective coronavirus vaccines: The coronavirus vaccines R&D roadmap," *Vaccine* 2023, 41, 16: 2645-2647. doi: https://doi.org/10.1016/j.vaccine.2023.02.053
    - "… we continue to face continued circulation and evolution of SARS-CoV-2 viruses that mutate to evade immune responses among hosts who have partial or waning vaccine coverage, further exacerbating the situation."

26. Han W et al., "Predicting the antigenic evolution of SARS-COV-2 with deep learning," Nat Comm 2023, 14: 3478. doi: https://doi.org/10.1038/s41467-023-39199-6
    - "We hypothesized that under high immune pressure, the virus would tend to escape the antibody neuralization over a short-term time scale, and therefore the forecasting problem transforms into a search problem: starting from an initial sequence, it searches for a variant sequence within some edit distance range that has an improved antibody escape potential without losing much ACE2-binding ability… These findings verify our assumptions: under the immune selection pressure, the virus evolves in the direction of immune escape, and our model can capture the antibody escape potential of the viral variants."

27. Harvey WT et al., "SARS-CoV-2 variants, spike mutations and immune escape," *Nat Rev Microbiol* 2021, 19: 409–424. doi: https://doi.org/10.1038/s41579-021-00573-0

- "Given that therapeutics (vaccines and antibody-based therapies) target mainly the SARS-CoV-2 spike protein, the selection pressures that favour the emergence of new variants carrying immune escape mutations generated in chronic infection will be similar to those selecting for mutations that allow reinfections within the wider population."

28. He P et al., "SARS-CoV-2 Delta and Omicron variants evade population antibody response by mutations in a single spike epitope," *Nat. Microbiol.* 2022, 7: 1635-1649. doi: https://doi.org/10.1038/s41564-022-01235-4
    - "Owing to immune pressure induced by natural infection and vaccination, numerous SARS-CoV-2 variants have emerged, these variants encoding spike proteins with substituted amino acids that function to evade antibody neutralization… Here we identify an important role for VH1-69 HCDR2 in anti-SARS-CoV-2 immunity… These mutation 'hot spots' should be continuously monitored and future studies should address the potential pathogenic consequences of VH1-69 antibody evasion by SARS-CoV-2."

29. Jankowiak M et al., "Inferring selection effects in SARS-CoV-2 with Bayesian Viral Allele Selection," *PLoS Genet.* 2022, doi: 10.1371/journal.pgen.1010540
    - "… we conduct an analysis that allows for vaccination-dependent selection effects and find tantalizing evidence that S:N501Y exhibits vaccination-dependent differential fitness… The elevated contribution of S-gene mutations (notably in the RBD) over non-S-gene mutations starting around November 2021 is apparent. Collectively these two results suggest that immune escape has become an increasingly prominent factor in SARS-CoV-2 evolution over time, likely a result of rising rates of convalescent and vaccine-induced immunity to Spike."

30. Jena D et al., "Impact of vaccination on SARS-CoV-2 evolution and immune escape variants," *Vaccine* 2024, 42, 21: https://doi.org/10.1016/j.vaccine.2024.07.054
    - "Our comparative analysis revealed a significant higher incidence of intra-host single nucleotides variants (iSNVs) in vaccinated cases compared to unvaccinated ones (p value<0.0001). Furthermore, we have found that specific mutational processes, including APOBEC (C > T) mediated and ADAR1 (A > G) mediated mutations, were found more prevalent in vaccinated cases. Vaccinated cases exhibited higher accumulation of nonsynonymous mutation than unvaccinated cases… Our findings suggest that vaccine plays an important role in the evolution of the virus genome."

31. Kennedy DA and AF Read, "Monitor for COVID-19 vaccine resistance evolution during clinical trials," *PLoS Biol.* 2020, 18, 11: e3001000. doi: https://doi.org/10.1371/journal.pbio.3001000

- "To avoid being caught off guard by the evolution of vaccine resistance, standard samples from clinical trials can be repurposed to assess the risk of resistance evolution even before a vaccine is licensed."

32. Konishi T, "Mutations in SARS-CoV-2 are on the increase against the acquired immunity," *PLoS One* 2022, 17, 7: e0271305. doi: 10.1371/journal.pone.0271305
    - "In Omicron, there was a high density of S mutations suggesting that there was selection pressure to avoid the acquired immunity imparted by monovalent vaccines... These findings suggest that the early mRNA vaccine has lost its effectiveness. Accordingly, the sixth peak in Japan is becoming extremely high without subsiding, which can be due to dependency of the government only on the vaccines."

33. Koyoma T et al., "Evasion of Vaccine-Induced Humoral Immunity by Emerging Sub-Variants of SARS-CoV-2," *Future Microbiol.* 2022, 17, 6: 417-424. doi: https://doi.org/10.2217/fmb-2022-0025
    - "... the selection pressure exerted by vaccines might pave the way for other escape mutants in the near future."

34. Kumar N et al., "Bayesian Molecular Dating Analyses Combined with Mutational Profiling Suggest an Independent Origin and Evolution of SARS-CoV-2 Omicron BA.1 and BA.2 Sub-Lineages," *Viruses* 2022, 14, 12: 2764. doi: 10.3390/v14122764
    - "Nonetheless, in the event of the emergence of multiple new mutations in the Omicron's spike protein, which are quite distinct in the BA.1 and BA.2 sub-lineages, as well as their estimated separate most recent common ancestor, it may be more plausible to conclude that a combination of RBD- and NTD-directed classes of antibody therapeutics at sub-optimal doses in COVID-19 patients or optimal doses in an immunocompromised patient or waned vaccine-induced immunity may have provided a conducive environment to accumulate multiple mutations in Omicron's spike protein."

35. Kumar SW et al., "Vaccine-elicited immune pressure and SARS-CoV-2 mutational dynamics in breakthrough infections," *Gene Rep.* 2024, 35: 101899. doi: https://doi.org/10.1016/j.genrep.2024.101899
    - "Vaccinated individuals exhibit significantly higher mutation rates, including immune escape mutations... Selection pressure may drive viral mutations for enhanced immune evasion."

36. Lewnard JA et al., "Increased vaccine sensitivity of an emerging SARS-CoV-2 variant," *Nat Commun* 2023, 14: 3854. doi: 10.1038/s41467-023-39567-2
    - "Immunological and evolutionary factors driving this apparent bifurcation in evasion of vaccine-derived and infection-derived responses for XBB/XBB.1.5 merit further investigation. Notably, vaccinations available in the US (mRNA-1273, BNT162b2, Ad.26.COV2.S, and NVX-CoV2373) target only the SARS-CoV-2

spike antigen. In contrast, infection with SARS-CoV-2 induces responses against an array of SARS-CoV-2 antigens, some of which may be independently associated with protection."

37. Li X, "Omicron: Call for updated vaccines," *J. Med. Virol.* 2022, 94, 4: 1261-1263. doi: https://doi.org/10.1002/jmv.27530
    - "The Omicron SARS-CoV-2 variant was potentially generated from a chronically infected COVID-19 patient vaccinated with an messenger RNA (mRNA)- or non-mRNA-based vaccine, offering the opportunity for the virus to evolve and mutate to evade the body's immune response. To understand the significance of this SARS-CoV-2 variant and what it means for the global response to the pandemic, vaccinologists should systematically evaluate the role of mRNA- and non-mRNA-based vaccines in the generation of novel SARS-CoV-2 variants, including variants of concerns (VOCs) and interest (VOIs), that occur via breakthrough vaccine-elicited immunity."

38. Lomoio U et al., "SARS-CoV-2 protein structure and sequence mutations: Evolutionary analysis and effects on virus variants," *PLoS One* 2023, 18, 7: e0283400. doi: https://doi.org/10.1371/journal.pone.0283400
    - "We explore patterns of changes in a temporal dimension and compare the cumulative distribution of vaccination with the characteristics of the variant. Although we cannot infer any causality regarding vaccination driving the evolution, we should note that the presence of vaccinations in a timeline is located in the middle of the first variants of SARS-CoV-2 and Omicron. Considering also the clinical characteristics of Omicron in terms of vaccine escape and neutralization of immune response, we can assume that the effect of all Omicron changes may be related to the structural changes also revealed by the above-reported measures."

39. López-Cortés GI et al., "The Spike Protein of SARS-CoV-2 Is Adapting Because of Selective Pressures," *Vaccines* 2022, 10, 6: 864. doi: 10.3390/vaccines10060864
    - "Our results hint that selective pressures are induced by mass vaccination throughout the world and by the persistence of recurrent infections in immunosuppressed individuals, who did not eliminate the infection and ended up facilitating the selection of viruses whose characteristics are different from the previous VOCs, less pathogenic but with higher transmissibility."

40. Magazine N et al., "Mutations and Evolution of the SARS-CoV-2 Spike Protein," *Viruses* 2022, 14, 3): 640. doi: https://doi.org/10.3390/v14030640
    - "Taken together with the fact that many of these mutations occur within the Omicron variant (which appeared only after vaccinations became widely distributed), it is possible that resistance to neutralizing antibodies (particularly those found in postvaccinated sera) targeting the NTD play a large role in the positive selection for SARS-CoV-2… Mutations within the S protein of the

circulating variants of SARS-CoV-2 are increasing at a significant rate and are likely to occur more often as selective pressures from host immunity gained in previous infections and/or vaccinations continue to drive rapid evolution."

41. Mahroum N et al., "Vaccine-induced strain replacement: theory and real-life implications," *Future Microbiol.* 2024, 19, 11: 1017-1026. doi: 10.1080/17460913.2024.2345003
    - "... increasing fitness of nonvaccine strains and metabolic shifts in the subtypes have been described. Classical examples include pneumococcal infections and viral diseases, such as the human papilloma virus... The recent SARS-CoV-2 virus responsible for the COVID-19 pandemic has been correlated to the vaccine-induced pathogen strain replacement."

42. Martin DP et al., "Selection Analysis Identifies Clusters of Unusual Mutational Changes in Omicron Lineage BA.1 That Likely Impact Spike Function," *Mol Biol Evol* 2022, 39, 4: msac061. doi: https://doi.org/10.1093/molbev/msac061
    - "Given the evident epidemic growth advantages of Omicron overall previously known SARS-CoV-2 lineages, it is crucial to determine both how such complex and highly adaptive mutation constellations were assembled within the Omicron S-gene, and why, despite unprecedented global genomic surveillance efforts, the early stages of this assembly process went completely undetected."

43. McLeod DV and S Gandon, "Effects of epistasis and recombination between vaccine-escape and virulence alleles on the dynamics of pathogen adaptation," *Nat Ecol Evol* 2022, 6: 786–793. doi: https://doi.org/10.1038/s41559-022-01709-y
    - "We show that vaccines blocking infection, reducing transmission and/or increasing clearance generate positive epistasis between the vaccine-escape and virulence alleles, favouring strains that carry both mutations, whereas vaccines reducing virulence mortality generate negative epistasis, favouring strains that carry either mutation but not both."

44. Meganck RM et al., "SARS-CoV-2 variant of concern fitness and adaptation in primary human airway epithelia," *Cell Rep.* 2024, 43, 4: 114076. doi: 10.1016/j.celrep.2024.114076
    - "... the Omicron variant emerged in November of 2021, at which point ~4 billion people are believed to have been vaccinated and more were likely to have been previously infected. The greater level of population immunity likely constituted a selective pressure on the virus. The newly emerged Omicron BA.1 strains contained a greater proportion of viral mutations located in the spike protein, the major antigenic target of SARS-CoV-2 adaptive immune responses, as compared to previous variants."

45. Messali S et al., “Emergence of S gene-based quasispecies explains an optimal adaptation of Omicron BA.5 subvariant in the immunocompetent vaccinated human host,” *J Med Virol*. 2023, 95, 1: e28167. doi: 10.1002/jmv.28167
    - “The low frequency of quasispecies observed in BA.2.3- and BA.5-infected patients supports the hypothesis that these omicron sub-lineages are adapted to vaccine-elicited immune responses.”

46. Mussò N et al., “SARS-CoV-2’s high rate of genetic mutation under immune selective pressure: from oropharyngeal B.1.1.7 to intrapulmonary B.1.533 in a vaccinated patient,” *Int. J. Infect. Dis*. 2022, 118: 169-172. doi: 10.1016/j.ijid.2022.02.044
    - “The immune reaction was a combination of vaccine and immune response after infection with SARS-CoV-2, but the presence of antibodies did not lead to the disruption of the viral RNA before this could cause pulmonary infection; on the contrary, it accelerated the normal process of “intra-host specific rearrangement,” as shown by the presence of a new intra-pulmonary lineage characterized by 5 worldwide low-expressed SNPs…”

47. Nabel KA et al., “Structural basis for continued antibody evasion by the SARS-CoV-2 receptor binding domain,” Science 2021, 375, 6578. doi: 10.1126/science.abl6251
    - “As severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) replicates under selective pressure from natural and vaccine-induced immunity, variants of concern (VOCs) continue to emerge. Through adaptative evolution, these variants acquire mutations in the spike protein receptor binding domain (RBD) that binds the cellular receptor angiotensin-converting enzyme 2 (ACE2)… We find that accumulation of large numbers of RBD mutations is facilitated by structural plasticity at the RBD–ACE2 interface and further erodes the activity of therapeutic antibodies and serum from vaccine recipients. Furthermore, acquisition of an N-linked glycan on the SARS-CoV-2 RBD is an additional neutralization escape pathway that should be closely monitored during viral antigenic drift.”

48. Oliviera JR et al., “Immunodominant antibody responses directed to SARS-CoV-2 hotspot mutation sites and risk of immune escape,” *Front. Immunol*. 2023, 13 (Sec. Viral Immunology). doi: https://doi.org/10.3389/fimmu.2022.1010105
    - “Our results showed that amongst convalescents a more focused response, with fewer peptides being recognized, was associated with higher neutralization titers. We reason that immune pressure following vaccination contributed to epitope spreading and likely surge of omicron that presents several mutations at RBD and the capacity of escaping antibody neutralization.”

49. Planas D et al., “Distinct evolution of SARS-CoV-2 Omicron XBB and BA.2.86/JN.1 lineages combining increased fitness and antibody evasion,” *Nat. Commun*. 2024, 15: 2254. doi: https://doi.org/10.1038/s41467-024-46490-7

- "The variants are closely related and carry an additional and limited set of mutations in the spike corresponding to a stepwise accumulation of changes. Convergent evolution may have been associated with this process… This convergent evolution is likely due to a similar selective pressure exerted by imprinted or hybrid immunity triggered by Omicron infection and/or vaccination."

50. Rolland M and PB Gilbert, "Sieve analysis to understand how SARS-CoV-2 diversity can impact vaccine protection," *PLoS Pathog*. 2021, 17, 3: e1009406. doi: https://doi.org/10.1371/journal.ppat.1009406
    - "The recent spread of outlier variants emphasizes the need to rapidly track the impact of vaccine-induced pressure on SARS-CoV-2 evolution… The variants B.1.1.7 (originally identified in the UK), B.1.351 (originally identified in South Africa), and P.1 (originally identified in Brazil) have more mutations than what was expected at this time in the pandemic, and a large fraction of these mutations are in the Spike, indicating likely selection pressure behind their emergence… . The selective pressure exerted by the vaccine together with limited vaccine coverage in the population has the potential to open ecological niches where rare variants with potentially unfavorable resistance profiles could outcompete circulating viruses."

51. Rouzine IM and G Rozhnova, "Evolutionary implications of SARS-CoV-2 vaccination for the future design of vaccination strategies," *Commun. Med* 2023, 3, 86. doi: https://doi.org/10.1038/s43856-023-00320-x
    - "Mass vaccination, as we show below, might increase this pressure and accelerate SARS-CoV-2 evolution in spike epitopes compared to natural infection."

52. Ruan W et al., "SARS-CoV-2 serotyping based on spike antigenicity and its implications for host immune evasion," *EBioMedicine* 2025, 114: 105634. doi: 10.1016/j.ebiom.2025.105634
    - "As SARS-CoV-2 continues to spread and evolve, new variants/sub-variants emerge, raising concerns about vaccine-induced immune escape. Here, we conducted a systematic analysis of the serology and immunogenicity of major circulating variants/sub-variants of SARS-CoV-2 since the outbreak."

53. Sanyaolu A et al., "SARS-CoV-2 Omicron variant (B.1.1.529): A concern with immune escape," *World J Virol* 2022, 11, 3:137–143. doi: 10.5501/wjv.v11.i3.137
    - "Finally, it has been proposed that natural selection can arise as a result of mutations that increase viral infectivity, antibody resistance, and vaccine breakthrough. Evolutionary descent of the Omicron lineages showed that mutations arose under selection pressure due to antibodies elicited by infection, vaccination, or both, in the human population on a large scale."

54. Servellita V et al., "Predominance of antibody-resistant SARS-CoV-2 variants in vaccine breakthrough cases from the San Francisco Bay Area, California," *Nat Microbiol* 2022, 7, 277-288. doi: https://doi.org/10.1038/s41564-021-01041-4
    - "The predominance of immune-evading variants among post-vaccination cases indicates possible selective pressure for antibody-resistant escape variants circulating locally over time in the vaccinated population."

55. Tan CW et al., "SARS-CoV-2 Omicron variant emerged under immune selection," *Nat Microbiol* 2022, 7: 1756–1761. doi: https://doi.org/10.1038/s41564-022-01246-1
    - "Using the same serum panels, we demonstrated even more potent NAb escape of mRNA vaccine-induced neutralizing antibodies by Omicron subvariants BA.2.11 and BA.5 with the additional L452R mutation and L452R/F486V/R493Q mutations, respectively… We propose that the SARS-CoV-2 Omicron variant emerged under immune selection imposed during 2 years of virus transmission in humans."

56. Tuekprakhon A et al., "Antibody escape of SARS-CoV-2 Omicron BA.4 and BA.5 from vaccine and BA.1 serum," *Cell* 2022, 185, 14: P2422-2433.E13. doi: 10.1016/j.cell.2022.06.005
    - "Although mutations in the VoC are spread throughout S, there are particular hotspots in the NTD and RBD, exactly where potent neutralizing antibodies bind, and they are likely being driven by escape from the antibody response following natural infection or vaccination."

57. Vanden Bossche G, floor letter to the Oregon State Legislature, "The Science behind the Catastrophic Consequences of Thoughtless Human Intervention in the Covid-19 Pandemic," March 13, 2021, https://olis.oregonlegislature.gov/liz/2021R1/Downloads/FloorLetter/3166
    - "Why are the Covid-19 vaccines likely to enhance viral infectiousness? It's because they are prophylactic vaccines – designed to build immunity in individuals before they get exposed to the pathogen/virus. They are not suitable at all for administration to people during a pandemic… Exerting high immune pressure without preventing viral replication and transmission is a recipe for selective viral immune escape."

58. van Dorp CH et al., "Estimating the strength of selection for new SARS-CoV-2 variants," *Nat Commun* 2021, 12: 7239. doi: 10.1038/s41467-021-27369-3
    - "… the gradual rollout of vaccination programs globally is changing the immunological landscape, possibly leading to the emergence of escape strains that are partially or fully resistant to existing vaccines… Integrating molecular epidemiology surveillance into SARS-CoV-2 pipelines is essential for not only monitoring the emergence of new strains, but for establishing an early warning system to monitor for escape mutations in the era of vaccine rollout."

59. van Egeren D et al., “Risk of rapid evolutionary escape from biomedical interventions targeting SARS-CoV-2 spike protein,” *PLoS One* 2021, 16, 4: e0250780. doi: https://doi.org/10.1371/journal.pone.0250780
    - “Our modeling suggests that SARS-CoV-2 mutants with one or two mildly deleterious mutations are expected to exist in high numbers due to neutral genetic variation, and consequently resistance to vaccines or other prophylactics that rely on one or two antibodies for protection can develop quickly -and repeatedly- under positive selection.”

60. Wang Q et al., “Alarming antibody evasion properties of rising SARS-CoV-2 BQ and XBB subvariants,” *Cell* 2023, 186, 2: P279-286.E8. doi: 10.1016/j.cell.2022.12.018
    - “Together, our findings indicate that BQ and XBB subvariants present serious threats to current COVID-19 vaccines, render inactive all authorized antibodies, and may have gained dominance in the population because of their advantage in evading antibodies.”

61. Wang R et al., “Emerging Vaccine-Breakthrough SARS-CoV-2 Variants,” *ACS Infect. Dis.* 2022, 8, 3: 546–556. doi: https://doi.org/10.1021/acsinfecdis.1c00557
    - “We show that prevailing variants can be quantitatively explained by infectivity-strengthening and vaccine-escape (co-)mutations on the spike protein RBD due to natural selection and/or vaccination-induced evolutionary pressure. We illustrate that infectivity strengthening mutations were the main mechanism for viral evolution, while vaccine-escape mutations become a dominating viral evolutionary mechanism among highly vaccinated populations… We foresee an urgent need to develop new virus combating strategies.”

62. Wang R et al., “Mechanisms of SARS-CoV-2 Evolution Revealing Vaccine-Resistant Mutations in Europe and America,” *J. Phys. Chem. Lett.* 2021, 12, 49: 11850–11857. doi: https://doi.org/10.1021/acs.jpclett.1c03380
    - “By tracking the evolutionary trajectories of vaccine-resistant mutations in more than 2.2 million SARS-CoV-2 genomes, we reveal that the occurrence and frequency of vaccine-resistant mutations correlate strongly with the vaccination rates in Europe and America.”

63. Wang Z et al., “mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants,” *Nature* 2021, 592: 616–622. doi: 10.1038/s41586-021-03324-6
    - “Nevertheless, emergence of these particular variants is consistent with the dominance of the class-1 and -2 antibody response in infected or vaccinated individuals. We speculate that these mutations emerged in response to immune selection in individuals with nonsterilizing immunity.”

64. Willett BJ et al., “SARS-CoV-2 Omicron is an immune escape variant with an altered cell entry pathway,” *Nat. Microbiol.* 2022, 7: 1161-1179. doi: https://doi.org/10.1038/s41564-022-01143-7

- "Immune evasion by Omicron may have contributed to the extremely high transmission rates in countries with high vaccination rates or natural immunity... These experiments indicate a fundamental change in the biology of Omicron (BA.1 and BA.2) spike. It has a reduced ability to form syncytia, most probably linked to changes in spike pre-processing at the S1/S2 boundary. Omicron spike is also optimized to preferential entry via the endosome, resulting in alterations in cellular tropism. This biological about-face may underpin the evident changes in Omicron transmission and pathogenesis."

65. Yang Z et al., "SARS-CoV-2 Variants Increase Kinetic Stability of Open Spike Conformations as an Evolutionary Strategy," *mBio* 2022, 13, 1. doi: https://doi.org/10.1128/mbio.03227-21
    - "Under the selection pressure imposed by adaptation to the human host and increasing vaccinations and convalescent patients, SARS-CoV-2 is evolving and has adopted numerous mutations on S variants. These promote virus spreading and immune evasion, partially by increasing the propensity of S to adopt receptor-binding competent open conformations."

66. Zayou L et al., "Dynamics of spike-specific neutralizing antibodies across five-year emerging SARS-CoV-2 variants of concern reveal conserved epitopes that protect against severe COVID-19," *Front. Immunol.* 2025, 16 (Sec. Vaccines and Molecular Therapeutics). doi: https://doi.org/10.3389/fimmu.2025.1503954
    - "The world will enter its sixth year of a persistent COVID-19 pandemic, fueled by the continuous emergence of heavily Spike-mutated and highly contagious SARS-CoV-2 variants and sub-variants that continue to: (i) escape immunity induced by the current Spike-alone-based vaccines; (ii) disrupt the efficacy of the COVID-19 booster paradigm; and (iii) outpace the development of variant-adapted bivalent Spike-alone vaccines."

67. Zhang L et al., "SARS-CoV-2 BA.2.86 enters lung cells and evades neutralizing antibodies with high efficiency," *Cell* 2024, 187, 3: P596-608.E17. doi: 10.1016/j.cell.2023.12.025
    - "The origin of the BA.2.86 lineage remains elusive at present and it cannot be excluded that the virus emerged due to evasion of vaccine-induced antibody responses."

68. Zhang Y et al., "Vaccination Shapes Within-Host SARS-CoV-2 Diversity of Omicron BA.2.2 Breakthrough Infection," *J. Infect. Dis.* 2024, 229, 6: 1711-1721. doi: 10.1093/infdis/jiad572
    - "The enrichment of mutations in the spike protein gene indicates selection pressure exerted by vaccination on the evolution of SARS-CoV-2."

69. Zhao H et al., "VOC-alarm: mutation-based prediction of SARS-CoV-2 variants of concern," *Bioinform.* 2022, 38, 14: 3549-3556. doi: 10.1093/bioinformatics/btac370

- "We compared the paces of the evolution that caused the speedy mutation of the VOCs in Stages I, III, V and VII (predicted for Omicron). From Alpha to Delta, the pace of evolution was significantly decreased… which might be related to the fast rollouts of vaccines in late 2020 and early 2021. However, from Delta to Delta plus and Omicron, the pace of evolution has been significantly increased… This might be associated with the adaptiveness of the new VOCs to the selective pressures caused by vaccines."

70. Zhou D et al., "Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and vaccine-induced sera," *Cell* 2021, 184, 9: p2348-2361.e6. doi: 10.1016/j.cell.2021.02.037
    - "The ACE2-binding surface is to some extent the Achilles heel of the virus as it can be blocked by some neutralizing antibodies; however, since it is so small, it also threatens immune escape, as small changes can throw off neutralizing antibodies, thereby reducing the ability of natural or vaccine-acquired immunity to contain viral replication. Selective pressure for changes in the ACE2 interaction surface can thus have two entirely separate drivers. First, as SARS-CoV-2 has recently crossed a zoonotic barrier, it may be expected that evolution of the ACE2 interaction surface may occur to increase affinity to ACE2 and thereby increase viral transmissibility. And second, conversely, changes to the ACE2 interaction surface may also reduce the protection afforded by previous infection or vaccination, potentially leading to escape from pre-existing immunity induced by natural infection or vaccines."