

Revisiting Immunology Textbooks: Considering Potential Insights Based on the Role of RNA-Guided Antiviral Defense

Javdat Muratkhodjaev, Saodat Muratkhodjaeva, Tamara Aripova

Funding: No specific funding was received for this work.

Potential competing interests: No potential competing interests to declare.

Abstract

Amidst the backdrop of the COVID-19 pandemic, this article proposes a cautious reassessment of conventional understandings surrounding the immune system's role in combating viral infections. Departing from the prevailing focus on antibodies and T cells, the manuscript introduces a hypothesis that underscores the potential significance of RNA-mediated antiviral defense mechanisms such as CRISPR-Cas and RNA interference. Through an examination of the vertebrate specialized immune system (SIS) and interferons, the article explores the concept of antibody-dependent enhancement of infection, positioning it as a subject necessitating further investigation rather than a definitive critique of vaccination strategies. Emphasizing the importance of RNA interference as an overlooked element of human antiviral defenses, the authors advocate for a broader and more exploratory discourse within the scientific community regarding the intricate nature of immune responses and vaccine efficacy.

Muratkhodjaev Javdat*, **Muratkhodjaeva Saodat**, and **Aripova Tamara**

Institute of Immunology and Human Genomics Academy of Sciences of Uzbekistan, 100060, Y.Gulamov str.74, Uzbekistan

*corr. author e-mail – javdat_m@yahoo.com

Keywords: antiviral immunity; RNA interference; CRISPR-Cas; interferon; antibody-dependent enhancement; natural immunity; specialized immune system.

1. Introduction

The elucidation of CRISPR-Cas and RNA interference mechanisms in the late 20th century, coupled with the current COVID-19 pandemic, has provided a fertile ground for the formulation of a novel theoretical framework concerning antiviral immunity. However, despite significant advancements, contemporary scientific literature and educational

materials often perpetuate outdated paradigms regarding immune system functionality. Specifically, the dichotomy of innate and acquired immunity, though historically entrenched, warrants critical examination. The conventional categorization erroneously implies that innate immunity exclusively orchestrates immediate inflammatory responses, devoid of specificity or long-term memory, while acquired immunity, confers specificity and immunological memory. Such oversimplifications may engender misconceptions regarding the efficacy of antiviral vaccines, which ostensibly rely solely on the generation of antibodies and T cells. This discourse endeavors to scrutinize the tenets of adaptive immunity, herein referred to as the specialized immune system (SIS).

2. Specialized Immune System (SIS)

The emergence of the specialized immune system (SIS), characterized by T- and B- lymphocytes, approximately 500 million years ago in vertebrates, marked a pivotal juncture in immunological evolution. Traditionally, SIS delineation hinges on lymphocytes expressing recombination-activating gene (RAG)-dependent antigen receptors and the Major Histocompatibility Complex (MHC) [1]. However, the evolutionary imperative underlying the advent of SIS remains a subject of conjecture. Intriguingly, while SIS confers a competitive edge in combating pathogenic incursions and surveilling endogenous cellular aberrations [2], its prominence is conspicuously confined to vertebrates. Remarkably, all flora and invertebrates, which make up 97% of existing fauna species, thrive without T cells and antibodies.

This incongruity underscores the exigency of comprehending the physiological exigencies precipitating SIS emergence. Foremost among these imperatives is the need to maintain organismal integrity in the face of rampant cellular mutagenesis, which engenders a formidable challenge in large-bodied organisms. Evidently, the escalating incidence of somatic mutations necessitates a robust surveillance apparatus to thwart neoplastic transformation—a niche aptly filled by the SIS.

The difficulty of maintaining the integrity of the human body is truly astounding. With approximately 37 trillion cells constantly undergoing mutations, the body's repair and regulation mechanisms play a critical role. The fact that up to a trillion mutations can occur every day highlights the challenges our bodies face in maintaining genetic stability [3]. With age, the accumulation of mutations becomes more pronounced, leading to the dominance of clones initiated by driver mutations in many tissues. It is amazing how the number of mutant cells can increase from less than 40 billion in youth to 130 billion by age 60 [4]. This highlights the dynamic nature of our bodies and the ongoing battle against genetic changes that can potentially lead to disease or cancer.

To effectuate comprehensive cellular regulation, T- and B-cells were engendered alongside the MHC antigenic repertoire. MHC class I molecules, ubiquitously expressed on nucleated cells, serve as cell passports, diligently cataloging an array of antigens wrought by cellular metabolism. The role of T cells in targeting and destroying cancer cells is well-established, but B cells also play a crucial role in this process by producing antibodies tailored to cancer neoantigens. These antibodies bind specifically to these neoantigens, effectively labeling the aberrant cells for subsequent elimination. Acting as a sort of "black tag" for the immune system, antibodies facilitate the identification and destruction of foreign or altered cells. This

mechanism exemplifies a remarkable evolutionary strategy in vertebrates, enabling the immune system to effectively target and eliminate potentially harmful cells without the need for individual surveillance of every cell in the body.

Indeed, the complex role of antibodies in immune surveillance and protection is striking. The Fab fragment, with its antigen-binding specificity, is critical for recognizing not only tumor antigens but also foreign invaders. On the other hand, the Fc fragment plays a key role in organizing the immune response, determining how the target associated with the antibody will be eliminated. The Fc fragment interacts with receptors of various immune cells, such as phagocytes (neutrophils and macrophages), triggering antibody-dependent phagocytosis. In addition, leukocytes, including NK cells, mast cells, eosinophils, basophils, and T lymphocytes, possess receptors for the Fc fragment, which mediates antibody-dependent cytotoxicity. This mechanism allows the immune system to target and destroy cells tagged with specific antibodies.

Moreover, the complement system, containing proteins such as C1q and C3b, interacts with the Fc fragment, initiating complement-dependent cytotoxicity. When these complement proteins bind to the Fc -end of the antibody, they trigger the assembly of the membrane-lytic complex, which leads to the destruction of the target cell.

The coordination between Fab and Fc fragments, as well as the interaction with various immune cells and complement proteins, highlights the complex nature of the immune system's defense mechanisms.

By marking cells with antibodies, the immune system can effectively identify and prioritize targets for destruction, whether invading pathogens or abnormal cells in the body. This targeted approach minimizes the risk of collateral damage to healthy tissue while effectively neutralizing threats to the body's health. This precise mechanism of immune surveillance plays a crucial role in safeguarding the body against a wide range of potential dangers, from cellular abnormalities to infections.

In the context of bacterial, protozoal, fungal, and helminthic infections, antibodies are indeed vital components of the immune response.

When the body encounters these types of pathogens, specialized immune cells detect their presence and trigger the production of antibodies specific to the antigens present on the surface of these pathogens. These antibodies serve as markers, allowing the immune system to identify and target the invading pathogens for destruction.

Once antibodies bind to the surface of the pathogens, they can activate various effector mechanisms to eliminate them. One key mechanism is opsonization, where antibodies coat the surface of the pathogens, making them more easily recognized and ingested by phagocytic cells such as macrophages and neutrophils. These phagocytes then engulf and destroy the opsonized pathogens.

Additionally, antibodies can activate the complement system, a group of proteins that work together to promote inflammation, opsonization, and lysis of pathogens. When antibodies bind to pathogens, they can trigger the activation of complement proteins, leading to the formation of membrane attack complexes that directly lyse the pathogens or mark them for phagocytosis.

In the case of helminthic infections, antibodies can also play a role in mediating immune responses such as eosinophil activation and antibody-dependent cell-mediated cytotoxicity (ADCC), which contribute to the clearance of these parasites.

Overall, the appearance of pathogen-specific antibodies is indeed a highly effective strategy in the immune response against bacterial, protozoal, fungal, and helminthic infections.

In the case of viral infections, the immune system also mounts a response by producing virus-specific antibodies. When a virus infects a host cell and replicates inside it, viral antigens may be presented on the surface of the infected cell. Antibodies can recognize these viral antigens and bind to them, marking the infected cells for destruction through various mechanisms.

One such mechanism is antibody-dependent cellular cytotoxicity (ADCC), where immune cells recognize and bind to the antibodies bound to the infected cells. Once bound, such cells release cytotoxic molecules, leading to the destruction of the infected cells. This process helps eliminate the reservoir of virus within the body and prevents further viral replication and spread. Furthermore, antibodies can also activate the complement system, leading to the formation of membrane attack complexes that directly lyse the infected cells or mark them for phagocytosis by immune cells.

While the destruction of infected cells may seem drastic, it is indeed a beneficial strategy for the organism as a whole. By sacrificing the infected cells, the immune system prevents the spread of the virus and limits the damage caused by the infection.

That's a crucial point to highlight. Viruses are essentially inert outside of host cells; they consist of genetic material (either DNA or RNA) surrounded by a protein coat. They lack the cellular machinery necessary for replication and metabolic activities, so they cannot multiply or cause pathology on their own. For a virus to infect a host cell and initiate replication, it must first bind to specific receptors on the surface of the host cell. These receptors serve as entry points for the virus, allowing it to gain entry into the cell's interior. Each virus has a specific set of receptors that it can bind to, and these receptors are often proteins or other molecules on the surface of host cells.

If a cell lacks the specific receptors required for viral entry, the virus cannot infect that cell. This is why certain viruses have a limited range of host cells and tissues that they can infect. The presence or absence of these receptors can determine the susceptibility of different cell types to viral infection. This selective binding to host cell receptors is an important step in the viral life cycle and is essential for the initiation of infection.

The presence of neutralizing antibodies in the blood is a key aspect of the immune response, particularly in the context of vaccination. These antibodies can bind to viruses and prevent them from infecting target cells, thereby reducing the effective concentration of the virus and limiting its ability to cause infection.

However, the resulting virus-antibody complex must be cleared from the body to effectively control the infection. One mechanism for clearing these complexes involves phagocytosis by immune cells that carry receptors for the Fc fragment of antibodies. These cells recognize the antibody-bound viruses and engulf them, leading to their destruction. In many

cases, this process is successful in clearing the virus from the body and resolving the infection. However, if phagocytosis is not effective and the virus remains viable inside these immune cells, it can lead to a phenomenon known as antibody-dependent enhancement (ADE) of infection.

In ADE, instead of neutralizing the virus, the antibody-virus complex facilitates the entry of the virus into immune cells that may not carry receptors for the virus. This can result in enhanced viral replication and spread, leading to more severe disease symptoms and potentially worsening the outcome of the infection.

Another mechanism of enhancement observed in certain viral infections, including SARS-CoV-2, the virus responsible for COVID-19. In this mechanism, the binding of antibodies to viral antigens can induce conformational changes in the viral proteins, making them more accessible or better able to bind to cellular receptors. For example, in the case of SARS-CoV-2, the spike (S) protein on the surface of the virus binds to the angiotensin-converting enzyme 2 (ACE2) receptor on host cells to facilitate viral entry. Some antibodies may bind to the S protein, and rather than neutralizing the virus, they may induce structural changes that enhance its ability to bind to the ACE2 receptor [5]. This phenomenon can potentially increase viral infectivity and contribute to disease severity.

This mechanism of enhancement through conformational changes in viral proteins highlights the complexity of the interaction between viruses, antibodies, and host cells. It underscores the importance of understanding the intricacies of the immune response to viral infections, especially in the context of vaccine development and therapeutic interventions.

The COVID-19 pandemic has indeed provided valuable insights into the dynamics of the immune response to SARS-CoV-2 infection, particularly regarding neutralizing antibodies and disease severity.

Studies have shown that there is a complex relationship between antibody levels and disease severity in COVID-19 patients. While neutralizing antibodies are typically associated with protection against viral infections, in some cases, higher levels of antibodies have been correlated with more severe disease outcomes in COVID-19 patients [6][7][8].

For example, research has found that hospitalized COVID-19 patients tend to have higher levels of antibodies, including those specific to the spike protein receptor-binding domain (RBD), compared to nonhospitalized individuals [9]. Additionally, there is evidence to suggest that the duration of the antibody response may be prolonged in patients with more severe disease [10].

Furthermore, the emergence of new variants of SARS-CoV-2 has raised concerns about the effectiveness of neutralizing antibodies generated by previous infection or vaccination against these variants. Some variants have shown resistance to neutralization by antibodies, leading to breakthrough infections in vaccinated individuals or reduced efficacy of convalescent plasma therapy.

Recent experimental work has clearly shown the futility of preventing infection with the SARS-Co-2 virus by high titers of neutralizing antibodies alone [11]. An excellent review of the use of mRNA vaccines clearly indicated the weak protective potential of these vaccines, far from the claimed 95% protection [12]. Moreover, the authors state that the use of these vaccines does more harm than good.

In summary, antibodies, while traditionally viewed as key players in combating viral infections, may inadvertently exacerbate viral infections through mechanisms such as antibody-dependent enhancement (ADE), wherein they facilitate viral entry into host cells. This phenomenon expands the range of target cells for viral infection rather than neutralizing the virus, thus challenging the conventional understanding of antibody-mediated immunity against viruses.

Given this complex interplay between antibodies and viral infections, there arises a pressing need to reassess the efficacy and safety of mandatory vaccination practices for viral disease prevention. While vaccines aim to stimulate the immune system to produce antibodies and memory T cells against specific pathogens, the potential risk of ADE underscores the importance of thoroughly evaluating vaccine candidates to mitigate this phenomenon.

Furthermore, the repeated administration of vaccines may have unintended consequences, such as depleting the pool of naïve B and T cells. This depletion could compromise the immune system's ability to mount effective responses against new antigens, including those generated during tumorigenesis. Therefore, careful consideration of the long-term effects of vaccination strategies is warranted to ensure optimal immune function and overall health outcomes.

3. RNA-guided immunity

If we posit that antibodies, and indeed the entire specialized immune system (SIS), are not primarily designed to combat viruses, an inevitable inquiry arises: how, then, do our cells confront the myriad of viral threats?

Viruses stand apart from other pathogenic microorganisms due to their unique characteristics: they lack independent metabolic processes and cannot replicate outside host cells. Consequently, it might be inferred that in the absence of cellular life forms, viruses would not exist. However, the striking structural and genetic parallels observed among viruses infecting the three domains of life suggest their emergence predates modern diversified cells [13][14]. This assertion finds support in structural-phylogenomic investigations comparing viral and cellular proteomes [15].

Presently, it is estimated that the number of viruses on Earth approximates 10^{31} , with each cell, whether nuclear-free, plant, or animal, harboring between 10 to 100 viruses. Such ubiquity underscores the perpetual evolutionary pressure exerted by viruses on cellular life forms. In such a hostile environment, how do cells manage to persist?

The answer lies in the evolutionary ingenuity of prokaryotic organisms, which appeared on Earth approximately 3.7 to 4.2 billion years ago [16][17]. Bacteria and archaea devised an intracellular defense mechanism that effectively retains a memory of prior encounters with viruses. This defense mechanism hinges on the synthesis of specialized sections of DNA, known as CRISPR arrays, upon encountering foreign viral or plasmid genomes. Subsequent interactions involve CRISPR-associated (Cas) proteins, which act as nucleases that digest viral nucleic acids and integrate specific viral sequences called spacers into bacterial DNA. Upon reinfection, small RNAs transcribed from these spacers target nucleases to foreign viral genomes and destroy them, thereby providing adaptive immunity [18][19]. The CRISPR-Cas system represents a genuine adaptive immune mechanism, preserving a repository of spacer sequences derived from encountered viral or plasmid genomes. Notably, the arrangement of spacers within the CRISPR array provides insights

into the historical encounters between bacteria and viruses.

Although the CRISPR-Cas system initially emerged in prokaryotes, it underwent adaptations to accommodate the complexities of multicellular organisms, including the presence of a nuclear membrane and terminal chromosomes. Notably, multicellular organisms, which arose much later in Earth's history, approximately 2.5 billion years ago ^{[20][21]}, harbor a comparable defense mechanism known as RNA interference.

RNA interference, initially identified in the nematode *Caenorhabditis elegans* in 1993^[22], functions as a mechanism for suppressing gene expression through the complementary binding of small interfering RNA (siRNA) within a multiprotein RNA-induced silencing complex (RISC) to the target coding RNA. This interaction results in the suppression of protein synthesis from the coding RNA ^[23]. The mechanism operates via two distinct pathways: complete complementarity between siRNA and coding RNA triggers cleavage by RISC-associated nucleases, while partial complementarity leads to delayed translation and chemical modifications hindering ribosome binding, ultimately silencing gene expression.

The versatility of RNA interference lies in its ability to regulate protein synthesis within cells by controlling the availability of various small interfering RNAs, thereby modulating gene expression—a pivotal aspect of cellular epigenetic control. Notably, the remarkable diversity observed among cell types within an organism, despite their shared genomic content, owes much to the regulatory role of RNA interference in shaping cellular identity and function.

The integration of RNA interference into the cellular machinery underscores its indispensable role in everyday cellular processes across all tissues and organs of multicellular organisms. Particularly noteworthy is the vast repertoire of microRNAs dedicated to thwarting the proliferation of viral proteins within human cells ^[24]. This intrinsic defense mechanism obviates the immediate necessity for incorporating viral spacers into the host DNA, a strategy observed in plant and invertebrate cells ^[25].

However, evidence suggests that the capacity for spacer incorporation is preserved within the human cellular framework, as demonstrated by direct experimental investigations and observations of individuals recovering from viral infections, including COVID-19 ^{[26][27][28][29]}. Notably, the administration of mRNA vaccines has also been shown to induce the formation of specific spacers, underscoring the dynamic interplay between innate antiviral mechanisms and exogenous immunization strategies ^[30]. In this way, new microRNAs can be formed that are specifically designed to combat this virus. In this way, new microRNAs can be formed that are specifically designed to combat this virus. We assume that this mechanism underlies what we call natural immunity, acquired by the body after suffering a viral infection and protecting it for many years.

When delving into human antiviral immunity, it is essential to underscore the mechanisms of protection during early childhood. Recent research has unveiled a remarkable aspect of this defense: breast milk harbors not only antibodies but also an extensive repertoire of microRNAs, numbering up to 1,400 different types ^[31].

Each of these microRNA molecules possesses the capacity to modulate the activity of an average of 15-20 genes, presenting a vast opportunity to regulate gene expression in infants. Studies have demonstrated that upon ingestion, these microRNAs are detectable in the bloodstream and distributed throughout the body's tissues, including the

brain [32][33]. Thus, when discussing antiviral immunity, it is imperative to highlight the significant role of this transfer of protection to the child through breastfeeding.

The presence of such a diverse array of microRNAs in breast milk underscores its multifaceted role in infant health and development, extending beyond mere nutrition. These microRNAs likely contribute to the establishment of the infant's immune system, providing a layer of defense against viral pathogens early in life. Moreover, they may exert long-lasting effects on gene expression patterns, influencing various aspects of immune function and overall health into adulthood.

In conclusion, throughout the course of coevolution with the viral environment, cellular life forms have evolved RNA-controlled antiviral defense systems. Prokaryotes employ the CRISPR-Cas system, while eukaryotes utilize RNA interference. Both systems share the common strategy of employing small RNAs to identify and guide specialized nucleases to dismantle the viral genome. Within the human body, each nuclear cell independently combats viral intrusion through RNA interference. This defense mechanism relies on a diverse pool of microRNAs that target viral nucleotide sequences. Additionally, when necessary, highly specific microRNAs can be generated for a particular virus following the integration of spacers into the DNA of the recovered cell.

4. Auxiliary Antiviral Defense Mechanisms: Interferons

In the intricate battle against viral invasion, highly organized organisms have evolved auxiliary defense mechanisms to swiftly respond to viral threats. The proliferation of densely clustered cells of the same type within these organisms creates an environment ripe for viral spread. Once a virus multiplies within one susceptible cell, it can readily infect neighboring cells, posing a significant challenge to innate RNA-guided defenses, which may struggle to cope with high viral loads. To counteract this risk, an early warning system has been established, utilizing interferons as alarm signals.

Interferons serve as critical components of this defense system, orchestrating a multifaceted response upon detection of viral intrusion. Each nucleated cell possesses interferon receptors, and upon binding to interferon, these receptors initiate a cascade of events that drive the cell into an alarm state [34]. In this heightened state of alert, protein and nucleic acid synthesis are suppressed, endocytosis and exocytosis are inhibited, thereby impeding both viral entry and exit [35].

Interestingly, interferon production is triggered within cells that have already been infiltrated by viruses. Special cytoplasmic receptors, known as RLR receptors, play a pivotal role in this process by recognizing viral double-stranded RNA [36][37]. Activation of these receptors sets off a series of intracellular mechanisms culminating in the synthesis of interferons and pro-inflammatory cytokines. Cells receiving the interferon signal enter an antiviral state, halting the synthesis of viral proteins and DNA and preventing further infection.

Analogous to a person reacting to a gas attack by holding their breath to avoid inhaling toxins, cells respond to interferon signals by halting essential cellular processes to prevent viral propagation. However, prolonged and excessive interferon synthesis can lead to a pro-inflammatory state, triggering a cytokine storm and cell apoptosis. The wide-reaching effects of interferons are evidenced by their significant impact on the functioning of over 12 thousand genes, as documented on the

Interferome website (<http://interferome.its.monash.edu.au/interferome/site/dbStat.jspx>). This broad influence underscores the lack of specificity in the interferon response, highlighting its divergence from a truly specific and memory-based defense system.

In summary, while interferons play a crucial role in initiating an early antiviral response, their broad and nonspecific effects underscore the need for more targeted and memory-based defense mechanisms to combat viral infections effectively.

5. A New Interpretation of Immunity

The insights provided above necessitate a reevaluation of the traditional definition of the immune system. The dichotomy between "innate" and "adaptive" immunity must be reconsidered, as every cell possesses the capacity to adaptively combat pathogens through RNA interference, rendering this division inadequate. Moreover, if we acknowledge the sentinel role of the Specialized Immune System (SIS) in safeguarding the body's integrity, we must recognize that its function extends beyond mere adaptation to diverse pathogens.

Analogously, if we liken an animal organism to a social structure, initially, each "citizen" cell addresses its individual threats, utilizing RNA interference. However, as the organism evolves, analogous to the emergence of police and military forces in society, leukocytes and phagocytes, aided by complement and other humoral factors, serve as defenders. Furthermore, specialized monitoring organizations akin to the KGB or FBI emerge to identify internal threats and dissidents. T-cells diligently scrutinize all cells for self-identity, while B-cells, with the assistance of antibodies, flag "undesirable" cells, directing leukocytes and phagocytes to eliminate them. While these comparisons may seem metaphorical, the accurate delineation and classification of immune system components represent a significant and pressing challenge for the future. By discarding the outdated paradigm of immunity, we can avoid providing a rationale for the development of various antiviral vaccines.

It is imperative to reiterate that every nuclear cell in our body possesses the capability to combat viruses via RNA interference. This adaptive system retains memory and, over years of co-evolution with viruses, accumulates a diverse pool of microRNAs primed to suppress the replication of a wide array of viruses. Moreover, in the event of failure, new microRNAs tailored to combat specific viruses are generated. This underpins the concept of natural immunity, which warrants further exploration.

In conclusion, a paradigm shift in our understanding of antiviral protection is urgently required. The current immunological framework must be revised to encompass the adaptive and intricate mechanisms employed by cells to combat viral threats effectively.

Statements and Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors declare that they have no competing interest

Funding

No funding was used to write this article.

Authors' contributions

Saodat wrote about RNA-dependent immunity, Tamara wrote chapters on the specialized immune system. Javdat was responsible for the general idea of the article, and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

Authors' information

All authors of the article are employees of the Institute of Immunology and Human Genomics of the Academy of Sciences of the Republic of Uzbekistan.

References

1. [^] Flajnik, M.F. A cold-blooded view of adaptive immunity. *Nat Rev Immunol* 18, 438–453 (2018). <https://doi.org/10.1038/s41577-018-0003-9>
2. [^] Hirano M, Das S, Guo P, Cooper MD. The evolution of adaptive immunity in vertebrates. *Adv Immunol.* 2011;109:125-157. doi:10.1016/B978-0-12-387664-5.00004-2
3. [^] Burgess, D.J. A body-wide view of somatic mutations. *Nat Rev Genet* 22, 689 (2021). <https://doi.org/10.1038/s41576-021-00420-1>
4. [^] Evans, E. J., & DeGregori, J. (2021). Cells with cancer-associated mutations overtake our tissues as we age. *Aging and Cancer*, 2(3), 82-97. <https://doi.org/10.1002/aac2.12037>

5. [^] An infectivity-enhancing site on the SARS-CoV-2 spike protein targeted by antibodies Liu, Yafei et al. *Cell*, Volume 184, Issue 13, 3452 - 3466.e18. <https://doi.org/10.1016/j.cell.2021.05.032>
6. [^] Dan JM, Mateus J, Kato Y, Hastie KM, Yu ED, Faliti CE et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science* (80-). 2021; 371. <https://doi.org/10.1126/science.abf4063>
7. [^] Röltgen K, Powell AE, Wirz OF, Stevens BA, Hogan CA, Najeeb J et al. Defining the features and duration of antibody responses to SARS-CoV-2 infection associated with disease severity and outcome. *Sci Immunol*. 2020; 5. <https://doi.org/10.1126/sciimmunol.abe0240>
8. [^] Wu J, Liang B, Chen C, Wang H, Fang Y, Shen S, et al. SARS-CoV-2 infection induces sustained humoral immune responses in convalescent patients following symptomatic COVID-19. *Nat Commun*. 2021;12:1813
9. [^] Lapuente, D., Winkler, T. H., & Tenbusch, M. (2024). B-cell and antibody responses to SARS-CoV-2: Infection, vaccination, and hybrid immunity. *Cellular & Molecular Immunology*, 21(2), 144-158. <https://doi.org/10.1038/s41423-023-01095-w>
10. [^] Chen, X., Pan, Z., Yue, S., Yu, F., Zhang, J., Yang, Y., Li, R., Liu, B., Yang, X., Gao, L., Li, Z., Lin, Y., Huang, Q., Xu, L., Tang, J., Hu, L., Zhao, J., Liu, P., Zhang, G.,... Ye, L. (2020). Disease severity dictates SARS-CoV-2-specific neutralizing antibody responses in COVID-19. *Signal Transduction and Targeted Therapy*, 5(1), 1-6. <https://doi.org/10.1038/s41392-020-00301-9>
11. [^] Yan, Jingyi et al. Distinct roles of vaccine-induced SARS-CoV-2-specific neutralizing antibodies and T cells in protection and disease. *Molecular Therapy*, Volume 32, Issue 2, P540-555, (FEBRUARY 07, 2024) doi.org/10.1016/j.ymthe.2024.01.007
12. [^] Mead M, Seneff S, Wolfinger R, et al. COVID-19 mRNA Vaccines: Lessons Learned from the Registrational Trials and Global Vaccination Campaign. *Cureus* 16(1): e52876. (January 24, 2024) [doi:10.7759/cureus.52876](https://doi.org/10.7759/cureus.52876)
13. [^] Benson, S. D., Bamford, J. K. H., Bamford, D. H., and Burnett, R. M. (2004). Does common architecture reveal a viral lineage spanning all three domains of life? *Mol. Cell* 16, 673–685. [doi: 10.1016/j.molcel.2004.11.016](https://doi.org/10.1016/j.molcel.2004.11.016)
14. [^] Krupović, M., and Bamford, D. H. (2008). Virus evolution: how far does the double beta-barrel viral lineage extend? *Nat. Rev. Microbiol.* 6, 941–948. [doi: 10.1038/nrmicro2033](https://doi.org/10.1038/nrmicro2033)
15. [^] Nasir, A., and Caetano-Anollés, G. (2015). A phylogenomic data-driven exploration of viral origins and evolution. *Sci. Adv.* 1:e1500527. [doi: 10.1126/sciadv.1500527](https://doi.org/10.1126/sciadv.1500527)
16. [^] Knoll, Andrew H.; Javaux, Emmanuelle J.; Hewitt, David; et al. "Eukaryotic organisms in Proterozoic oceans". *Philosophical Transactions of the Royal Society B.* 361 (1470): 1023–1038. (2006). [doi:10.1098/rstb.2006.1843](https://doi.org/10.1098/rstb.2006.1843).
17. [^] Fedonkin, Mikhail A. (March 31, 2003). "The origin of the Metazoa in the light of the Proterozoic fossil record" (PDF). *Paleontological Research*. 7 (1): 9–41. [doi:10.2517/prpsj.7.9](https://doi.org/10.2517/prpsj.7.9). ISSN 1342-8144. S2CID 55178329. Archived from the original (PDF) on 2009-02-26. Retrieved 2008-09-02.
18. [^] Rodolphe Barrangou. The Roles of CRISPR-Cas Systems in Adaptive Immunity and Beyond. *Current opinion in immunology*, 2015, Vol. 32, pp. 36-41, [doi:10.1016/j.coi.2014.12.008](https://doi.org/10.1016/j.coi.2014.12.008)
19. [^] Eugene V. Koonin, Kira S. Makarova. Mobile Genetic Elements and Evolution of CRISPR-Cas Systems: All the Way There and Back. *Genome Biology and Evolution*, 2017, Vol. 9 (10), pp.2812–2825, [doi: 10.1093/gbe/evx192](https://doi.org/10.1093/gbe/evx192)
20. [^] Rosing, M. T. 13C-Depleted Carbon Microparticles in >3700-Ma Sea-Floor Sedimentary Rocks from West Greenland.

Science, 283(5402), 674–676 (1999). doi:10.1126/science.283.5402.674

21. [^]Dodd, M., Papineau, D., Grenne, T. et al. Evidence for early life in Earth's oldest hydrothermal vent precipitates. *Nature* 543, 60–64 (2017). <https://doi.org/10.1038/nature21377>
22. [^]Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell*. 1993;75(5):843-854. doi:10.1016/0092-8674(93)90529-y
23. [^]Fire A., Xu S., Montgomery M., Kostas S., Driver S., Mello C. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature*, 1998, Vol. 391, 6669, pp. 806–811, doi:10.1038/35888
24. [^]Watanabe Y, Kishi A, Yachie N, Kanai A, Tomita M: Computational analysis of microRNA-mediated antiviral defense in humans. *FEBS Lett*. 2007, 581: 4603-4610. 10.1016/j.febslet.2007.08.049
25. [^]Jeang, KT. RNAi in the regulation of mammalian viral infections. *BMC Biol* 10, 58 (2012). <https://doi.org/10.1186/1741-7007-10-58>
26. [^]Liguo Zhang, Alexsia Richards, M. Inmaculada Barrasa, Stephen H. Hughes, Richard A. Young, Rudolf Jaenisch. Reverse-transcribed SARS-CoV-2 RNA can integrate into the genome of cultured human cells and can be expressed in patient-derived tissues. *Proceedings of the National Academy of Sciences* May 2021, 118 (21) e2105968118; DOI: 10.1073/pnas.2105968118
27. [^]Ying Yin, Xiao-zhao Liu, Ximiao He and Li-quan Zhou. Exogenous Coronavirus Interacts With Endogenous Retrotransposon in Human Cells. *Front Cell Infect Microbiol*. 2021 Feb 25;11:609160. doi: 10.3389/fcimb.2021.609160
28. [^]Liu, Y., Rao, J., Mi, Y., Chen, L., Feng, L., Li, Q., Geng, J., Yang, X., Zhan, X., Ren, L., Chen, J., & Zhang, X. (2022). SARS-CoV-2 RNAs are processed into 22-nt vsRNAs in Vero cells. *Frontiers in Immunology*, 13. <https://doi.org/10.3389/fimmu.2022.1008084>
29. [^]Dhuli K, Medori MC, Micheletti C, et al. Presence of viral spike protein and vaccinal spike protein in the blood serum of patients with long-COVID syndrome. *Eur Rev Med Pharmacol Sci*. 2023;27(6 Suppl):13-19. doi:10.26355/eurrev_202312_34685
30. [^]Markus Aldén, Francisko Olofsson Falla, Daowei Yang, Mohammad Barghouth, Cheng Luan, Magnus Rasmussen and Yang De Marinis. Intracellular Reverse Transcription of Pfizer BioNTech COVID-19 mRNA Vaccine BNT162b2 In Vitro in Human Liver Cell Line. *Curr. Issues Mol. Biol*. 2022, 44(3), 1115-1126; doi.org/10.3390/cimb44030073
31. [^]Benmoussa A, Provost P. Milk MicroRNAs in Health and Disease. *Compr Rev Food Sci Food Saf*. 2019, 18(3), pp.703-722, doi: 10.1111/1541-4337.12424.
32. [^]Manca S, Upadhyaya B, Mutai E, Desaulniers AT, Cederberg RA, White BR, Zemleni J. Milk exosomes are bioavailable and distinct microRNA cargos have unique tissue distribution patterns. *Sci Rep*. 2018, 8(1):11321, doi: 10.1038/s41598-018-29780-1.
33. [^]Zemleni J. Milk exosomes: beyond dietary microRNAs. *Genes Nutr*. 2017, 12, 12, doi: 10.1186/s12263-017-0562-6.
34. [^]Pestka S, Langer JA, Zoon KC, Samuel CE. Interferons and their actions. *Annu Rev Biochem*. 1987;56:727-777. doi:10.1146/annurev.bi.56.070187.003455
35. [^]Levy DE. Whence interferon? Variety in the production of interferon in response to viral infection. *J Exp Med*. 2002 Feb 18;195(4):F15-8. doi: 10.1084/jem.20020075
36. [^]Jiaxi Wu, Zhijian J Chen. Innate Immune Sensing and Signaling of Cytosolic Nucleic Acids. *Annu Rev Immunol*. 2014,

32, pp. 461-488, doi: 10.1146 /annurev-immunol-032713-120156

37. [^] Daniel B. Stetson and Ruslan Medzhitov. *Type I Interferons in Host Defense*. *Immunity*. 2006, Vol. 25, 3, pp.373-381, doi: 10.1016/j.immuni.2006.08.007.