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The Immunopathology of Covid-19

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ABSTRACT

SARS-CoV-2 is a virus causing Covid-19, with a genome encoding proteins for replication. The innate immune system responds rapidly, employing pattern recognition receptors (PRRs) detecting viral presence. Mucosal tissues offer initial defense, with interferons inhibiting viral replication. Inflammatory cytokines and chemokines attract immune cells, sometimes resulting in cytokine storm. However, excessive inflammation may worsen disease outcomes. The adaptive immune system, comprising CD4+ T cells, CD8+ T cells, and B cells, organize targeted responses. CD4+ T cells activate immune cells and coordinate responses, while CD8+ T cells eliminate infected cells using cytotoxic molecules. B cells produce antibodies that neutralize the virus. Memory B and T cells provide long-term immunity, aiding rapid response upon reinfection. Recovered individuals often exhibit reduced reinfection risks due to memory immunity. Vaccines, like mRNA-based and viral vector-based types, stimulate similar immune responses, bolstering immunity and reducing severe illness.

Introduction

Covid-19, caused by SARS-CoV-2, is a rapidly growing virus first detected in 2019 in Wuhan, China. Henceforth, the number of cases associated with Covid-19 has grown exponentially, impacting people from over 100 countries worldwide. According to the World Health Organization (WHO), as of 2022, there have been more than 500 million cases with at least 6 million deaths of Covid-19 globally. As Covid-19 continues to evolve and emerge as new variants, it has become difficult in controlling the spread of the rapid disease. Scientists are continuing to research the virology of SARS-CoV-2, and they are also studying the roles our immune system, specifically the innate and adaptive immune systems, play in developing countermeasures against the SARS-CoV-2 virus. Understanding the virology of SARS-CoV-2 and our immune responses is critical for developing reliable treatment measures and preventing the spread of Covid-19. This research will extensively analyze the virology of the SARS-CoV-2 virus and the innate and adaptive immune systems' functions against the virus.

Pathogenesis of Covid-19

SARS-CoV-2, the causative agent of Covid-19, belongs to the family of single-stranded positive-sense RNA viruses. Its genome, spanning approximately 30 kilobases (kb), consists of 14 open reading frames (ORFs), responsible for encoding essential structural and non-structural proteins (NSPs) necessary for viral replication and survival. At the core of the viral genome, the 5’ untranslated region (UTR) serves as the initiation site for translation, while the 3’UTR marks its termination. Among the two main ORFs, ORF1a and ORF1b, which collectively account for about 65% of the viral genome, polyproteins pp1a and pp1ab are encoded. Proteolytic breakdown of these polyproteins by specific proteases yields 16 NSPs responsible for transcription, replication, and evasion of host defenses. The remaining 35% of the genome consists of four ORFs that encode structural proteins (spike, envelope, membrane, and nucleocapsid protein), organizing the virion assembly process, and eight ORFs that regulate viral infection via the production of accessory proteins.

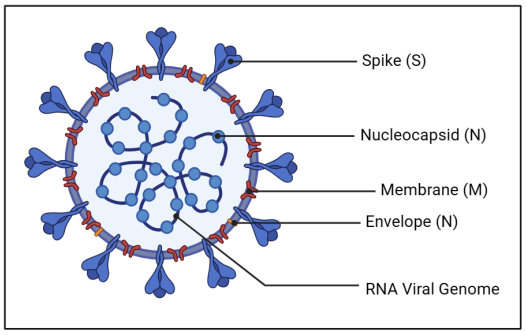

Figure 1. The virion structure of SARS-CoV-2.

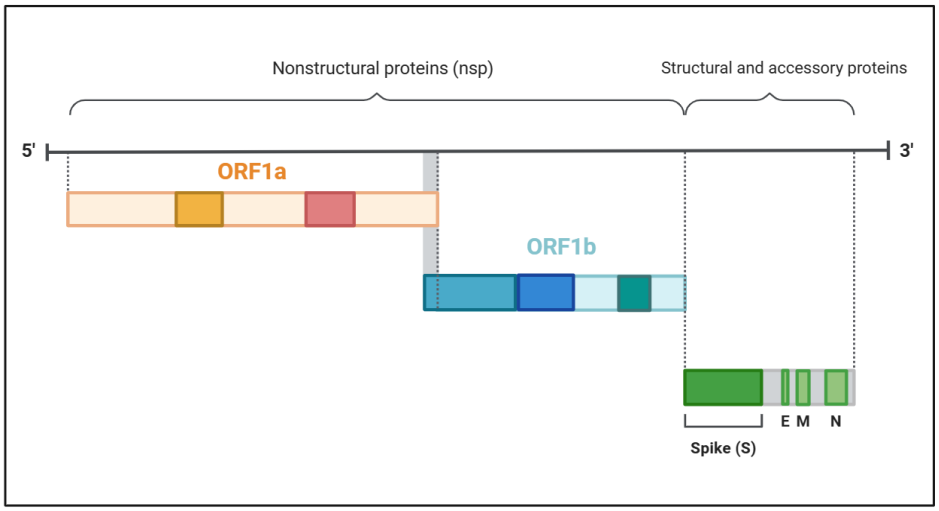

Figure 2. The genomic organization of SARS-CoV-2.

Cell Entry

The primary target of SARS-CoV-2 is the lung epithelium, where it uses the angiotensin-converting enzyme 2 (ACE-2) receptor for host cell binding. SARS-CoV-2 releases glycosylation of S (spike) proteins that drive the virus entry into the host cell surface. The transmembrane serine protease 2 (TMPRSS2) is the major protease that SARS-CoV-2 uses to activate the S protein and complete the binding process. TMPRSS2 allows the cleaving of the S protein into subunits S1 and S2. The S1 subunit includes the receptor-binding domain (RBD) region in the S protein to bind to the ACE-2 receptor, and the the S2 portion triggers endocytosis through the deployment of spike fusion peptides, catalyzing viral cell fusion in the host cell membranes. Thus, the viral replication of the SARS-CoV-2 virus begins.

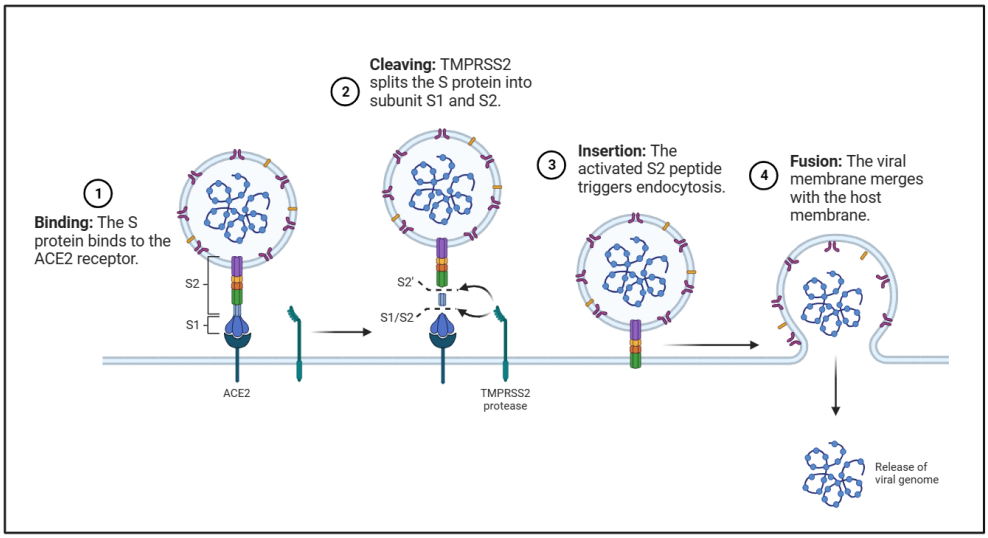

Figure 3. The S protein interacts with the ACE2 receptor and undergoes cleavage at specific sites known as S1/S2 and S2′ by the TMPRSS2 protease. This results in the activation of the S2 domain and the fusion between the viral membrane and host membrane.

Transmission and Replication

Following successful cell entry, the viral genome, comprising RNA, is injected into the host cell membrane. This genomic RNA serves as a blueprint for the RNA-dependent RNA polymerase (RdRp), a crucial enzyme responsible for viral replication. The genomic RNA is then translated into polyprotein pp1a and pp1ab, organized by the host cell mechanisms. These polyproteins are subsequently organized into replication/transcription complexes (RTC) within the host cell. It is at these RTCs where the subgenomic RNA synthesis takes place, leading to the encoding of structural proteins and accessory proteins essential for the construction of new virus particles.

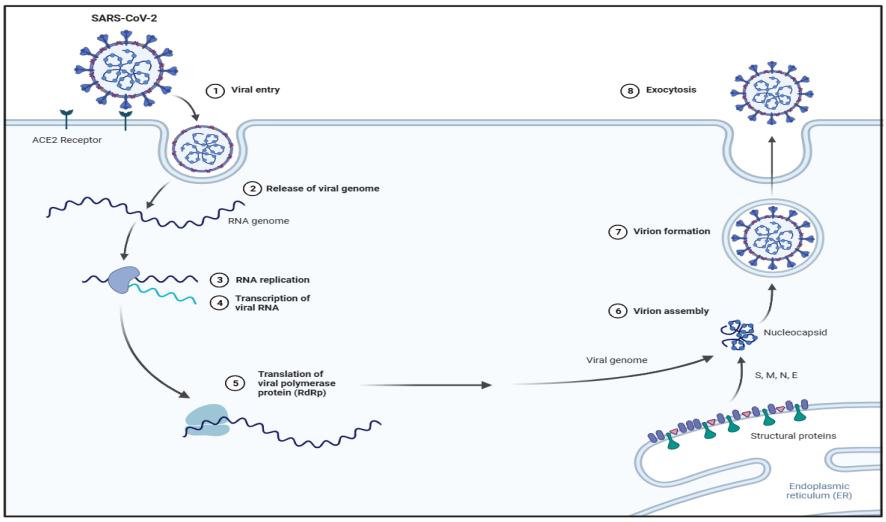

Figure 4. After the SARS-CoV-2 virus is incorporated into the host cell, the ribosome translates the viral polymerase protein. The RdRp enzyme carries the new RNA where it encodes the structural proteins necessary for virion assembly.

Innate Immune System

The innate immune system is the first line of defense when any pathogen like SARS-CoV-2 enters our body. The innate immune cells use pattern recognition receptors (PRRs) to detect pathogen-associated molecular patterns (PAMP). PRRs recognize RNA viruses such as the coronavirus using cytosolic and endosomal RNA sensors including retinoic acid-inducible gene I (RIG-I) and toll-like receptors (TLRs). RNA virus recognition results in the activation of cytokines, chemokines, and interferons.

Mucosal surfaces have the first contact and protect against the virus via mucosa-associated lymphoid tissues (MALT). Since SARS-CoV-2 enters through the respiratory tract, oral mucosa and conjunctival epithelium, mucosal immunoglobulin A (IgA) plays an important role in defending these barriers. As SARS-CoV-2 manages to infect epithelial cells, interferons are produced in the virus-infected cells to allow a more robust innate immune response to occur. The interferon (IFN) response is a group of signaling proteins created in the presence of viruses. Many respiratory diseases, such as Covid-19 rely on IFN types 1 and 3 to play an important role in mediating the infection caused by SARS-CoV-2 viruses. IFN types 1 via IFNα/β receptor (IFNAR) can produce an antiviral state by the Janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling pathway. Upon IFNAR signaling, STAT 1, STAT 2, and interferon regulatory factor (IRF) 9 form a complex that translocates to the nucleus to stimulate the transcription of IFN-stimulated genes (ISGs). These ISG proteins, including IFN-induced transmembrane (IFITMs) proteins 1, 2, and 3 control and limit SARS-CoV-2 infection. Even though type 1 IFN is effective against SARS-CoV-2 infections, SARS-CoV-2 employs various strategies to suppress interferon defenses. For SARS-CoV-2, non-structural proteins including ORF6 and nsp1 interfere with the IFN signaling, inhibiting STAT molecules and IRF to the nucleus. Type 3 IFNs are induced earlier and are limited to epithelial cells, neutrophils, and specific activated immune cells (dendritic cells, macrophages, and B cells). Type 3 IFNs have a specialized role in limiting the spread of the virus in the respiratory tract to the lungs.

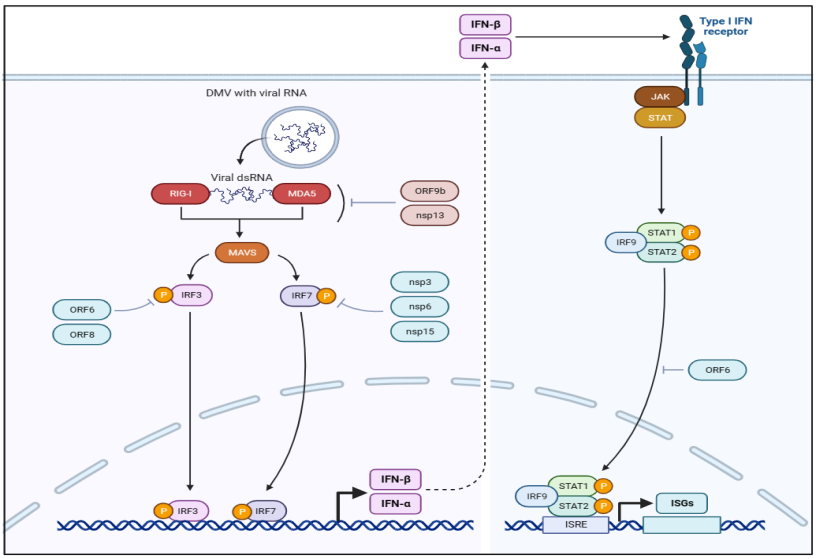

Figure 5. SARS-CoV-2 utilizes its ORF9b and nsp13 proteins to disrupt the function of RIG-I and MDA5, which are part of the RIG-I-like receptor (RLR) family. This disruption leads to a hindrance in the downstream

signaling processes that are normally facilitated by IRF3 and IRF7. Additionally, the virus strategically modulates the MAVS adaptor protein, which typically plays a key role in transmitting antiviral signals initiated by RLRs. Furthermore, specific viral proteins including ORF6, ORF8, nsp3, and nsp6 counteract the production of both IFN-B and IFN-A, subsequently impeding the activation of the JAK-STAT pathway. Moreover, SARS-CoV-2 interferes with the proper phosphorylation and activation of important molecules like STAT1, STAT2, and IRF9, resulting in a further reduction of the expression of interferon-stimulated genes (ISGs) that are critical for effective antiviral defense mechanisms.

The induction of dendritic cells, macrophages, and neutrophils start the immune reaction. When these immune cells are activated, they produce pro-inflammatory cytokines, which contribute to the cytokine release syndrome (CRS), also known as cytokine storm. In particular, ACE2-expressing macrophages carrying SARS-CoV-2 nucleoprotein antigen resulted in excessive production of interleukin (IL) 6, causing extreme inflammation. In SARS, the intensity of IL-6 production was considerably higher than in other viral respiratory infections (influenza and parainfluenza) suggesting that blocking pro-inflammatory cytokines could be a possible way to develop therapeutic treatments for Covid-19. Moreover, it has been observed that some patients do not develop the cytokine storm as a rapid response to coronavirus; several patients remained afebrile with the coronavirus disease. It is presumed that the adaptive immune system plays a role in limiting the infection for asymptomatic patients. Therefore, developing countermeasures during the asymptomatic or early stage of the disease may be the most effective way to boost immune responses.

High levels of chemokine in patients also caused severe cases of lung pathology. Macrophages produce chemokines such as monocyte chemotactic protein (MCP) and chemokine C–C ligand 2 (CCL2) to recruit leukocytes like neutrophils. High numbers of neutrophils with increased neutrophil-to-lymphocyte ratio (NLR) were detected in blood-vessels which suggest that inflammatory responses in the lungs may be more devastating than the disease itself.

Innate immunity, although crucial for the initial response to Covid-19, individuals are susceptible to more severe symptoms because of the low efficacy responses. The innate immune system plays an important role in detecting viruses like SARS-CoV-2 using tools such as PRRs, and the responses it produces will significantly affect the disease outcome. The innate immune system expressed high levels of pro-inflammatory cytokines, caused by the cytokine storm, which correlated with disease progression to the respiratory tract. Furthermore, various innate cytokines such as macrophages and neutrophils only deteriorated the condition of innate immunity. So far, the innate immune system plays a pivotal role in disease progression because of the excessive inflammatory response, and as a result, causing more harm than good.

Adaptive Immune System

The second line of defense is the adaptive immune system, consisting of specialized immune cells to attack a specific target and develop immunological memory to remember the specificity of antigens. The adaptive response produces three major cell types: CD4+ T cells, CD8+ T cells, and B cells in response to SARS-CoV-2 infection. Each specialized cell has their own important roles in controlling the SARS-CoV-2 virus and are necessary to robust immunity. With the help of B cells, CD4+ T cells, and CD8+ T cells, the production of antibodies and antigen-specific cytotoxic T-lymphocytes can achieve both cellular and humoral immunity within the Covid-19 disease.

CD4+ T Cells

CD4+ T cells play a pivotal role in coordinating the immune response against viral infections, including SARS-CoV-2. These specialized T cells have the ability to recognize specific antigens using their T cell receptors (TCRs) and are essential for organizing an effective immune defense. CD4+ T cells are found within the broader

population of T cells and are crucial for the activation and regulation of other immune cells, such as B cells and CD8+ T cells, as well as the secretion of cytokines that facilitate cell recruitment and communication within the immune system. One subset of CD4+ T cells, known as T helper 1 (Th1) cells, possesses potent anti-pathogen functions. Th1 cells are particularly involved in cell-mediated immune responses. Upon encountering viral antigens, Th1 cells produce interferon-gamma (IFNγ) and other cytokines that promote the activation of cytotoxic CD8+ T cells. These cytotoxic T cells are responsible for the direct elimination of virus-infected cells through the induction of apoptosis, a programmed cell death process. By initiating the cytotoxic response, Th1 cells contribute to the control and clearance of the viral infection. Another subset of CD4+ T cells, called T follicular helper cells (Tfh cells), plays a critical role in initiating and maintaining humoral immunity. Tfh cells are instrumental in the activation of B cells, which are responsible for producing antibodies. Tfh cells stimulate B cells to differentiate into antibody-secreting plasma cells. Additionally, Tfh cells facilitate the formation of memory B cells, which can provide long-term protection and rapid antibody production upon subsequent exposure to the virus. This collaborative effort between Tfh cells and B cells ensures the generation of a robust and sustained humoral immune response. In severe cases of COVID-19, a significant presence of CCR6+ circulating Tfh (cTfh) cells has been observed, suggesting a potential association with Th17 cell-mediated immunopathology. Th17 cells are a subset of CD4+ T cells that produce interleukin-17 (IL-17) cytokines. However, the levels of IL-17 cytokines in severe COVID-19 cases have been reported to be low. On the other hand, there appears to be a robust production of CCR6+ IL-22, which is generated by SARS-CoV-2-specific CD4+ T cells. IL-22 has been associated with tissue repair processes, particularly in the context of lung epithelial cell repair during SARS-CoV-2 infection.

CD8+ T Cells

During SARS-CoV-2 infection, CD8+ T cells become activated and differentiate into effector cells, ready to eliminate the infected cells. These effector CD8+ T cells release a repertoire of molecules with potent cytotoxic effects, including interferon-gamma (IFNγ), granzyme B, perforin, and CD107a. IFNγ, a key cytokine produced by CD8+ T cells, is crucial for controlling viral replication. It helps to limit the spread of the virus by inhibiting viral replication within infected cells and activating other immune cells to combat the infection. Granzyme B and perforin, on the other hand, induce apoptosis in infected cells. Granzyme B activates caspases, leading to programmed cell death, while perforin creates pores in the target cell's membrane, allowing granzyme B to enter and initiate cell death pathways. CD107a, also known as lysosomal-associated membrane protein 1 (LAMP-1), is involved in the degranulation process of CD8+ T cells, facilitating the release of cytotoxic molecules to eliminate infected cells. Multiple studies have demonstrated the importance of SARS-CoV-2-specific CD8+ T cells in determining the outcomes of COVID-19. These CD8+ T cells specifically recognize viral antigens presented on the surface of infected cells, including spike, nucleocapsid, membrane, and ORF3a proteins. By targeting these viral proteins, CD8+ T cells contribute to the elimination of infected cells and the control of viral replication.

One remarkable characteristic of CD8+ T cell responses during acute COVID-19 is their rapid development. In some cases, virus-specific CD8+ T cells have been detected as early as day 1 after symptom onset. This highlights the early immune defense mounted by CD8+ T cells against SARS-CoV-2. Their prompt activation and differentiation into effector cells reflect their crucial role in the initial containment and clearance of the virus.

B Cells and Antibodies

Upon encountering SARS-CoV-2, antigen-presenting cells, prominently dendritic cells, capture and present viral antigens. These antigen-presenting cells then migrate to the lymph nodes, where they encounter CD4+ T

cells. CD4+ T cells, equipped with specific TCRs, recognize these viral antigens, thus initiating their activation. Subsequently, CD4+ T cells differentiate into Tfh cells, key players in guiding B cell activation. Within the germinal centers of lymph nodes, Tfh cells engage with B cells that display the same viral antigens. This interaction triggers a series of complex signaling events that result in the activation and differentiation of B cells into plasma cells. These specialized plasma cells become antibody factories, producing vast quantities of antibodies tailored to target the invading SARS-CoV-2. The antibodies generated during B cell differentiation play a pivotal role in neutralizing SARS-CoV-2. Neutralizing antibodies bind specifically to viral antigens, preventing the virus from entering host cells and interfering with its replication cycle. By doing so, they act as a potent first line of defense, impeding viral spread and controlling the infection. Furthermore, these antibodies also contribute to antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP). ADCC involves the binding of antibodies to infected cells, which, in turn, activates natural killer (NK) cells to kill the antibody-bound cells. Similarly, ADCP relies on the attachment of antibodies to infected cells, facilitating their uptake and destruction by phagocytic immune cells. In addition to the initial effector response, the adaptive immune system generates memory T cells that persist long after the resolution of the acute infection. Memory CD4+ and CD8+ T cells are instrumental in conferring long-term immunity against SARS-CoV-2. These memory cells possess an enhanced capacity to recognize the virus upon re-exposure, resulting in a rapid and robust immune response that can effectively control viral replication and prevent severe disease. Memory CD4+ T cells, also known as central memory T cells (Tcm), reside in the lymphoid tissues, such as lymph nodes and spleen, where they continuously survey for the presence of viral antigens. In contrast, memory CD8+ T cells, termed effector memory T cells (Tem), are distributed throughout peripheral tissues, including the lungs, gastrointestinal tract, and other mucosal surfaces. This strategic distribution allows them to promptly encounter and eliminate infected cells in tissues, where the virus often establishes initial infection.

Long-Term Immunity

One of the most critical aspects of the adaptive immune response to SARS-CoV-2 is the development of long-term immunity, which plays a pivotal role in protecting individuals from reinfections. Upon resolution of the acute infection, the immune system generates memory B cells and memory T cells that persist in the body, providing a reservoir of immunological memory. This memory enables a faster and more robust immune response upon re-exposure to the virus, preventing severe disease and reducing the risk of reinfection.

Memory B cells, a subset of B cells, retain the ability to recognize the specific antigens presented by SARS-CoV-2. These memory B cells reside in lymphoid tissues and continuously survey for the presence of viral antigens. Upon encountering the virus again, memory B cells undergo rapid activation and differentiation into plasma cells. These plasma cells rapidly produce large quantities of antibodies tailored to neutralize the invading virus. The presence of memory B cells and the rapid antibody response they trigger contribute significantly to the control of viral replication and the prevention of severe illness during reinfections.

Memory T cells, including both CD4+ and CD8+ memory T cells, are crucial in conferring long-term immunity against SARS-CoV-2. Central memory CD4+ T cells (Tcm) reside in lymphoid tissues, such as lymph nodes and spleen, where they continually monitor for viral antigens. Effector memory CD8+ T cells (Tem), on the other hand, are distributed throughout peripheral tissues, including the lungs, gastrointestinal tract, and other mucosal surfaces. This strategic distribution allows Tem cells to promptly encounter and eliminate infected cells in tissues, where the virus often establishes initial infection. During reinfections, memory T cells play a crucial role in recognizing and targeting virus-infected cells. CD8+ memory T cells specifically recognize viral antigens presented on the surface of infected cells, including proteins such as spike, nucleocapsid, membrane, and ORF3a. The recognition of these antigens triggers the activation of CD8+ T cells, leading to the release of cytotoxic molecules.

Research has shown that individuals who have recovered from a SARS-CoV-2 infection tend to have a reduced risk of reinfection compared to those who have not been previously infected. Moreover, the presence of memory B and T cells in recovered individuals may provide cross-reactive immunity against emerging SARS-CoV-2 variants. However, it is essential to note that the longevity and effectiveness of memory immunity may vary among individuals and may be influenced by factors such as age, underlying health conditions, and the severity of the initial infection.

The effectiveness of long-term immunity against reinfections is a crucial factor in shaping vaccination strategies. Vaccines for Covid-19, such as mRNA-based vaccines and viral vector-based vaccines, aim to mimic natural infection and induce robust memory B and T cell responses. By doing so, they enhance the body's ability to mount an effective immune response upon exposure to the virus, preventing severe disease and reducing transmission.

Conclusion

In conclusion, the immune response to SARS-CoV-2 is a multi-layered defense mechanism comprising the innate and adaptive immune systems. The innate immune system acts as the first line of defense, rapidly identifying the virus through pattern recognition receptors (PRRs) and initiating a cascade of responses that include the production of interferons, cytokines, and chemokines. Mucosal surfaces play a pivotal role in the early defense, with mucosa-associated lymphoid tissues (MALT) guarding entry points like the respiratory tract.

The adaptive immune system, consisting of CD4+ T cells, CD8+ T cells, and B cells, organizes a more targeted and specific response. CD4+ T cells coordinate immune cells and produce cytokines, enhancing the immune reaction. CD8+ T cells eliminate infected cells directly, curbing viral spread. B cells generate antibodies that neutralize the virus. Importantly, memory B and T cells ensure long-term immunity, facilitating a quicker and stronger response upon re-exposure.

This intricate interplay between the innate and adaptive immune systems is crucial in mitigating SARS-CoV-2 infections. It offers insights into the mechanisms of immunity and potential avenues for therapeutic interventions, including vaccine development. By deciphering the immune response's complexities, researchers aim to harness its full potential to combat Covid-19 effectively and inform strategies that bolster immune defenses against emerging variants. As the virus continues to evolve, a comprehensive understanding of the immune response remains important in our ongoing battle against the global health challenges posed by SARS-CoV-2.

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References

Boechat, J. L., Chora, I., Morais, A., & Delgado, L. (2021). The immune response to SARS-CoV-2 and COVID-19 immunopathology - Current perspectives. Pulmonology, 27(5), 423–437. https://doi.org/10.1016/j.pulmoe.2021.03.008

Alberts B, Johnson A, Lewis J, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002. Chapter 24, The Adaptive Immune System. Available from: https://www.ncbi.nlm.nih.gov/books/NBK21070/

Sette, A., & Crotty, S. (2021). Adaptive immunity to SARS-CoV-2 and COVID-19. Cell, 184(4), 861–880. https://doi.org/10.1016/j.cell.2021.01.007

Felgenhauer, U., Schoen, A., Gad, H. H., Hartmann, R., Schaubmar, A. R., Failing, K., Drosten, C., & Weber, F. (2020). Inhibition of SARS-CoV-2 by type I and type III interferons. The Journal of biological chemistry, 295(41), 13958–13964. https://doi.org/10.1074/jbc.AC120.013788

Adil, M. T., Rahman, R., Whitelaw, D., Jain, V., Al-Taan, O., Rashid, F., Munasinghe, A., & Jambulingam, P. (2021). SARS-CoV-2 and the pandemic of COVID-19. Postgraduate medical journal, 97(1144), 110–116. https://doi.org/10.1136/postgradmedj-2020-138386

Beyerstedt, S., Casaro, E. B., & Rangel, É. B. (2021). COVID-19: angiotensin-converting enzyme 2 (ACE2) expression and tissue susceptibility to SARS-CoV-2 infection. European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology, 40(5), 905–919. https://doi.org/10.1007/s10096-020-04138-6

Anka, A. U., Tahir, M. I., Abubakar, S. D., Alsabbagh, M., Zian, Z., Hamedifar, H., Sabzevari, A., & Azizi, G. (2021). Coronavirus disease 2019 (COVID-19): An overview of immunopathology, serological diagnosis and management. Scandinavian journal of immunology, 93(4), e12998. https://doi.org/10.1111/sji.12998

Sanyal S. (2020). How SARS-CoV-2 (COVID-19) spreads within infected hosts - what we know so far. Emerging topics in life sciences, 4(4), 371–378. https://doi.org/10.1042/ETLS20200165

Mousavizadeh, L., & Ghasemi, S. (2021). Genotype and phenotype of COVID-19: Their roles in pathogenesis. Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi, 54(2), 159–163. https://doi.org/10.1016/j.jmii.2020.03.022

Fehr, A. R., & Perlman, S. (2015). Coronaviruses: an overview of their replication and pathogenesis. Methods in molecular biology (Clifton, N.J.), 1282, 1–23. https://doi.org/10.1007/978-1-4939-2438-7_1

Mohamadian, M., Chiti, H., Shoghli, A., Biglari, S., Parsamanesh, N., & Esmaeilzadeh, A. (2021). COVID-19: Virology, biology and novel laboratory diagnosis. The journal of gene medicine, 23(2), e3303. https://doi.org/10.1002/jgm.3303

Merad, M., Blish, C. A., Sallusto, F., & Iwasaki, A. (2022). The immunology and immunopathology of COVID-19. Science (New York, N.Y.), 375(6585), 1122–1127. https://doi.org/10.1126/science.abm8108

Hosseini, A., Hashemi, V., Shomali, N., Asghari, F., Gharibi, T., Akbari, M., Gholizadeh, S., & Jafari, A. (2020). Innate and adaptive immune responses against coronavirus. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapy, 132, 110859. https://doi.org/10.1016/j.biopha.2020.110859