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Review Article

Immune dynamics of SARS-CoV-2 virus evolution

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ABSTRACT

In December 2019, the first case of COVID-19 surfaced in Wuhan, China. The relatively unknown SARS-CoV-2 virus led to the global 2020–2021 pandemic claiming thousands of lives. One of the major reasons for the prolonged duration of the pandemic consisting of multiple waves, due to sporadic surges in the number of cases, is the emerging variants. Such variants of the classic Wuhan strain hold multiple mutations that increase the viral fitness, improve transmissibility, aid in immune escape, and overall increase the virulence of the virus. Hence, studying and understanding the viral evolution and the interaction dynamics of the virus with the human immune system becomes vital. To that end, here, we review some of the immune aspects associated with SARS-CoV-2 and COVID-19 with a focus on immune responses to variants of concern. The article breaks down the normal immune response elicited against the virus and its variants along with various interesting concepts of antibody-dependent enhancement, immune escape, immune suppression, and immunophenomics while also highlighting the next frontiers in dealing with the virus. The unprecedented research into understanding the immunological underpinnings of the COVID-19 global pandemic will pave the way for evidence-based strategies for the management of this and any future widespread infectious diseases.

Keywords: COVID-19, Variants, Mutation, Immune escape, Immune response, Immunophenomics, Immune suppression

INTRODUCTION

In 2020 and 2021 as the COVID-19 pandemic carried on, several variants of SARS-CoV-2 emerged with some being classified as variants of concern (VOC). Due to mutations within the virus genome, multiple forms or "variants" of the virus can arise.

All variants fall under the following broad categories according to the SARS-CoV-2 Interagency Group classification based on the effectiveness of medical countermeasures, the severity of disease, and the transmissibility:

Variants being monitored (VBM)

VBM includes those variants for which authorized data about causing severe disease or increased transmission exists but these are no longer detected or are found to be transmitting at a very low level. Hence, these are not posing an imminent risk to public health.

Variant of interest (VOI)

VOI includes changes in the genome that specifically affect the phenotypic characteristics of the virus such as changes to receptor binding and reduced efficacy of treatments which

consequently make them more virulent and the disease more severe. A VOI might require one or more appropriate public health actions, including enhanced sequence surveillance, enhanced laboratory characterization, or epidemiological investigations to assess how easily the virus spreads to others, the severity of disease, the efficacy of therapeutics, and exploring whether currently approved or authorized vaccines offer protection. The viral variant genome should hold mutations with either suspected or confirmed phenotypic manifestations along with either it displaying the ability of community transmission or the WHO classifying it as a VOI. As mentioned before, common characteristics of the variants of interest include the presence of specific mutations predicted to increase transmission or immune escape while affecting the diagnostics as well as treatment, proven ability to increase positive case numbers, and a limited expansion relative to VOCs.[1,2]

VOC

Mutations that have biological significance, for instance, those that can enhance the transmissibility of the virus, cause the virus to be more virulent. Variants for which evidence of the presence of such mutations exists are classified as VOC. Along with an increase in transmissibility, multiple other attributes of a VOC include the variant causing increased hospitalization or deaths, increasing the severity of the disease it causes, displaying a considerably decreased effect to neutralizing antibodies *in vitro* and *in vivo*, prior treatments, and available vaccines against the variant displaying a reduced efficacy (interrelated to the previous cause), or increased false negatives using the employed diagnostic method.[2]

Here, we review some of the immune aspects associated with SARS-CoV-2 and COVID-19 with a focus on immune responses to VOCs. The unprecedented research into understanding the immunological underpinnings of the COVID-19 global pandemic will pave the way for evidence-based strategies for the management of this and any future widespread infectious diseases.

VOC DURING THE COVID-19 PANDEMIC

The variants currently classified as VOC have been listed below (updated on 20 November 2021):

Alpha (B.1.1.7 lineage- UK variant- 20I/501Y.V1): Major mutations observed include Δ69/70 (N-terminal domain deletion mutation speculated to allosterically alter the conformation of the S1 subunit),[3] Δ144Y (N-terminal domain deletion mutation),[4] and N501Y (RBD substitution mutation which increases binding to the ACE 2 receptor increasing cellular entry).[3,5] Further, A570D (RBD substitution mutation),[6] D614G (RBD substitution mutation),[7], and P681H (N-terminal domain non-synonymous mutation)[8] are the other major mutations observed with this strain along with E484K (RBD substitution mutation enabling the viral immune escape)[9,10] and S494P (RBD substitution mutation reported to enhance the binding affinity to the ACE2 receptor).[11,12] The variant has been found to have 50–70% increased transmissibility while inducing a relatively more severe diseased state with reduced neutralization efficacy of the neutralizing antibodies.[2,13]

Beta (B.1.351 lineage - South African variant-20H/501Y.V2): K417N (RBD substitution mutation reported to decrease the antibody binding to the virus allowing immune evasion),[14] E484K, N501Y, and D614G mutations are the spike mutations associated with the South African variant. It is noteworthy that variants harboring these along with N501Y mutation have been found to display significantly decreased antibody binding.[14] The variant is found to have 50% increased viral transmission with some evidence for resistance to the neutralizing antibodies.[2]

Gamma (P.1 lineage - Brazilian/Japanese variant - 20J/501Y.V3): K417N/T (K417T [Japanese strain]-RBD missense mutation),[14] E484K, N501Y, and D614G are the mutations associated with this strain of SARS-CoV-2. The variant is associated with increased transmissibility and decreased neutralization efficacy by neutralizing antibodies.[2] It has been traced by the molecular clocking method to 6th November 2020 as the emergent P.1 mutant in Manaus, Brazil,[15] which was also discovered in Japan.[16]

Delta (B.1.617.2 lineage - Indian variant- 21A): The variant B.1.617.2 holds the following S glycoprotein mutations - T19R (Threonine to Arginine substitution mutation at position 19), R158G (Arginine to Glycine), L452R (RBD substitution mutation reported to allow a stronger binding to the ACE2 receptor while also facilitating immune escape),[17] T478K (Threonine to Lysine substitution mutation predicted to increase transmission), D614G (Aspartic acid to Glycine), P681R (Proline to Arginine), and D950N (Aspartic acid to Asparagine) while also displaying a deletion at the 156 and 157 amino acid positions within the spike proteins.[18] These mutations cause the variant to display increased transmissibility, potentially reduced neutralization within vaccinated individuals, as it also displays decreased neutralization when treated with EUA monoclonal antibodies.[2,18] However, it has been reported that vaccines show a good enough efficacy in neutralizing the variant.[19] It first appeared in India in October 2020, bearing partial responsibility for the second wave in India,[20,21] and the third wave in the UK while also affecting many other countries, including Europe, the United States, and other countries.[1,22,23] This variant displays a relatively higher number of vaccine breakthrough cases with a greater viral load. It also displayed an approximately eight-fold decreased sensitivity to vaccine-induced antibodies.[20]

Apart from the above-mentioned variants, multiple other variants have been identified, many of which have been classified as "Variants of Interest (VOI)." Mu (B.1.621) and lambda (C.37) are the variants currently (as of November 20, 2021) classified as variants of interest.[1]

Omicron (B.1.1.529 lineage - South African variant): Very recently (November 26, 2021), this new VOC has been discovered and named by the WHO. The sample from "Patient Zero" for this variant was confirmed on November 9, 2021, and after a surge in cases for this variant observed for 3 weeks, it was reported to the WHO on November 24, 2021. This variant is quite alarming since it shows numerous mutations in the Spike (S) protein which could enhance its ability to transmit, reinfection, escape the host immune system, and also cause vaccine failure. For now, its presence can be confirmed only when the RT-PCR test cannot detect nor amplify any one of the three genes targeted. This phenomenon is known as "S gene dropout or S gene target failure."[24]

UNDERSTANDING HOST IMMUNE RESPONSE

Immunity against COVID-19

Against SARS-CoV-2, alveolar macrophages and alveolar type 2 epithelial cells have pattern recognition receptors (PRR) such as Toll-like receptors (TLR), NOD-like receptors, RIG-1-like receptors (RLR), and C-type lectin receptors which are sensitive to Pathogen Associated Molecular Patterns (PAMPs) associated with COVID-19. The infected host cells, that is, alveolar Type 2 epithelial cells undergo a normal viral replication cycle which leads to pyroptosis (inflammatory apoptosis) and causes a release of PAMPs such as viral RNA, oligomers of apoptosis-associated speck (ASC)-like protein containing a caspase recruitment domain, damage-associated molecular patterns, adenosine triphosphate, etc. Stimulation of the receptors in affected host cells and the detection of PRRs by alveolar macrophages, endothelial cells, and epithelial cells leads to the release of interferons (IFNs), pro-inflammatory cytokines, and chemokines. This causes a localized inflammation, increased vascular permeation, and migration of immune cells such as dendritic cells (DCs), T-cells, natural killer (NK) cells, macrophages, and neutrophils. Normally, all this leads to the removal of the infected and apoptotic cells by NK cells, killer T-cells, and alveolar macrophages along with antibody-mediated virus inactivation and removal by alveolar macrophages, causing very little inflammation and overall damage to lungs. In the case of COVID-19, all these events are dysregulated and lead to a phenomenon called the "cytokine storm" and eventually cause acute respiratory distress syndrome (ARDS) and multiple organ damage.[25,26]

INNATE IMMUNITY

Innate immune cells such as macrophages, DCs, and macrophages after interaction of their PRRs, that is, TLRs and RLRs with PAMPs of COVID-19 stimulate many cell-signaling pathways and lead to the production of transcription factors such as NF-κB, AP-1, and IFNs regulatory factors such as IRF3 and IRF7 which give rise to pro-inflammatory cytokines (TNF-α, IL-6, IL-12, etc.) and Type 1 IFN-α and IFN-β, respectively, as well as IFN-γ. This leads to local inflammation, induction of T-cells as well as NK cells, and inhibition of viral replication. Eosinophils and NK cells are also responsible for antiviral activity by mechanisms of endocytosis using reactive nitrogen species, NKG2D signaling, and cell-mediated cytotoxicity, respectively. The role of macrophages can be understood with the interaction of inflammasome with an ORF-8-like protein in SARS-CoV-2. This leads to the production of pro-caspase-1 and consequently caspase-1, leading to the activation of IL-18 and IL-1β, both responsible for local inflammation, pyroptosis of the target host cells, and production of IFN-γ, involving the adaptive immune cells. In pyroptosis, after programmed cell death, a release of chemokines and cytokines responsible for the migration of immune cells is observed. IL-1 is responsible for inciting the "cytokine storm" seen in ARDS due to SARS-CoV-2.[27-29]

Neutrophils are phagocytic, inflammatory, and migratory cells to clear up an infection. The engulfed pathogens are usually killed by the reactive oxygen and nitrogen species and enzymes like myeloperoxidase produced in the endosome. Enormous amounts of neutrophil infiltration have been observed in the case of autopsied COVID-19 patients. Pathology and mortality by neutrophils in COVID-19 are found to be due to an excess of neutrophil extracellular traps (NETosis) which cause thrombosis, inflammation, sepsis, and respiratory failure. NETs include the released microbicidal proteins, myeloperoxidase, extracellular DNA, and elastase by neutrophils to tackle an infection by trapping the pathogen. Triggers for the production of NETs include IL-6, IL-1β, stimulated platelets, vascular endothelial cells, and lastly, infected epithelial cells.[27-29] Complement action against the infected cells can potentially take place by complement factors such as C5a and C3a.[30]

ADAPTIVE IMMUNITY

With the help of antigen-presenting cells (APCs) such as macrophages and DCs, T-cells of CD4+ (helper) and CD8+ (killer) lineages are activated. In terms of humoral immunity, we see that CD4+ cells activate B-cells to give rise to plasma cells that produce anti-SARS-CoV-2 antibodies that bind to and inactivate the virus.[85,86] IgG and IgM isotypes of immunoglobulins are produced with the

former being produced later and responsible for generating immunological memory. Helper T-cells aid in immune action by the production of pro-inflammatory cytokines and cell mediators. They also activate T_{H1} cells which ensure a cell-mediated response. Helper T-cells mostly react to the spike protein and killer T-cells show their action against the M, N, and other open viral proteins.[30]

Cell-mediated immunity against COVID-19 is ensured with the help of CD8+ T-cells as they eliminate virus-infected cells, which present COVID-19 antigens on their surface, by the release of toxins such as granzyme B, perforin, and granulysin after interacting with these cells leading to apoptosis. These cells are stimulated and present in peripheral blood a week after the onset of the first symptoms.[25-28]

Infection by SARS-CoV-2 takes approximately 4–10 days to show the first symptoms. Further, the next 7–10 days are crucial for the infected host. This is typical of viral infections. But for COVID-19, this is the crucial time where it is determined whether the host's health will deteriorate or recover. This time is required to stimulate T-cells in this infection lies in the later 7–10 days stage. It is very much dependent upon the T-cell response during this duration and hence it is plausible that the action of T-cells against the virus decides the severity of COVID-19 in a patient. Hence, the stronger the T-cell response, the chances of recovery might increase. A UK-based study showed that convalescent patients having mild cases have reported higher proportions of SARS-CoV-2-specific CD8+ T cells. This aids in the understanding of protective immunity and highlights the potential of including non-spike proteins within future COVID-19 vaccine design. The functionality of the T-cell subsets stimulated against SARS-CoV-2 was evaluated after procuring their intracellular cytokine production profiles. Both CD4+ and cytotoxic T cells show multi-cytokine production of IL-2, IFN-γ, and TNF in various combinations which were common for both severe and mild cases. In mild cases, a higher frequency of CD8+ T-cells was observed against M and NP proteins over S protein whereas for the severe cases it was contrary-S protein, ORF3, ORF8, and membrane protein. Furthermore, only the S protein was able to activate the helper T cells to then eventually bring in the humoral immunity but it is speculated that these cells could do more harm in severe cases. Overall, the study did determine that severe COVID-19 cases induced a better T-cell response and immunological memory.[31]

In the case of humoral immunity, helper T-cells and B-cells are activated a week after the first symptoms occur. In the case of SARS-CoV, anti-nucleocapsid (N) protein antibodies are first developed followed by antibodies against the spike (S) protein formed about 4–8 days after the occurrence of symptoms. Neutralizing anti-S antibodies are observed post 2 weeks of symptom appearance and overall neutralizing antibodies are seen after 3 weeks. This is claimed to be seen much earlier in the case of SARS-CoV-2 due to the higher viral titers [Figure 1].[25]

CYTOKINE STORM

COVID-19 primarily attacks the host respiratory system, causing pneumonia, lymphopenia and ARDS, acute lung injury, etc., in severe cases. The immune response against the pathogen arises after contact with exposed outer epitope surfaces as well as according to the antigenic conformation. Adaptive and innate immune responses begin with a comprehensive PRR-PAMP interaction.

The SARS-CoV-2 genome comprises a total of 12 ORFs. Among these one-third of the genome encodes various structural proteins such as Spike (S), Membrane (M), Envelope (E), Nucleocapsid (N) along with some non-structural proteins (NSPs).[32] These structural proteins or the whole virus elicit the host immune response by binding with host TLRs, mainly TLR 3, 7, and 8. TLR signaling in immune cells induces the production of cytokines such as interleukins (ILs) and chemokines. The deadly effect of cytokine secretion, in the case of ARDS, is uncontrollable inflammation. Large amounts of chemokines secreted (CCL2, CXCL9, CXCL10, CCL3, CXCL8, CCL5, etc.) and pro-inflammatory cytokines (TNF-α, TGF-β, IFN-α, IFN-γ, IL-6, IL-12, IL-18, IL-33, IL-1β, etc.) by immune effector cells recruit B-cells and T-cells, resulting in greater cytokine production.[12] This is the "cytokine storm" as illustrated in [Figure 2a].

Among all cytokines, IL-6 has a special role in respiratory inflammation as well as against coronavirus pathogenicity. IL-6 is a pleiotropic cytokine that regulates hematopoiesis, metabolism, and organ development. It has been reported that COVID-19 patients have increased Angiotensin II due to ACE2 downregulation than healthy individuals causing an accumulation of Angiotensin II. This stimulates NADPH-oxidase (in the Angiotensin 1 receptor axis) and reactive oxygen species production consequently resulting in more IL-6 through transcriptional activation and induction in inflammation-mediated immunopathology.[33]

These immunopathogenic studies illustrate that though the pyrogenic effects of cytokines aid in the reduction of the viral load, their uncontrollable production can destroy healthy cells as well. Hence, in such situations, the elicited immune response does more harm than good.

ANTIBODY-DEPENDENT ENHANCEMENT (ADE)

ADE is a unique host immune system phenomenon wherein antibodies bound to the viral epitope promote the internalization of the virus through the F_c receptor, increasing the disease severity.[34] It is observed in reinfections

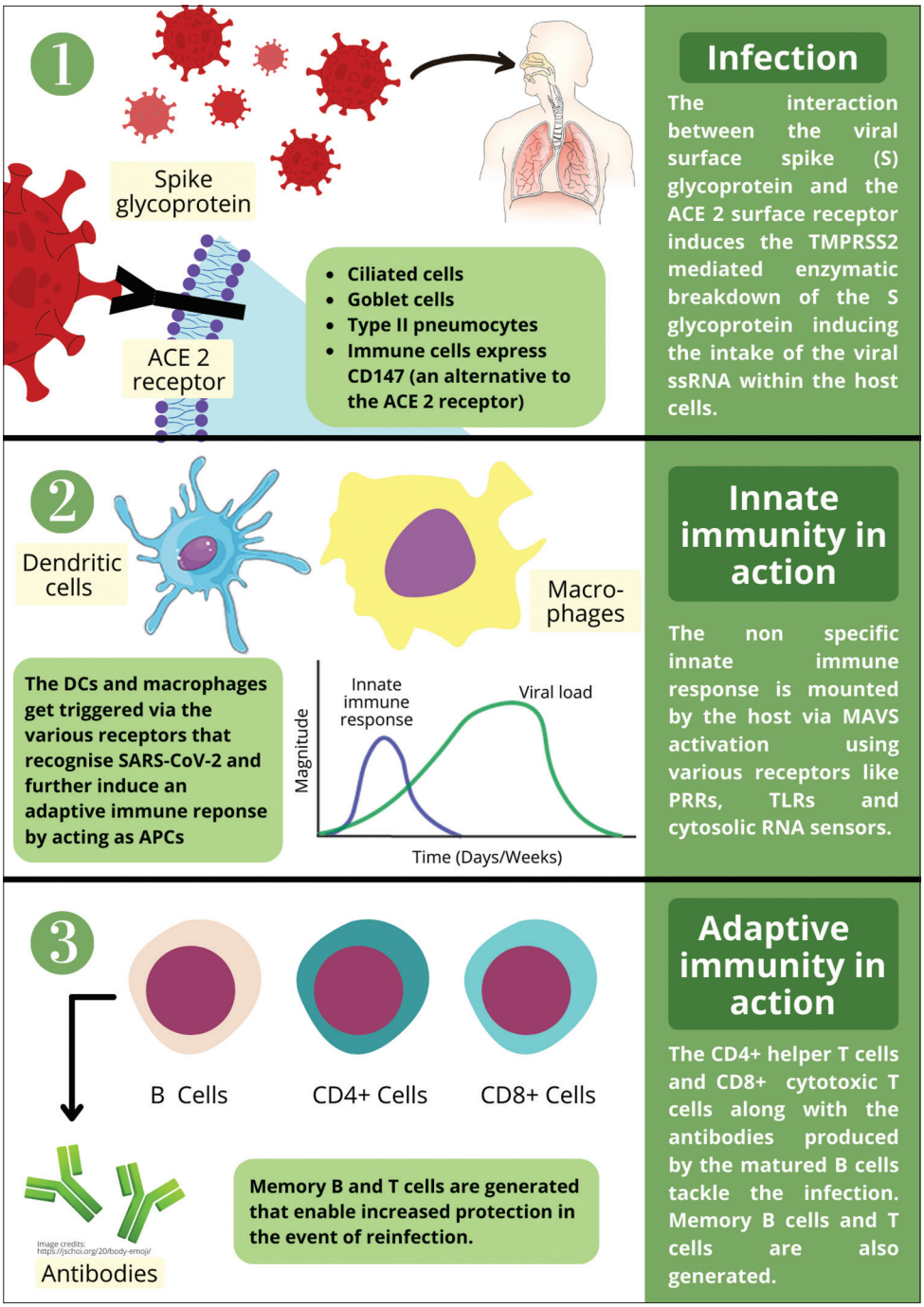

Figure 1: The overall events that take place in the host immune system against SARS-CoV-2 virus.[85,86] ACE 2: Angiotensin converting enzyme 2, TMPRSS2: Transmembrane protease serine 2 precursor, APC: Antigen presenting cells, MAVS: Mitochondrial antiviral-signaling protein, PRR: Pattern recognition receptors, TLR: Toll-like receptor, CD4/8: Cluster of differentiation 4/8.

and vaccinated individuals, wherein non-neutralizing antibodies and neutralizing antibodies at less-than-optimal concentrations against a particular strain of the virus, enhance the disease caused by a variant.

ADE has also been linked to causing multisystem inflammatory syndrome in children, and hence, the development of antibody independent SARS-CoV-2 vaccines, that are T-cells based, has been proposed.[35] ADE has been observed in the case of SARS-CoV and MERS, leading to valid concerns about ADE in the case of SARS-CoV-2.[36] Multiple studies have indicated that the classic strain of SARS-CoV-2 has not yet displayed ADE in the event of reinfection. Although, it is important to

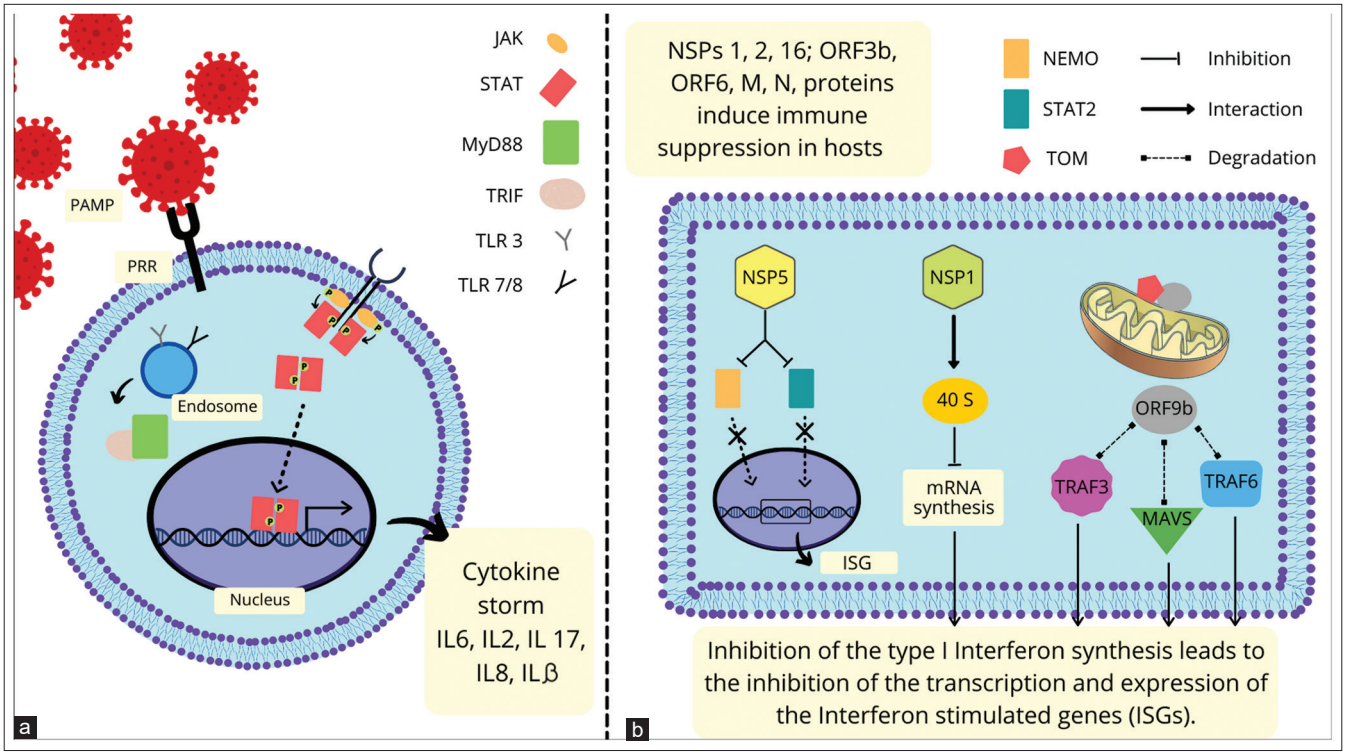

Figure 2: (a) On the left shows the events in the immune cell that lead to production of excessive pro-inflammatory cytokines and cause the dreaded "*Cytokine Storm*." PRR: Pattern recognition receptors, PAMP: Pathogen-associated molecular patterns, JAK: Janus kinases, STAT: Signal transducer and activator of transcription, IL: Interleukin, MyD88: Myeloid differentiation primary response 88, TRIF: TIR-domain-containing adapter-inducing interferon-β, TLR3/TLR7/8: Toll-like receptor. **(b)** On the right shows various mechanisms utilized by SARS-CoV-2 in immune cells which lead to immunosuppression. NSP: Non-structural protein, NEMO: NFκ-B essential modulator, STAT: Signal transducer and activator of transcription, ISGs: Interferon stimulated genes, TRAF: Tumor necrosis factor receptor associated factor, MAVS: Mitochondrial antiviral signaling protein, TOM: Translocase at the outer membrane of mitochondria, IFN: Interferon.

point out that ADE has been observed in the case of dengue infections, even after a 20-year interval between the primary infection and the reinfection.[37]

An *in vitro* study utilizing convalescent sera to study ADE in Raji cells, K562 cells, and primary B cells revealed that ADE was mediated by specific antibodies targeted to the RBD of the spike protein with different binding patterns relative to the neutralizing antibodies; the antibody titer played a significant role.[38] A case study of a 25 years old man with possible reinfection of a SARS-CoV-2 variant within 2 months of the primary infection indicated that the immunity developed from the initial infection was not completely effective in preventing the second infection; it is also noteworthy that the reinfection led to relatively severe disease.[39]

Another study reported that antibodies produced against a specific site within the N-terminal domain of the spike protein enhanced the viral infectivity through an F_c-receptor-independent ADE mechanism.[40] On the contrary, many reports have displayed minimal to no evidence of ADE *in vivo* concerning SARS-CoV-2.[36,41-43] A study by Zhou *et al.* reported that ADE was prominent *in vitro* and suggested that more than just the expression of F_c receptors is essential for ADE.[44] Hence, a reliable conclusion cannot yet be drawn, and more data are required to elucidate the possibility of ADE due to reinfections and vaccinations.

An increasing concern is the growing number of variants and their susceptibility to the currently employed vaccines. Vaccinations have proved effective to some extent against the VOCs, but more research to understand and characterize the ADE effect due to the vaccines will be required.

A recent study by a group of researchers described the presence of high-affinity antibody P4A1in COVID-19 convalescent patients. P4A1 interacts with and covers a major portion of the receptor-binding motif of the spike receptor-binding domain as shown by high-resolution complex structure analysis. They engineered the P4A1 antibody whose effect subsequently reduces the potential risk for ADE of infection and extends its half-life.[45]

IMMUNE ESCAPE

Immune escape or immune evasion, in simple terms, is a stealth strategy adopted by invading pathogens, including viruses, wherein the mutations within the viral antigen allow escape from the neutralizing antibodies produced either due to an initial exposure or vaccinations.[46]

The currently administered vaccines (as of November 25, 2021) include inactivated virus vaccines (Covaxin, Sinovac, Sinopharm), viral vector vaccines (AstraZeneca-Covishield & Vaxzevria, Sputnik V), RNA based vaccines (Pfizer, Moderna), and protein subunit vaccines (Novavax) all of which aim to induce the production of neutralizing antibodies against the spike protein of the Wuhan-1 strain of the virus.[47,48]

The four major VOCs: Alpha (B.1.1.7 lineage- UK variant-20I/501Y.V1), Beta (B.1.351 lineage- South African variant-20H/501Y.V2), Gamma (P.1 lineage-Brazilian/Japanese variant-20J/501Y.V3), and Delta (B.1.617.2 lineage- Indian variant-G/478K.V1) have displayed significant immune evasion abilities *in vitro* and/or *in silico* against monoclonal antibodies as well as convalescent plasma (CP).[2,47,49] Considering specific mutations, the E484 mutations in the RBD (which affect both ACE2 binding as well as antibody recognition) have been shown to confer a significant immune escape capability by multiple studies: Reduced neutralization to convalescent sera;[50] complete escape from neutralization to convalescent sera;[51] reduced neutralization to both convalescent sera and monoclonal antibodies.[40] The E484K and N501Y mutations in the case of the Beta and Gamma variants, and the L452R mutation in the case of the Epsilon variant (a variant previously classified as a VOI), have been found to mediate immune escape.[47,49] The immune evasion ability of the variants has significantly decreased the vaccine efficacy,[52] especially in the case of Beta and Gamma variants, more so in the case of the former, which has been demonstrated using pseudovirus-based neutralization assays.[53,54] Moderna and Pfizer have already begun studies to develop booster doses and vaccines against the emerging variants displaying immune escape, both estimated to be complete in 2022.[55] The four major VOCs: Alpha (B.1.1.7 lineage - UK variant - 20I/501Y.V1), Beta (B.1.351 lineage - South African variant-20H/501Y.V2), Gamma (P.1 lineage - Brazilian/Japanese variant - 20J/501Y.V3), and Delta (B.1.617.2 lineage - Indian variant - G/478K.V1) have displayed significant immune evasion abilities *in vitro* and/or *in silico* against monoclonal antibodies as well as CP.[1,2,47,49] Considering specific mutations, the E484 mutations in the RBD (which affect both ACE2 binding as well as antibody recognition) have been shown to confer a significant immune escape capability by multiple studies: reduced neutralization to convalescent sera;[50] complete escape from neutralization to convalescent sera;[51] reduced neutralization to both convalescent sera and monoclonal antibodies.[40] The E484K and N501Y mutations in the case of the Beta and Gamma variants and the L452R mutation in the case of the Epsilon variant (a variant previously classified as a VOI) have been found to mediate immune escape.[47,49,56] The immune evasion ability of the variants has significantly decreased the vaccine efficacy,[52] especially in the case of Beta and Gamma variants, more so in the case of the former, which has been demonstrated using pseudovirus-based neutralization assays.[53] Moderna and Pfizer have already begun studies to develop booster doses and vaccines against the emerging variants displaying immune escape, both estimated to complete in 2022.[55,57]

The reduced vaccine efficacies are still considerably over 50% and the E484 mutation present in many of the variants has not rendered the neutralizing antibodies completely ineffective.[9] Another study has displayed the prevention of escape mutant emergence by the use of multivalent nanobodies targeted to the receptor-binding domain of the spike protein.[58] Yet another study revealed that the Beta (B.1.351) variant displayed increased resistance to the neutralization capability of the convalescent serum by a factor of 2, while in the case of sera obtained from individuals vaccinated with mRNA vaccines, it showed an increased resistance by a factor of 2.5–3.3.[53] The N439K substitution mutation containing variants are also found to have considerably increased immune escape ability due to their elevated ACE2 affinity when tested with monoclonal antibodies specifically.[59]

Variants have also evolved to evade the host T-cells due to mutations within the nucleocapsid (N) protein and ORF3a proteins. A study showed that several variants, with mutations within the nucleocapsid and ORF3a proteins, within the SARS-CoV-2 T-cell epitopes (five CD8+, two CD4+) lead to a reduced response from T-cells. They also reported that T-cell evasion was not significant in the case of spike protein variants. The evaluated variants held mutations including P365S, Q213K, T362I, and P13L. These mutations have also been found in the Alpha variant, indicating that it may have the potential for T-cell immune evasion.[60] A recent study has also pointed out that sera obtained from persistently infected immunocompromised people displayed lower neutralization titers indicating the presence of immune escape variants within such immunocompromised patients. The authors concluded by speculating that such variants could give rise to SARS-CoV-2 strains with the potential to enhance the ongoing pandemic.[61]

IMMUNE SUPPRESSION

SARS-CoV-2, by its structural proteins, such as NSP1, NSP3, NSP16, ORF3b, ORF6, and M and N proteins, induce immunosuppression in the host by various mechanisms. One of them entails the inhibition of the production of IFNs

by interfering in the signaling pathways that enable their production, inhibiting the transcription factors involved in the same, and lastly by dysregulating effector mechanisms that enable IFN production. M protein affects Type 1 IFN production, which involves IFN-α. Even the production of Type 3 IFNs like IFN-λ is affected. Both of these are essential in COVID-19 to inhibit viral replication and prevent severe clinical outcomes.[27,62,63] Furthermore, inhibition of IFN-β expression may occur because of a novel short protein produced by the orf3b region of the SARS-CoV-2 genome. It is essential for curbing viral pathogenicity by inhibiting its replication.[64]

Another way to induce immunosuppression is by inducing lymphopenia, that is, low blood count of lymphocytes till about 800 cells/μL of blood which causes poor prognosis and reduces the chances of survival in the patients.[65] This is speculated to be due to T-cell apoptosis and reduced T-cell stimulation is caused because of impaired DC migration. It also affects function in other APCs.[30] Another study reported that SARS-CoV-2 disrupts the helper T-cell function by reducing the production of IFN-γ and TNF-α. It causes excess stimulation of killer T-cells by enabling high production of granzyme B and perforin. Furthermore, the eventual exhaustion of killer T-cells occurs because of the high expression of TIGIT and HLA-DR on their surface. Moreover, all these events cause a reduction in the functional diversity of T-cells. These events increase the severity of COVID-19 in patients.[66]

Furthermore, NK cells are an important part of antiviral immunity, which requires a high expression of the NKG2D receptor for its activation. However, this virus causes an upregulation of the inhibitory receptor, NKG2A, which hampers the function of NK cells.[30]

The previous studies have shown that an alternative form of ORF, that is, ORF-9b in the N gene of SARS-CoV-2 can target mitochondrial outer membrane protein and significantly inhibit IFN-I production. This inhibition can potentially take place due to HSP90 binding with TOM 70 and as a result inducing IFN-I production. ORF-9b has also been reported to interact with TOM 70 and induce autophagy. Hence, ORF-9b competes with HSP90 to bind with TOM70; such interaction of ORF-9b-TOM70 is responsible for lactic acidosis.[67] ORF-9b protein is also found to be responsible for the degradation of MAVS, TRAF3, and TRAF6, which reduces the host IFN response. A recent study also shows that the NSPs encoded by ORF-1a and ORF-1b of Coronavirus can antagonize immune response in hosts. NP5, the Major protease referred to as "Mpro," directly inhibits Type-1 IFN production by cleaving NEMO and STAT2. NSP5 targets glutamine residues on the P1 site of STAT2, thus inhibiting transcription of ISGs. It has been demonstrated previously that NSP1 degrades exogenous host mRNA by interacting with host 40S ribosome and can inhibit the synthesis of Type-1 IFN [Figure 2b].[68]

A team of researchers from South Africa has linked the Beta variant (501Y.V2) to the upsurge of cases in the country's Eastern Cape Province.[69] This variant was isolated and was attempted to be treated with convalescent sera.[70] This attempt seems to have failed since the sera could not efficiently neutralize the variant. This has kept scientists divided about herd immunity being developed from natural infection.[23]

IMMUNOCOMPROMISED PATIENTS: COMPLICATIONS

The second wave of the pandemic in India brought along with it a lot of opportunistic fungal co-infections. These infections are COVID-19-associated pulmonary aspergillosis or invasive pulmonary aspergillosis (IPA) caused by *Aspergillus*, CAM (COVID-19-associated mucormycosis), or "black fungus" caused by *mucormycetes* such as *Mucor, Rhizopus, Lichtheimia* and *Apophysomyces*, Candidiasis or "white fungus" caused by *Candida*. This is due to the administration of corticosteroids as treatment in patients with severe COVID-19 who get immunocompromised as a result. These conditions are hard to detect since the symptoms are similar to COVID-19. IPA infection remains restricted to the lungs, whereas CAM spreads to the brain, eyes, nose, and lungs through the sinuses. CAM is treated by antifungals such as Isavuconazole, Amphotericin B, and Posaconazole.[71-73] In IPA, the infection is exacerbated due to IL-10, whose overproduction causes stunted T_{H1} responses and stimulated T_{H2} responses, leading to a decrease in the number of macrophages. This infection is fatal due to difficulty in diagnosis and the fact that lung function gets compromised.[74] Serology and molecular diagnostic tests are either unavailable or still developing. Two biomarkers are available for IPA: Galactomannan, β-D-glucan. COVID-19 hampers all the factors by which CAM can be controlled: Surgery, hyperglycemia, and early administration of liposomal Amphotericin B. This leads to a 45–90% patient mortality rate.[73,75]

IMMUNOPHENOMICS

The emergence of new variants of SARS-CoV-2, with greater transmissibility, has further highlighted the critical role that IFNs plays in influencing SARS-CoV-2 evolution.

The human genome encodes a diverse array of IFNs- Type-I IFNs such as IFNα and its subtypes, IFNβ, IFNω and the Type-III IFNs such as IFNλ1, IFNλ2, and IFNλ3. The previous studies have stated that IFNα2 and IFNβ have promising outcomes against SARS-CoV-2, but the current emerging variants are showing strong inhibition against cytokines.

Guo *et al*. used five different isolates from prominent lineages (USA-WA1/2020-lineage A from the US, N501Y mutated form of Alpha lineage, i.e., B.1.1.7 from the United Kingdom,

Beta lineage, i.e., B.1.351 from South Africa) and infected them on alveolar type II epithelial cell line A549, which were already pre-incubated with 17 recombinant IFNs. The result demonstrated that IFNα8, IFNβ, and IFNω were most potent followed by IFNα5, IFNα17, and IFNα14 against USA-WA1/2020, but not Gamma (P.1), Beta (B.1.351) or Alpha (B.1.1.7) strains. Moreover, maximum inhibition was not obtained with either IFNβ or IFNλ1 against the Alpha (B.1.1.7) variant, and Beta (B.1.351) variant was also more resistant against both of these IFNs compared to the lineage B isolates tested in a separate experiment. Resistance against IFNs may produce a high viral load and, in turn, induce pathogenicity. In addition to spike protein, non-structural proteins such as NSP3, NSP6, and NSP12 exhibited high variation and were shown to antagonize IFN signaling.[45]

T-CELLS, VACCINES, AND POLYCLONALS: THE NEXT FRONTIER

In the case of SARS-CoV-2 variants such as the Beta (B.1.351) variant, wherein the antibodies generated from the previous strains show less efficacy, scientists are now diverting toward the use of killer T-cells, which is, CD8+ lineage. This is because killer T-cells can recognize a wider range of epitopes from COVID-19 proteins in terms of number (15–20) and types (not only spike protein but also other expressed proteins with a slower mutation rate) which proves that they can potentially be a much better alternative than antibodies to deal with the emerging COVID-19 variants. The logic for the latter fact is that these cells recognize processed and presented viral antigens on the surface of infected host cells.[76] Their feasibility is also proved by the fact that T-cell response against the VOCs (Alpha, Beta, Gamma) and former VOI (Epsilon CAL.20C/B.1.427 and B.1.429) as compared to the original strain seems mostly unfazed especially after recovery or administering Pfizer and Moderna vaccines; decreased only by 10–22%. This could most likely be because of the conservation of T-cell epitopes in these variants; 97% of CD8+ and 93% of CD4+ T-cell epitopes have been revealed to be conserved. VOCs seems to have affected a very negligible percentage of T-cell epitopes: 7% for CD4+ and 3% for CD8+ T cell epitopes.[77]

Scientists are also debating whether to make a multivalent vaccine consisting of both the original and mutated versions of the antigen, that is, spike protein to combat the South African (Beta) variant.[78] Ongoing clinical trials exist to find evidence for the effectiveness of mixing vaccines for the sake of boosting the immune responses against COVID-19 and speeding up the vaccination program. One of these involves giving the AstraZeneca-Oxford Viral vector vaccine (initial dose) with the Pfizer RNA vaccine (booster dose) at the same time in one shot. The trial started on February 4, 2021, and is being managed by experts at the University of Oxford, UK. This combination strategy is known as "Heterologous Prime-Boost" and was utilized even for tackling the Ebola epidemic. This is opposed to the usual strategy of "Homologous Prime-Boost" wherein repeated shots of the same antigen are given with the same type of vaccine delivery method. Oxford is also planning to do the same with the Russian Sputnik V vaccine which is a viral vector vaccine. The latter vaccine is a viable candidate because it consists of different viral vectors amongst its subsequent doses making it a "Heterologous Prime Boost" vaccine. Such an approach of using different vectors leads to the prevention of a decrease in the intensity of the elicited immune responses. This vaccine has also shown 90% efficacy against COVID-19 original strain.[79] As of now, the "Heterologous Prime-Boost" team from Oxford University, which is headed by Dr. Matthew Snape, has released its initial safety and reactogenicity data regarding this vaccine strategy named "Com-COV." Reactogenicity involves the physiological reactions after administering the vaccine to the people involved in the clinical trials. The systemic reactions such as headache, myalgia, fever, and local reactions such as injection-site pain or induration redness or swelling are monitored here.[80]

In June 2021, the primary immunological reactions from the clinical trial population for Com-COV were released. This was a comparative study between the two strategies: "Heterologous Prime-Boost" versus "Homologous Prime-Boost." Here, in case of systemic reactions for the latter strategy, for the group that got administered with the Pfizer vaccine, they appeared after receiving the booster dose. While for the group that received the AstraZeneca vaccine had them after the prime dose. For the former strategy, these reactions were only seen after the booster dose but to a greater degree.[81]

RNA vaccines are a relatively more viable option despite the complexity and production cost incurred since they produce antibodies that recognize more complex epitopes than the ones predicted and can potentially help to mount a more robust immune response whenever a new variant is introduced into the host. This vaccine type combats the immunological phenomena in which the host generates a robust immune response only when vaccines of original strain and first variant are given for a particular antigen. As more variants are introduced, the immune responses have been observed to become less significant.[78] As opposed to pre-clinical studies, RNA vaccines are unable to generate a proper killer T-cell response as compared to the AstraZeneca-Oxford vaccine.[76] An animal study has shown that this can be circumvented if the vaccine is combined with the AstraZeneca-Oxford vaccine and is given as a single shot. This combined shot showed better killer T-cell stimulation than that of individual shots given as a part of "Homologous Prime-Boost."[82]

Scientists hypothesize that CP, consisting of polyclonal antibodies generated in patients who have recovered from

a COVID-19 variant infection, can effectively combat new variants which might emerge in future.[83]

Approaches based on nanotechnology are being utilized to ensure longevity of COVID vaccines. Administering the vaccine antigens using nanoparticles seems to generate a stronger humoral immune response by influencing the B-cell differentiation in the germinal centers of secondary lymphoid organs such as lymph nodes. This is possible by influencing the interaction of Follicular helper T-cell (T_{FH}) with B-cells. This was tested for encapsulated HIV gp120 with gp120 fusion protein in a glycosylated nanoparticle with favorable results. More neutralizing antibodies were seen to be stimulated against such a multimeric and particulate antigen than an antigen of monomeric nature. Furthermore, glycosylation of either the antigen or nanocarrier seems to have contributed to the longevity of the response. Multivalent vaccines are known as String-of-beads vaccines that express various pathogenic epitopes which are held together by spacers. Using particular and compatible nanocarriers such as immunoproteasome, these various epitopes will be sent to surrounding lymph nodes. After engulfment by DCs, every epitope will be released by a mechanism of antigen processing and will be presented to adaptive immune cells. The efficacy of such a vaccine strategy would rely on epitope release by using suitable spacers and the best amalgamation of both T-cell and B-cell epitopes.[84]

CONCLUSION

We have summarized various immune aspects associated with SARS-CoV2 and highlighted the clinical relevance and immense potential for future research. We hope that the knowledge gained from studying the immune aspects of the SARS-CoV-2 pandemic and the long-term immunological sequelae will widen our understanding and better prepare us to tackle infectious and chronic conditions affecting the immune system in the future.

Authors contribution

Madhura Kamat, Vishakha Kurlawala have contributed equally in this study.

Declaration of patient consent

Patient consent is not required as there are no patients in this study.

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Conflicts of interest

There are no conflicts of interest.

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