




Role of IFN and Complements System: Innate Immunity in SARS-CoV-2

This article was published in the following Dove Press journal:
Journal of Inflammation Research

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Abstract: The critical role of the innate immune system has been confirmed in driving local and systemic inflammation and the cytokine release storm in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This dysregulated immune response is focused on interferon (IFN) and complement activation, which are crucial for the development of metabolic inflammation, local lung tissue damage, and systemic multi-organ failure. IFNs control viral infections by inducing expression of IFN-stimulated genes (ISGs) that restrict distinct steps of viral replication. Therefore, in this review article, we propose the mechanism of SARS-CoV-2-associated acute respiratory disease syndrome, and assess treatment options by considering IFNs and by targeting IFN-antagonist SARS-CoV-2 virulent gene products. Furthermore, we elaborate on the mechanism of the amplified complement-mediated inflammatory cytokine storm, and propose an antiviral and immunotherapeutic strategy against coronavirus disease 2019 (COVID-19).

Keywords: SARS-CoV-2, innate immune system, cytokines, complement, IFNs, virulent

Background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an enveloped novel virus with a single-strand RNA (+SSRNA) genome in the subfamily *Coronaviridae*.^{1,2} The virus is a club-shaped spherical virion with spike projections from the virus membrane.^{1,3,4} The organization and expression of the genome are similar for all coronaviruses, in which the 16 non-structural proteins (Nsp1–Nsp16), encoded by open reading frame (ORF)1a/b at its 5'-terminal, are followed by the structural protein spike (S), envelope (E), membrane (M), and nucleocapsid (N), which are encoded by another ORF at its 3'-terminal.⁵ Clinically, patients with SARS-CoV-2 present with fever, cough, shortness of breath, and acute respiratory distress syndrome (ARDS).^{6–8} However, some patients have been diagnosed positive by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) or nucleic acid test results without relevant clinical symptoms or who are only minimally symptomatic.⁹ Current research evidence shows that asymptomatic individuals have the same infectivity as those with symptomatic infections and can spread the virus efficiently.¹⁰ In turn, the emergence of these silent transmissions of SARS-CoV-2 has caused difficulties in controlling the COVID-19 pandemic.¹¹ Both innate and adaptive immune responses are activated by SARS-CoV-2 infection to overcome the problem.¹² However, uncontrolled inflammatory innate immune responses may lead to harmful tissue damage. Commonly, the symptoms resulted from the consequent infiltration of inflammatory cells and the release of proinflammatory cytokines.¹² These include interleukin (IL)-1 β , IL-6, and

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IL-8, which are responsible for fever and fibrosis and/or plural effusion of the lung, which provoke severe acute respiratory syndrome.^{13,14} In addition, CXCL9 and CXCL16, chemoattractants of T cells and/or natural killer (NK) cells, CCL8 and CCL2, recruiting monocytes/macrophages, CXCL8, and other classic neutrophil chemoattractants are profoundly elevated. Therefore, we suggest that the presence of these cells may be a primary driver of the signature pathology observed in coronavirus disease 2019 (COVID-19) patients. This review describes the interaction between SARS-CoV-2 and innate immunity, and discusses the escaping mechanism against interferons (IFNs), predominantly Nsp5, as well as the pathology of the disease, related to exuberant activation of the complement system. Therefore, a detailed understanding of the interactions of the virus with innate immunity and the immunopathology behind COVID-19 could lead to new approaches aimed at improving immunotherapy for SARS-CoV-2.

Innate Immune Response to SARS-CoV-2s,

For effective recognition of viral infection and successive activation of an antiviral state, innate immune responses are vital and essential host defense mechanisms.¹⁵ The innate immune response is the first-line inborn host defense mechanism against non-specific pathogens. This innate immunity to viruses involves complex interactions between soluble factors and cells. These cells understand and sense the foreign microbes through the presence of pattern recognition receptors (PRRs), commonly named toll-like receptors (TLRs). TLRs, a family of sensor proteins, assist the immune system in discriminating between “self” and “non-self”.¹⁶ The TLR detects a specific and conserved microbial domain called the pathogen-associated molecular pattern (PAMP), which acts as a ligand with elements such as lipids, lipoproteins, proteins, RNAs, and DNA of the microbe’s structure.^{17,18} TLR family members TLR-3, TLR-7, TLR-8, and TLR-9 are involved exclusively in the membrane’s intracellular compartments, such as endosomes, in the recognition of viral nucleotides. The TLR signaling cascade is mediated by the TIR (toll/IL-1 receptor) domain, which contains an adaptor molecule, such as myeloid differentiation primary response gene 88 (MyD88), TIR-domain containing adaptor protein (TIRAP), or TIR-domain-containing adapter (TRIF).^{18–20} The TIR complex plays a role in the inflammatory immune response via the production of proinflammatory cytokines, type I IFN, and up-regulation of costimulatory

molecules.^{21,22} In a small COVID-19 patient cohort, levels of IFN- α and IFN-stimulated gene (ISG) were associated with viral load as well as disease severity.²³ Taking this a step further, these studies indicate that severe infections lead to high IFN signatures but fail to clear and bring down the viral load. In contrast, owing to their virulence gene product, the expression and IFN- α/β response are decreased (Figure 1). In the same way as SARS-CoV,²⁰ SRAS-CoV-2 uses its spike glycoprotein to bind with the angiotensin-converting enzyme-2 (ACE2) receptor to gain entry to cells,²⁴ suggesting a similar cellular tropism and route of entry. This receptor is expressed not only in cardiopulmonary tissues, particularly in type I alveolar cells, but also in some hematopoietic cells, including monocytes, dendritic cells, and macrophages.¹⁹ Prior to the translocation of IFN regulator factors (IRF-7) and nuclear factor- κ B (NF- κ B) from the cytoplasm to the nucleus, the single-stranded viral RNA should be recognized by TLR-7 with the following recruited signal transducing adaptor proteins, MyD88, playing a key role. Hence, it promotes the expression of type I IFN (primarily IFN- α and IFN- β s), chemokines, and inflammatory cytokines (Figure 2).²⁵ The major antiviral chemical mediators in the innate immune response are IFNs, which act to limit the spread of the virus and enhance macrophage phagocytosis of the pathogen, as well as NK cell restriction of infected target cells.

Interferon

Interferons are a large family of genetically and functionally related proteins. These molecules were discovered in the 1950s by Isaacs and Lindemann. They are recognized as essential first-line biological protein defense against viral infection in mammals.^{26,27} The term “IFN” was coined to describe a special substance, possibly produced by cells, which is centrally important in the innate immune response to the virus through “interfering” with virus replication, with antiviral activity, antiproliferative activity, stimulation of cytotoxic T cells and NK cells, and immune modulation.^{28,29} IFNs are divided into three types, named type I, type II, and type III.²⁰ Type I IFNs are the largest IFN family, including IFN- α (13 subtypes) encoded at chromosome 9, IFN- β encoded at chromosome 12, IFN- ϵ , IFN- κ , and IFN- ω .^{26,30,31} IFN- δ , IFN- τ , and IFN- ζ (or limitin) have also been identified as type I IFNs in swine, ruminants, and mice, respectively.³² Furthermore, four IFN-like cytokines have been reported: limitin (found only in mice), IL-28A, IL-28B, and IL-29, found in humans and other mammals. Almost all cell types are capable of producing IFN- α/β ; however, plasmacytoid dendritic cells

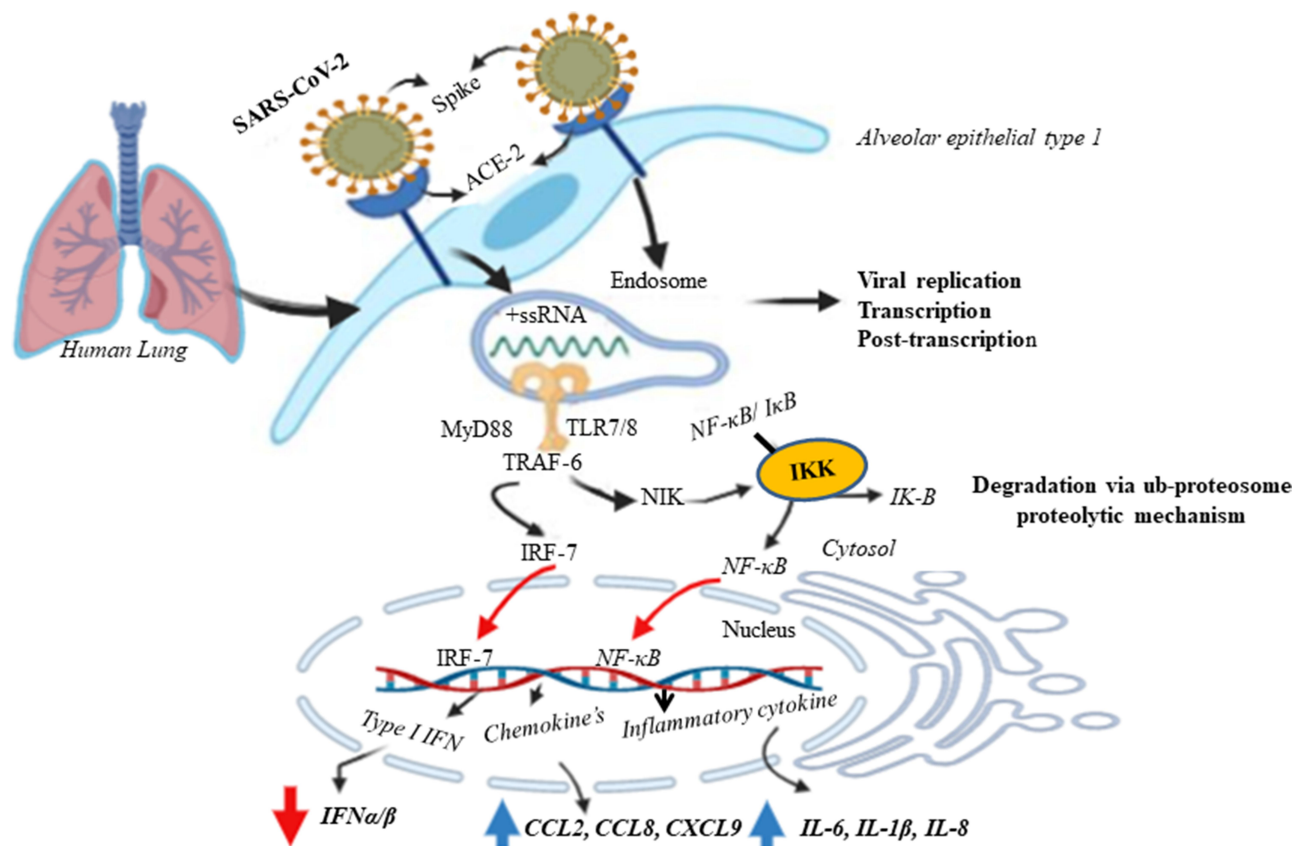


Figure 1 Possible mechanism of SARS-CoV-2 mediated innate immune response at the lung alveolar cell. The SARS-CoV-2 infects alveolar epithelial cells (mainly Alveolar epithelial type 1) through ACE2 receptor. Recognition of ssRNA by TLR-7 in the endosomal membrane recruits MyD88 to the receptor, which induces proinflammatory cytokines and type I IFNs through MyD88-TRAF6-NIK-IKK-NF-κB pathway and MyD88-TRAF-6-IRF-7 respectively. The destruction of epithelial cells and the increase of cell permeability lead to the release of virus. Phosphorylated IRF-7 and translocate to the nucleus results expression of type I IFN genes and release IFNα/β. IκappaB kinase (IKKs) directly phosphorylate the inhibitory IκB family members, which normally sequester NF-κB as inactive form (NF-κB/IκB) in the cytosol. However, phosphorylation of IκBs leads to their polyubiquitination and degradation via ubiquitin proteasome proteolytic mechanism. The activation of the NF-κB pathway results in the induction of aberrant inflammatory cytokines and chemokine's secretion by alveolar cells including IL-6, IL-1β, IL-8, CCL2, CCL8 and CXCL9.

(pDC) are specialist type I IFN-producing cells, particularly producing IFN- α , β , and ω during the course of an infection.

The activation of the IFN system is the most important defense for containing the initial stages of a viral infection. IFN is mostly produced when TLRs on the cell surface (TLR-1, TLR-2, TLR-4, TLR-5, TLR-6, and TLR-10) are induced by lipopolysaccharides of Gram-negative bacteria, and virion proteins.³³ On the other hand, some TLRs are anchored to the endosomal membrane (TLR-3, TLR-7, TLR-8, and TLR-9), which is stimulated by PAMPs such as dsRNA, ssRNA virus, and viral dsDNA. Upon the attachment or binding of viral RNAs to TLR-7/8, it can induce genes encoding for inflammatory cytokines and type I IFNs via NF- κ B and IRF-7 (Figure 3).^{16,33,34} In addition, cytoplasmic viral RNA genomes or viral replicative intermediates can be recognized by the family of RNA helicases, such as retinoic acid-induced gene-1 (RIG-I) and melanoma differentiation-associated gene-5 (MDA-5), which ultimately can control genes encoded for IFN production.³⁵⁻³⁷ In the

foundation of the innate immune response system, type I IFNs are first line of defense against viral infection. Immediately after the body encounters a viral infection, host cell PRRs, such as TLRs, RLR, Mda5, protein kinase R (PKR), and 2'-5'oligoadenylate synthetase (OAS), serve in discriminating self from non-self ligands at a cellular level, which stimulates the cells to produce and secrete IFN. The IFN signaling cascades are initiated upon its binding with its receptor, IFRAR1/2, leading to cytoplasmic kinase activation that allows the recruitment of JAK-1 and TYK, which phosphorylate STAT1 and STAT2, respectively. Both transcription factors then form heterodimers with IRF-9, and translocate to act on the IFN-stimulated response element (ISRE) and promote the binding of RNA polymerase II to express ISG.²⁰ After translation followed by posttranslational modification, the product of ISG brings an antiviral effect and immunomodulation, and by inhibiting replication it can limit both the spread and the load of the virus (Figure 3).

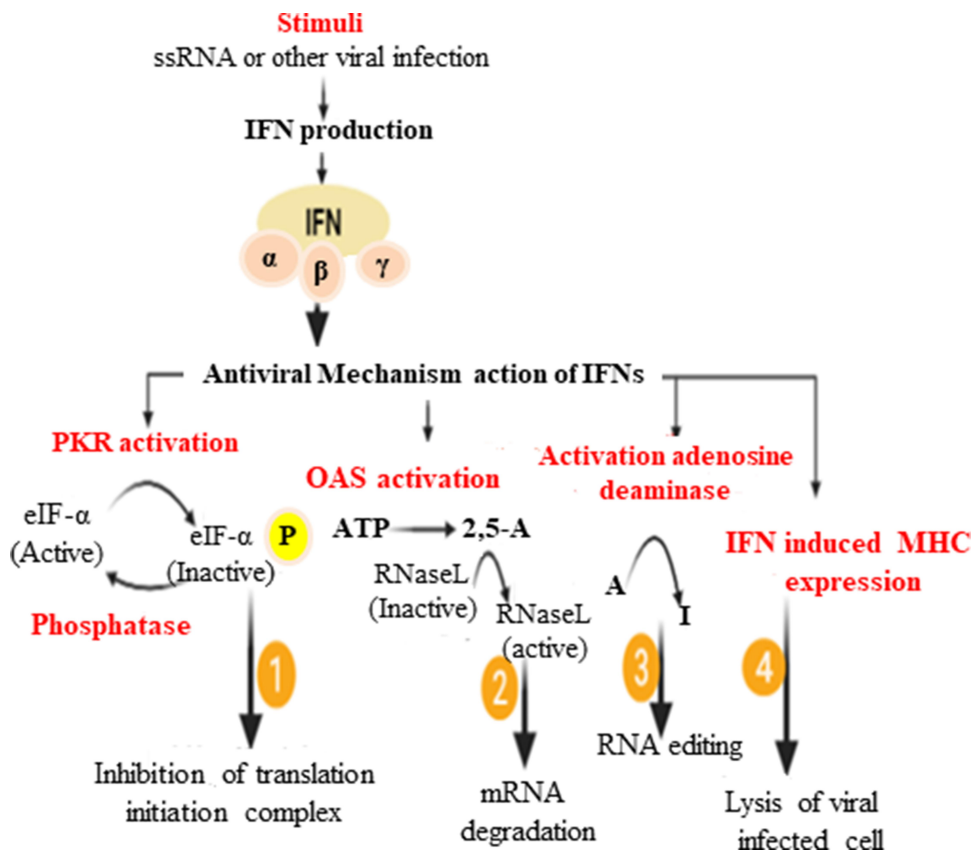


Figure 2 Schematic representation of how interferons induce antiviral state in host cells.

Interferon as a Treatment Option for COVID-19

IFNs are rapidly secreted and induce a wide range of effects that not only act upon innate immune cells but also modulate the adaptive immune system. Type I IFNs- α/β are broad-spectrum antivirals, both exhibiting direct inhibitory effects on viral replication and supporting an immune response to clear the viral infection.³⁸ A study conducted in China showed that treatment with IFN- $\alpha 2b$ (manufactured using recombinant DNA technology) with or without Arbidol[®] (an inhibitor of Nsp12 cofactors called Nsp7/8) significantly reduced the duration of detectable virus in the upper respiratory tract. At the same time, IFN- $\alpha 2b$ reduced the duration of elevated blood levels of the inflammatory markers IL-6 and C-reactive protein. Surprisingly, from the onset of symptoms, the mean time to viral clearance was 27.9, 21.1, and 20.3 days for patients treated with Arbidol (umifenovir) alone, IFN- $\alpha 2b$ alone, and a combination of Arbidol and IFN- $\alpha 2b$, respectively.³⁹ Therefore, these research findings illustrate that IFN treatment accelerated viral clearance by approximately 7 days.^{39,40} Moreover, for better clinical outcomes of patients with cytokine storm, the IL-6 receptor inhibitor

tocilizumab (branded as Actemra), a recombinant human IL-6 monoclonal antibody, is useful as a therapeutic adjunct.^{41,42}

Complement Activation in SARS-CoV-2 Infection

The complement system is a key player in innate immunity and consists of both soluble factors and cell surface receptors that interact to sense and respond against invading pathogens.⁴³ It can also act as a bridge between innate and adaptive immunity to enhance humoral immunity and T-cell function.⁴⁴ Complements recognize non-self-structures which present on the surface of pathogens, and initiate activation of zymogens via proteolytic cascades, resulting in inflammation, opsonization, and/or cell lysis.⁴⁵ However, the defensive function of the complement system can exacerbate immune, inflammatory, and degenerative responses in various pathological conditions. In general, in response to foreign pathogen, there are three major complement activation pathways. The first one is the classical pathway, characterized by C1q-mediated antigen-antibody complexes. The second is the lectin pathway, initiated by mannose-binding lectin (MBL), which recognizes the microbial surface glycan domain, called mannose. Mannose-associated serine protease (MASP)

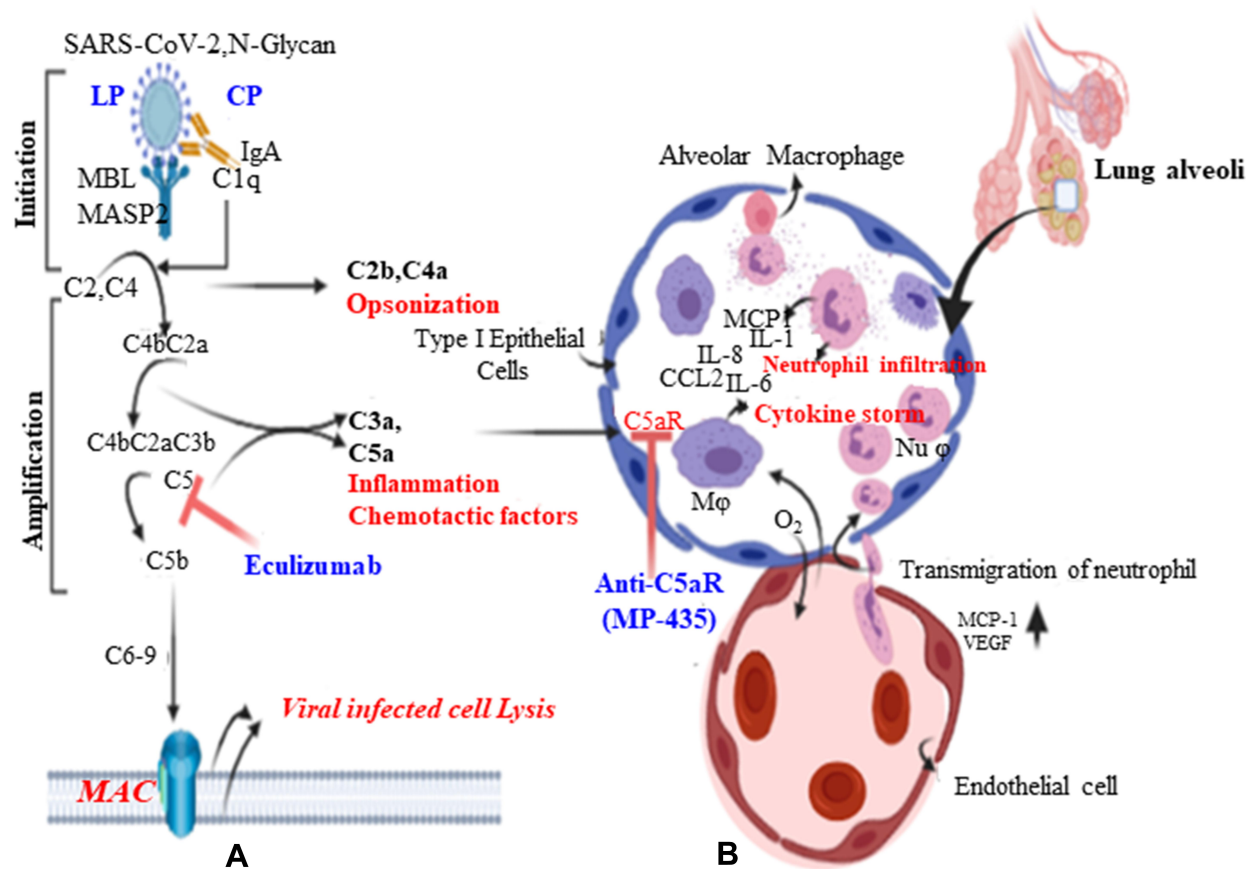


Figure 3 Complement pathways and pathology of ARDS. A:lectin(LP) and classical(CP) pathways use C3 as common intermediate. Exuberant activation of complement pathways results over production of chemo attractants, C3a and C5a (anaphylatoxins) and promote inflammation. Eculizumab humanized monoclonal antibody target against C5 preventing breakdown into C5a (anaphylatoxin action of complement component) and C5b, which is an integral component of the membrane attack complex(MAC). B: Overproduction of C3a and C5a which acts on type I epithelium of the air sack (pulmonary edema and fibrosis or hyaline membrane) as well as the endothelial wall of the vasculature (increased vascular permeability), results recruitment of inflammatory cells mainly monocytes and neutrophil, and release of many inflammatory cytokines known as inflammatory cytokine storm.

cleaves complement (C)4 and 2. C4 and C2 cleavage products form the classical and lectin pathway C3 convertase (C4bC2a), which cleaves C3 into C3b and C3a. A second molecule of C3b can associate with C4bC2a to form C5 convertase (C4bC2aC3b) of the classical and lectin pathways.⁴⁵ The third is the alternative pathway, mediated by spontaneous cleavage of C3 binding with the pathogen's cell surface components.⁴⁴⁻⁴⁶ While complements play a central role in innate immunity and provide a bridge with the adaptive immune response against pathogens, their over-activation may cause excessive alveolar air sac infiltration by neutrophils, and monocytes secondary to excessive production of C3a and C5a (Figure 4). Subsequently, this leads to collateral lung injury and exacerbating factors for SARS-CoV-associated ARDS. Since SARS-CoV-2 is a new virus, the body does not have memory antibodies or specific IgG to detect the spike glycoprotein through activation of adaptive immunity in the early phase of COVID-19 infection.^{47,48}

Since SARS-CoV-2 is a respiratory disease, the primary antibody secreted might be IgA, which is not reliable for activating the classical complement pathway, unless based on an aggregated antibody. Instead, the virus may form IgA complexes or SARS-CoV-S-N-glycan (N-330) MBL binding to activate the lectin complement cascade defense against the invading virus in mucosal immunity.⁴⁹

Overriding activation of complement pathways leads to the formation of bioactive molecules, mainly C3a and C5a. C5a is the most potent chemoattractant involved in the migration and recruitment of inflammatory cells in the activation of phagocytic cells and the release of granular enzymes and free radicals.⁵⁰ Since SARS-CoV-2 has a similar identity to SARS-CoV, C3a products of the complement cascade exacerbate SARS-CoV-associated ARDS.⁵¹ A study showed that C3 knockout mice infected with SARS-CoV had less respiratory dysfunction, decreased infiltration of neutrophils and monocytes, and low levels of cytokines and chemokines in both

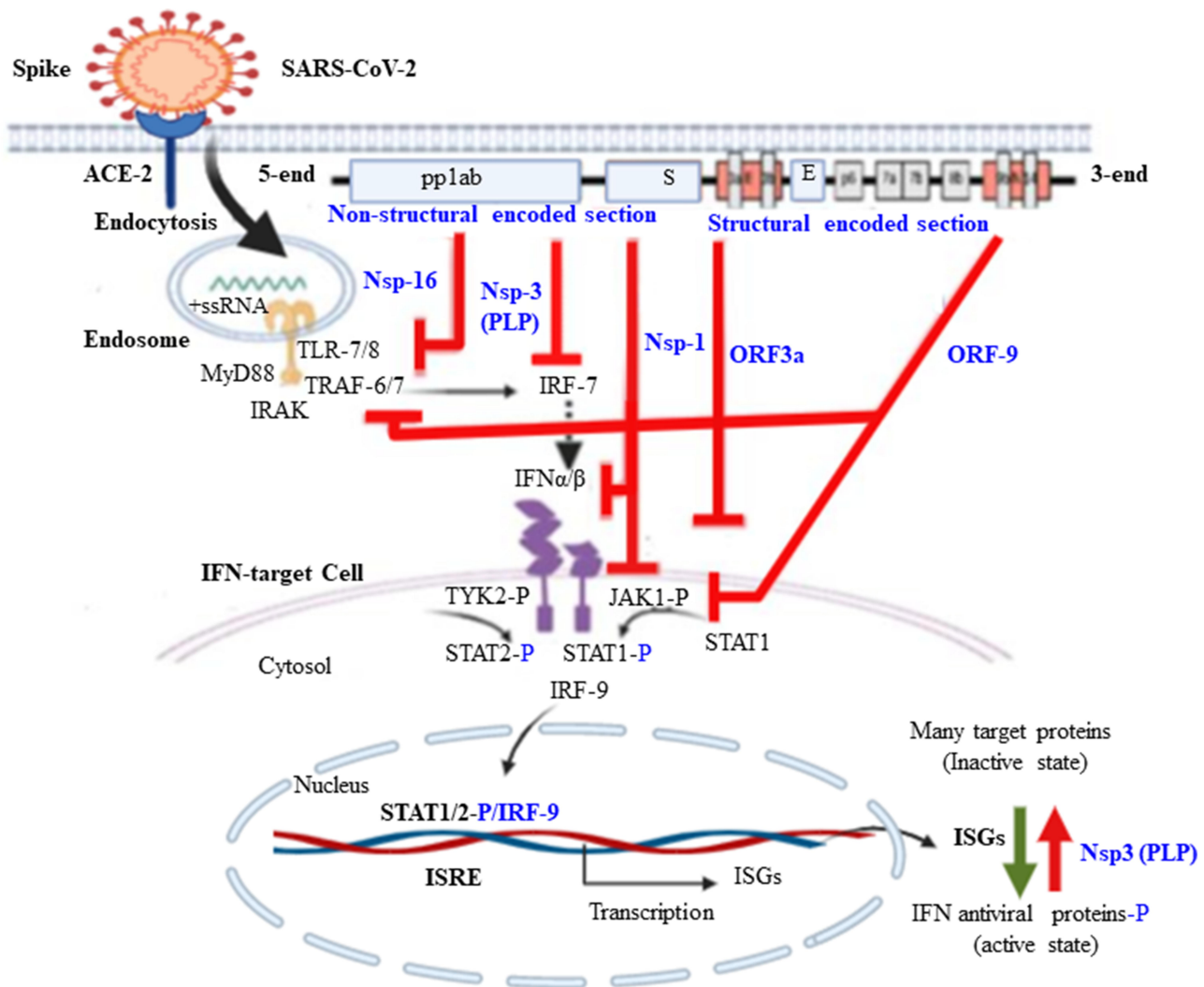


Figure 4 Proposed mechanism of SARS-CoV-2 induced inhibition of IFN production and its signaling during action on target cells. Some SARS-CoV-2 genes act as virulence factors that antagonize innate immune response by inhibiting IFN gene expressions such as Nsp16, Nsp3, and ORF9. Other gene components of the SARS virus act as inhibitors of INF signaling on different target cells mainly ORF9, ORF3a and Nsp1.

lungs as well as sera. However, they showed similar viral load in the lung alveoli.⁵¹ SARS-CoV-2-mediated C5a triggers inflammatory cell activation and cytokine release. This results in epithelial cell degradation and increased vascular permeability. Together, large numbers of inflammatory infiltrates, fluid, and blood cells shift to the alveoli and lung pleura, resulting in dyspnea and respiratory insufficiency. Many scholars have shown that C5a signaling contributes greatly to inflammation and lung damage, and is targeted at treating highly pathogenic virus infections. Moreover, recently C5aR targeting has shown more promising beneficial effects than C5 in small animal models of ARDS by reducing proinflammatory cytokines.⁵²⁻⁵⁴ In addition, different research output elaborates that C5 is a bottleneck for both the release of C5a as an inflammatory mediator and the membrane attack

complex (MAC) (C5b-C9) as a defense against viral infection. Thus, anti-C5a and anti-C5aR only block the effects of C5a without affecting the formation of the MAC, thus protecting against not only viral infection but also superimposed bacterial infections of the lung.^{55,56} Eculizumab is an anti-C5 antibody; however, C5aR antagonists such as MP-435 target the action of C5a alone and do not inhibit MAC formation (Figure 4).⁵⁷ Therefore, receptor antagonists (anti-C5aR) may lessen a patient's susceptibility to bacterial infection, so caution is needed when anti-C5 monoclonal antibodies are used.

SARS-CoV-2 Subversion of Innate Immunity

Viruses have evolved a number of strategies to evade the immune response.^{58,59} Even though SARS-CoV-2 and other related viruses, such as SARS-CoV and Middle East

respiratory syndrome coronavirus (MERS-CoV), are sensitive to the IFN- α/β immune response system, this novel coronavirus is still highly pathogenic. Recent and previous research experience on SARS-CoV showed that the genome contains virulence factors that target core molecules to evade the host response. One of the major mechanisms used by SARS-CoV-2 to escape from host innate immunity is encoded COVID-19 proteins inhibiting IFN production and signaling cascade. This allows the virus to replicate, transcribe, and be transmitted. The detailed understanding of the mechanism of hindrance of IFN production and actions, promoting COVID-19 replication and proliferation, is essential in planning therapeutic options and controls against viral infection.

Nsp16 encodes 2'-O-methyltransferase, which can methylate the RNA cap at 2'-O-positions.⁶⁰ The first strategies inhibit type I IFN production by viral genes encoding for Nsp16 or S-adenosyl-L-methionine (Ado Met)-dependent 2'-O-methylase. This involves post-transcriptional modification of the ssRNA at the 5'-terminal and changing 5'-triphosphate groups into methylated 5'-cap-1 formation, and mimicking the host cell's mRNA. Therefore, the viral RNA is capable of mimicking the host RNA and is not detected by MDA-5 and TLR-7/8, allowing the virus to escape innate immune recognition.⁶¹ Yet, any non-self-recognizable RNA (having 5'-triphosphate groups) in the cytoplasm can be specifically recognized by RIG-I or TLR-7/8 or Mda5, which then amplify type I IFN production.^{62,63} In contrast, eukaryotic mRNA, which is not recognized by retinoic acid-inducible protein I (RIG-I) or MDA-5, usually has a 5'-cap structure methylated at the N7 position of the capping guanosine residue (cap 0), the resulting ribose-2'-O position (cap 1), and sometimes at adjoining residues (cap 2) during posttranscriptional modification.⁶⁴ Also, Nsp10 works with Nsp16 to bind to SAM and m7GpppA-RNA as substrated for its 2'-O-MTase activity.^{64,65} Thus, eukaryotic mRNA 5'-cap structures are known to increase mRNA stability, protecting against 5'-exonuclease. This facilitates the binding of translational initiation factor proteins with both 40s and 60s ribosomes, and enhances translational efficacy.⁶⁶

Nsp3 (1922 aa) is the largest multidomain protein among all the Nsps of β -CoV. It is cleaved off from ORF1a/ORF1ab by the papain-like protease domain or PL2pro domain that is within Nsp3 itself.^{67,68} It is a central player during the formation of viral replication associated with other Nsps, especially Nsp4 and Nsp6.⁶⁹ SARS-CoV-2 Nsp3 contains 16 domains, namely

ubiquitin-like domain 1 (Ubl1), hypervariable region (HVR) or acidic domain, macrodomain I/II/III (MacI/II/III), domain preceding Ubl2 and PL2pro (DPUP), ubiquitin-like domain 2 (Ubl2), papain-like protease 2 domain (PL2pro), nucleic acid binding domain (NAB), beta coronavirus-specific marker domain (β SM), transmembrane domain 1 (TM1), Nsp3 ecto-domain (3Ecto), transmembrane domain 2 (TM2), amphipathic helix region (AH1), and domains specific to *Nidoviriales* and *Coronaviridae* (Y1 and CoV-Y).⁶⁸ After Nsp16, Nsp3 acts as the second most important virulent gene product, mainly by inhibiting RIG-I (cytoplasmic cascade) and TLR-3/7 (endosomal)-dependent IFN- α/β production, probably by interaction of the papain-like protease (PLP) domain with protein stimulators of IFN gene (STING). PLP of MERS-CoV down-regulates the gene encoding for IFN- β via its deubiquitinating activity.⁷⁰ Moreover, even though the mechanism is not yet clear, different scholars have indicated that the PLP of SARS-CoV inhibits the host antiviral innate immune response by inhibiting phosphorylation, dimerization, and nuclear translocation of IRF-7 or -3, likely by forming a complex with IRF-3.^{71,72}

Nsp1 is found in the closest portion of the 5' end encoded by ORF1a/b. This Nsp is considered as the third virulent factor, by inhibiting IFN- β mRNA from being translated into the protein product and by promoting degradation of overexpressed host endogenous mRNA. Its mechanism is to bind with 40s ribosomal subunits and cause conformational change (loss of function), and prevent 80s formation, resulting in translational inhibition. Furthermore, the Nsp1-40s ribosomal complex induces the modification of 5'-capped mRNA and renders the template RNA translationally incompetent.^{64,73,74} Nsp1 also inhibits the action of IFN- α/β on its signaling cascade intermediates, mainly on the JAK-STAT pathway via blocking its phosphorylation (Figure 1).⁷²

The SARS-CoV-1 or -2 genome consists of nine unique ORFs. Of these, ORF3a is the largest and encodes a protein of 274 amino acids.⁷⁵ The 3a protein is part of the virus particle and is expressed abundantly in infected cells.⁷⁶ The major virulence of this sectioned viral protein is achieved by down-regulation of the expression of the type I IFN receptor (IFNAR), leading to a blockade on type I IFN signaling.^{72,77} IFNs are known to be crucial regulators of the development of Treg cells. In the regulatory immune system of patients with COVID-19, Treg cell counts have been shown to correlate inversely with disease severity.⁷⁸

Non-Structural Protein as a Potential Therapeutic Target for SARS-CoV-2

The genomes of replicases are involved during viral RNA gene expression. They are also considered as virulent gene products of the viruses to enable escape from the host immune response.^{70,79} The multidomain Nsp3 (PLP), Nsp5 (3CLpro), Nsp12 (the main RNA-dependent RNA polymerase), Nsp16 (2'-O-methyltransferase), and Nsp13 (ATP-dependent RNA helicase) are the most important popular drug targets because of their vital biological function (Figure 5).⁷⁰

Since COVID-19 does not have a confirmed drug option, many broad-spectrum antibiotics and antiviral drugs are under clinical trial to reduce and treat the virus.^{80–86} Drugs that have shown high binding affinity to Nsp3 include ribavirin, valganciclovir, thymidine, and levodropropizine. In addition, FDA-approved drugs (remdesivir, saquinavir, and darunavir) and two small molecules (flavone and coumarin derivatives) are potential inhibitors of 3CLpro.⁸⁷ Moreover, antibacterial drugs (lymecyclin, demeclocycline, and chlorhexidine) also target 3CLpro.⁷⁹

COVID-19 uses unique 2'-O-methyltransferase (2'-O-MTase) as capping machinery of its RNA at the 5'-end. As a result of this, the viral genome mimics the host mRNA and escapes from innate immune sensing factors, such as RIG-I and MDA-5, and type I IFN antiviral activities.^{61,88,89} A known HIV inhibitor drug, dolutegravir (DG) showed an estimated binding free energy of 9.4 kcal/mol, and inhibits 2'-O-MTase, while bictegravir conferred an estimated binding free energy (DG) of 8.4 kcal/mol against 2'-O-MTase.

Moreover, natural ligand (SAM) analogues, namely trabodenoson, binodenoson, and regadenoson, may be potent inhibitors of the substrate-binding pockets of Nsp16.^{86,89}

Nsp1 inhibits host gene expression via targeting the 40s ribosomal subunit, which induces host mRNA degradation and IFN-dependent signaling. Therefore, Nsp1 is a promising drug target, and its inhibition would make SARS-CoV more susceptible to the host's antiviral defenses. This implies that the innate immune activities via type I IFN would become free of being inhibited by Nsp1 and work actively against the virus.^{90,91}

The other protein that is most conserved in SARS-CoV-2 is RNA-dependent RNA polymerase (Nsp12), which is particularly involved in low-fidelity replication and transcription at the time of viral priming.^{92,93} In addition, Nsp7 and Nsp8 complexes act as cosubstrates to promote the binding of Nsp12 to RNA and boost enzyme activity at the time of transcription.^{79,92} The other potential alternative COVID-19 broad-spectrum antiviral drug therapy, Arbidol (an indole derivative), also targets Nsp7/8 complex and blocks viral replication by inhibiting SARS-CoV-2 fusion to the host cell.^{79,80}

Remdesivir (RDV) is a nucleoside analogue and shows the most promising broad-spectrum antiviral activity in SARS-CoV-2.⁴¹ This drug was originally developed by Gilead Sciences (USA) for treating Ebola virus disease. Subsequent studies showed that RDV is a monophosphoramidate prodrug that has metabolized into C-adenosine nucleoside analogue by targeting RNA-dependent RNA polymerase (RdRp) as a chain terminator.^{80,85,94–96} Similarly, favipiravir is a guanine

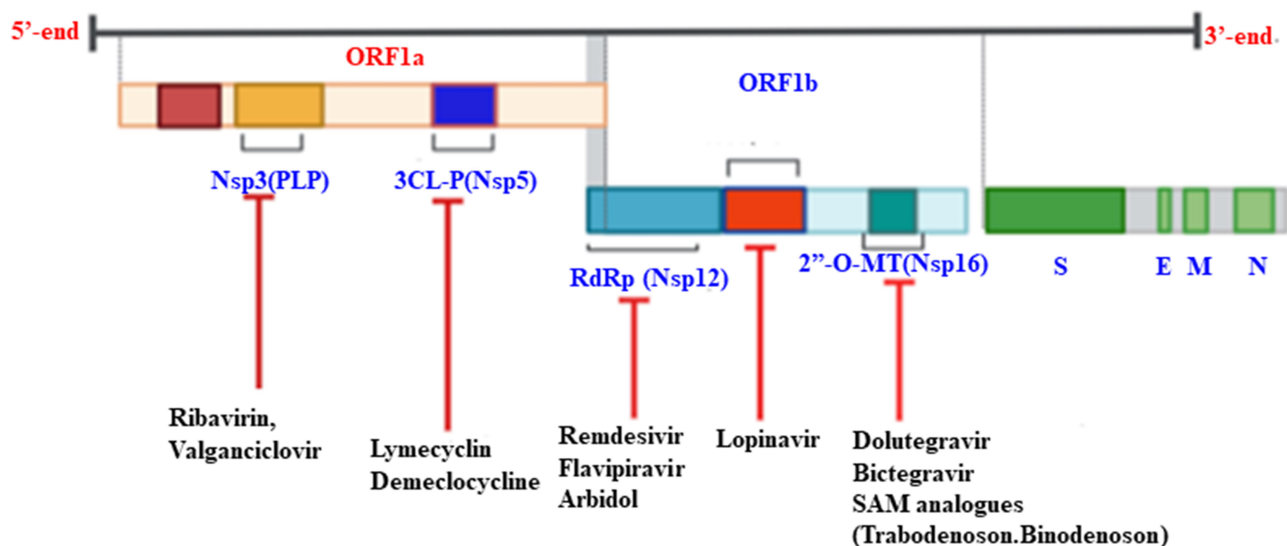


Figure 5 Therapeutic targets of non-structural proteins of SARS-CoV-2.

Table 1 Summary of Some Non-Structural Protein Targeting Drugs of COVID-19 Registered Under Clinicaltrials.gov

Drug(s)	Class	Site of Study	Clinical Trial Status	Available Clinical Trial Outcomes
Remdesvir (GS-5734)	Ebola virus (EBOV)	USA, Birmingham	Completed (Phase III)	Time to recovery by day
Favipiravir	Antiviral for flu and Ebola	USA, Arizona	Recruiting (Phase IV)	Time to clinical improvement until 14 days later
Lopinavir and ritonavir	Antiretroviral (PI)	Canada (Nova Scotia Health Authority)	Recruiting	No clear benefit*
Ribavirin	Antiviral and anti-HCV	USA, Colorado	Recruiting	NA
Trabodenson	SAM ligand analogue	USA	Recruiting	NA

Note: *WHO discontinued this clinical trial.

Abbreviations: PI, protease inhibitor; HCV, hepatitis C virus; NA, not available.

analogue, also used as an inhibitor of RNA-dependent RNA polymerase, but it has been less approved than remdesvir (Table 1).^{79,97}

The other multifunctional non-structural protein is Nsp13 (helicase), for unwinding of dsDNA as well as RNA from 5'–3' polarity, coupled with NTP (ATP) as an energy source.^{98,99} Because this SARS-CoV helicase enzyme is absolutely necessary for subsequent viral replication and proliferation, it is thought to be an attractive target of anti-SARS-CoV-2 drugs. Lopinavir (known as a protease inhibitor of HIV infection) possibly targets Nsp3b/3c and helicase, with ritonavir being used to increase the half-life of lopinavir via inhibition of cytochrome 450 (Table 1).^{94,97} Collectively, targeting the Nsp of the novel coronavirus helps to boost the innate immune recognition ability as well as the activity of the type I IFN antiviral signaling cascade.

Conclusions and Future Directions

In innate immunity, IFNs are key players against viral infection; however, SARS-CoV-2 infecting cells must overcome the IFN-mediated antiviral response to replicate and propagate to new host cells. Because of different evasion mechanisms of the SARS-CoV-2 genome, containing virulent accessory as well as non-structural gene products, such as Nsp16, Nsp3, Nsp1 ORF3a, and ORF9, designing drugs that inhibit these gene products is vital for boosting the action of IFNs against COVID-19. Dysregulated complement systems are the most likely cause of the pathogenesis of ARDS or cytokine storms in COVID-19. We have much research evidence to show that anti-C5a and/or C5aR antibody can provide promising therapeutic options by reducing

histopathological injury of lung alveoli and decreasing inflammatory cytokines. Furthermore, we suggest IFN- α 2b and Arbidol as potential therapeutic options to reduce the viral load as well as its pathological sequelae. SARS-CoV-2 induces an inflammatory cytokine storm linked with an exuberant elevation of C5a and downregulation of IFN α / β secretion, and its action is associated with the disease severity. Therefore, it is reasonable to hypothesize that blocking of C5aR, IL-6, and virulent Nsp of SARS-CoV-2 might be an effective therapeutic avenue for COVID-19-mediated ARDS and its complications.

Abbreviations

C, complement; Nsp, non-structural protein; ORF, open reading frame; IL-6, interleukin-6; MERS, Middle East respiratory syndrome; SARS, severe acute respiratory syndrome; COVID-19, coronavirus disease 2019.

Acknowledgments

We would like to forward our gratitude to the authors of the articles that we used to generate this review report.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

For this review, we did not receive any grants from any funding agencies.

Disclosure

The authors declare that they have no any competing interest.

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