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Review

The role of SARS-CoV-2 accessory proteins in immune evasion

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ABSTRACT

Many questions on the SARS-CoV-2 pathogenesis remain to answer. The SARS-CoV-2 genome encodes some accessory proteins that are essential for infection. Notably, accessory proteins of SARS-CoV-2 play significant roles in affecting immune escape and viral pathogenesis. Therefore SARS-CoV-2 accessory proteins could be considered putative drug targets. IFN-I and IFN-III responses are the primary mechanisms of innate antiviral immunity in infection clearance. Previous research has shown that SARS-CoV-2 suppresses IFN-β by infecting host cells via ORF3a, ORF3b, ORF6, ORF7a, ORF7b, ORF8, and ORF9b. Furthermore, ORF3a, ORF7a, and ORF7b have a role in blocking IFNα signaling, and ORF8 represses IFNβ signaling. The ORF3a, ORF7a, and ORF7b disrupt the STAT1/2 phosphorylation. ORF3a, ORF6, ORF7a, and ORF7b could prevent the ISRE promoter activity. The main SARS-CoV-2 accessory proteins involved in immune evasion are discussed here for comprehensive learning on viral entry, replication, and transmission in vaccines and antiviral development.

1. Introduction

Accessory proteins are essential virulence factors in a variety of pathogenesis pathways during SARS-CoV-2 infection. How they contribute to pathogenesis is unclear. The majority of the roles that these accessory proteins are thought to play relate to immune evasion techniques, such as the regulation of cytokine synthesis by ORF9c or the inhibition of type I IFN activity by ORF3b, ORF6, ORF7a, ORF8, or ORF9b. These auxiliary proteins also influence other important cellular processes as ORF3a's involvement in autophagy or apoptosis, ORF3d's function in mitochondrial function, and ORF9b's function in inflammasome activation. The expression of and interactions with cellular components of each sgRNA are influenced by their various regulatory mechanisms. However, there are still a number of issues that need to be looked into. The roles of accessory proteins have been proposed as potential experimental targets for the development of novel therapeutics or drug repurposing. ORF3a has a strong relationship to viral pathogenicity because it alters the biological physiology of the host cell. Inflammasomes and NF-kB signaling are stimulated, which not only promotes cytokine storms but also regulates host cell death and autophagy. Additionally, the viroporin ORF3a, which is distinct from the envelope (E) protein, functions as an ion channel to aid in the release of viral particles. The host heme oxygenase HMOX1, to which ORF3a binds, is essential for the anti-inflammatory effects of the NLRPS pathway.

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; ORF, Open reading frame; RBD, receptor binding domain; ACE2, Angiotensin-converting enzyme 2 (ACE2); IFN, Interferon; STAT1/ 2, Signal transducer and activator of transcription 1/ 2; JAK 1, Janus kinase 1; ISG, interferon-stimulated gene; NPC, nuclear pore complex; ISRE, Interferon-sensitive response element; MAVS, Mitochondrial antiviral signaling; TOM70, Translocase of outer mitochondrial membrane 70; IKK, inhibitor of nuclear factor kappa B kinase-related kinase; TRAFs, tripartite-motif protein 25 (type I and type II IFN-inducible E3 ligase); RIG-1, retinoic acid-inducible gene I (Type-1 Interferon Pathway); TRIM 25, Tripartite Motif Containing 25; IRF3, Interferon regulatory factor; MAPK, Mitogen-Activated Protein Kinase; NLS, nuclear localization signal; KPNA2, karyopherin alpha 2.

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Medications that stop the connection between ORF3a and HMOX1 are an effective method of treating COVID-19. Additionally, plasma from a COVID-19 patient who was recovering contained anti-ORF3a antibodies. ORF7a induces an immune reaction in host cells. It not only promotes NF-kB activation and results in the production of proinflammatory cytokines and chemokines, but it also inhibits STAT2 phosphorylation, which hinders the formation of type-I interferon. This variety would be beneficial in the development of broad-spectrum antiviral drugs against this protein. The structure allows for the development of therapies that specifically target ORF8 to stop it from interacting with a variety of host proteins, including lysyl oxidase (LOX), the interleukin 17 receptor (IL17RA), and growth/differentiation factor 15 (GDF15). Furthermore, the host's strong antibody responses to the ORF8 protein have become the primary serological indicator of SARS-CoV-2 infection for screening. Convalescent COVID-19 patients' plasma has also been found to have ORF9b-specific antibodies [1–5].

The SARS-CoV-2 genome encodes and produces different accessory proteins, including ORF3a, ORF3b, ORF6, ORF7a, ORF7b, ORF8, ORF9b, ORF9c, and ORF10 [6]. In the second replication phase of SARS-CoV-2, the positive strand sub-genomic mRNA through negative-sense RNA as a template transcript of the ORFs [7]. As a result of their intrinsic nature, accessory proteins, like the primary and extrinsic functional spike proteins covering the receptor binding domain (RBD), and sites for protease cleavage, cannot be considered positive selection targets. The SARS-CoV-2 accessory proteins ORF6, ORF3b, ORF7a, ORF7b, ORF8, ORF9b, and ORF10 are considered interferon antagonists and have notable function in the SARS-CoV-2 pathogenesis [4,8]. Furthermore, these proteins can impair the host's immune response through various mechanisms. An early interferon (IFN) response is required to induce a potent antiviral state. The delayed IFN response and proinflammatory cytokine expression during COVID-19 infection highlight host inflammatory responses. SARS-CoV-2 proteins suppress host immune responses indirectly by interfering with RNA maturation, mRNA translation, protein trafficking, and nuclear trafficking (Table 1). Our comprehension of the host vs. SARS-CoV-2 interaction will influence the development of viral antagonists as potential therapeutic targets (Table 2). During the acute COVID-19, the IFNs I and IFNs II immune responses are in the first line; however, the SARS-CoV-2 to bypassing them to induce active infection. Nonstructural, structural, and accessory proteins of SARS-CoV-2 play critical roles in accelerating the life cycle and evading host immune responses [9]. The accessory proteins, such as ORF3a, ORF3b, ORF6, ORF7a, ORF7b, ORF8, ORF9b, and ORF10, which among coronaviruses have the lowest degree of homology, are essential for controlling the host's response to COVID-19 infection. ORF3a, ORF7a, and ORF7b inhibit IFNα signaling, and ORF8 suppresses IFNβ signaling. STAT1/2 phosphorylation can be interfered with by ORF3a, ORF7a, and ORF7b. Many SARS-CoV-2 accessory proteins prevent the phosphorylation of STAT1 and STAT2. ORF3a, ORF6, ORF7a, and ORF7b inhibit STAT1 phosphorylation, and ORF3a and ORF6 can inhibit STAT2 phosphorylation. ORF3a, ORF6, ORF7a, and ORF7b have suppressed more than 40% of ISRE promoter activity [10]. (Fig. 1). During COVID-19, the SARS-COV-2 accessory proteins have a notable function in cellular pathogenesis. The majority of mentioned accessory proteins' roles are associated with immune evasions, such as ORF9c inhibiting cytokine secretion or ORF3b, ORF6, ORF7a, ORF8, ORF9b, ORF9c, and ORF10 affecting IFNs I. Furthermore, ORF3a and ORF3b alter critical cellular mechanisms, including apoptosis and autophagy, ORF3b alters mitochondrial activity, and ORF9b activates the inflammasome. The Omicron (B.1.1.529) variant of SARS-CoV-2 through RBD of spike attached to the ACE2 receptor and evasive the antibody response in variant complexes. [11]. In addition, ORF3a plays a role in apoptosis. ORF9 b and ORF9c interact with cellular organelles and inhibit the antiviral response. [12]. Studies reported that virus virulence is potentially the results of protein evolution and the triple South African and Brazilian mutants may affect already developed vaccine. [13]. Future studies will reveal the safety and efficacy of prophylactic vaccine against coronaviruses [14].

Table 1
A list of the strategies used by SARS-CoV-2 to suppress innate immune responses.

Viral Protein	Mechanism of Evasion	SARS-CoV-2/ Host Interactions	Ref.
ORF3a	1. The phosphorylation of STAT1/STAT2 is inhibited. 2. A decrease in IFN-promoter activity mediated by MAVS.	TRIM59	[10, 15–21]
ORF3b	1. Restricting the nuclear translocation of IRF3. 2. A decrease in IFN-promoter activity mediated by MAVS. 3. Restricting IRF3 nuclear translocation and phosphorylation.	TRIM59	
ORF6	1. Restricting the import and export of nuclear materials. 2. A decrease in IFN-promoter activity mediated by MAVS. 3. Restricting IRF3 nuclear translocation and phosphorylation. 4. Restricting the phosphorylation of STAT1/ STAT2. 5. The suppression of nuclear translocation of STAT1/ STAT2.	KPNA1/2, Nup98, Rae1	
ORF7a/ 7b and ORF8	1. The phosphorylation of STAT1 and STAT2 is inhibited. 2. The MAVS signaling complex is inhibited. 3. A decrease in IFN-promoter activity mediated by MAVS.	KPNA1/2, Nup98, Rae1	
ORF9b	1. Reduction of NEMO's K63-linked poly-Ub. 2. Prevents TOM70-HSP90 communication. 3. Restricting the phosphorylation of TBK1. 4. Reduction of IFN-promoter activity mediated by MAVS	TOM70, RIG-I, MDA5, MAVS, TBK1, STING	
Conclusion	- Despite being unimportant for viral replication, the COVID-19 accessory proteins are crucial for infection and pathogenesis. - SARS-CoV-2 ORF3a blocks STAT1 phosphorylation and elevates IFNAR1 ubiquitination to limit IFN signaling. - Only 22 amino acids constitute SARS-CoV-2 ORF3b, which may more effectively prevent the induction of IFN. - To stop the nuclear export of host mRNA and the nuclear import of different host factors, including IRF3 and STAT nuclear import, SARS-CoV-2 ORF6 binds directly to Nup98 and Rae1. - The phosphorylation of STAT1 but not STAT2 may be inhibited by SARS-CoV-2 ORF7a, whereas STAT1 and STAT2 phosphorylation may be suppressed by SARS-CoV-2 ORF7b. - SARS-CoV-2 ORF8 can reduce the level of IFN-mRNA and SeV-induced promoter activation. - SARS-CoV-2 ORF9b targets a variety of molecules to suppress the host's innate immune response.		

The SARS-CoV-2 accessory proteins may influence host immune responses. The lowest and the highest ORF3a distinctive variants have been discovered in Oceania and South America, respectively. These results suggest that widespread changes in accessory proteins influence SARS-CoV-2 pathogenicity and immune response [4]. This demonstrates that, prior to the first human infection in China, the direct ancestor of SARS-CoV-2 faced virtually no selective pressure to alter the immunity of its intermediate host.

Table 2
A summary of the results of the IFN therapy clinical trials.

Therapy	Results	Ref.
With or without Arbidol, IFN-α2b	• Patients receiving inhaled IFN-2b had a considerably quicker time to RT-PCR negative results. • The time when the virus is detectable in the upper respiratory tract is significantly reduced. • Reduced inflammatory marker levels in the blood (IL-6, C-reactive protein). • In sum, Arbidol and IFN-2b combined accelerated pneumonia absorption but did not increase COVID-19 RNA clearance or hospitalization in this group compared to IFN-2b monotherapy. These findings require confirmation in a more significant prospective randomized setting.	[18, 22–28]
Inhaled IFN-α2b	• The lower danger of illness development. • Shorter stay in the hospital. • There is proof that IFN-2b therapy inhibits the onset of lung problems linked to COVID-19. Clinical markers related to the progression of pulmonary diseases include low CD8 + T cell counts, low levels of circulating albumin, high levels of platelets, and increased levels of circulating interleukin-10, IL-6, and C-reactive protein (CRP).	
Subcutaneous injection of IFN-α2b	• Early viral removal • The Clinical condition is improving. • Decreased time spent using additional oxygen. • In sum, IFN alpha-2b combined therapy dramatically decreased the time the virus was detectable in the upper respiratory tract. Patients with COVID-19 who received IFN alpha-2b subcutaneously along with lopinavir/ritonavir experienced shorter hospital stays and quicker viral clearance, which calls for further clinical research.	
Inhaled IFN-β1a	• Decrease in the risk of suffering a fatal illness or passing away. • The Synairgen PLC-produced novel recombinant IFN-β1a formulation (SNG001) was designed for nebulized direct delivery to the lungs. It is produced by mammalian cells and has a more focused activity than interferon beta-1b. When administered systemically to patients with multiple sclerosis, this drug has previously demonstrated long-term safety and effectiveness.	
Subcutaneous injection of IFN-β1b	• Reduction in the transmission of SARS-CoV-2. • A faster time to full relieving symptoms. • Less time spent in the hospital. IFN beta-1b effectively decreased the time to clinical improvement in patients with severe COVID-19 without significantly increasing side effects. ICU admission and the need for invasive mechanical breathing decreased when IFN beta-1b was administered.	
Subcutaneous injection of IFN-β1a or/ and IFN-β1b combination	• Significantly quicker time to clinical improvement with IFN β − 1a. • No discernible difference with IFN β − 1b. • Combining IFN beta 1a/b has many antiviral effects, such as inducing cytotoxic T-cell responses, preventing the translation of viral mRNA, eradicating viral RNA, RNA editing, and altering the production of nitric oxide.	
Subcutaneous injection of PEG IFN-λ	• Shorten the length of the virus-shedding period and prevent clinical worsening. PEG-interferon lambda improved viral reduction, particularly in COVID-19 outpatients with high baseline viral levels, and increased the proportion of patients who had viral clearance by day 7 of treatment. It may be able to delay the onset of viral shedding and halt the progression of the infection.	

2. ORF3a

SARS-CoV-2 ORF3a is the largest accessory protein with 275 aa residues and has 72.7% matching with the SARS-CoV ORF3a [29]. ORF3a is essential for virus replication and pathogenesis. It functions as a viroporin (ion channel) and may increase virus release. ORF3a is involved in several processes, including immune evasion, pathogenesis, cell distribution, and protein internalization into various subcellular compartments [8]. It has been shown that this accessory protein interacts with the host's immune system by activating the pro-IL-1β expression gene and IL-1 secretion, resulting in NF-kB signaling and NLRP3 inflammasomes, as well as increased cytokine storm generation [30]. SARS-CoV-2 ORF3a has been demonstrated to activate caspase-3 and induce apoptosis in Vero, HepG2, and HEK293T cells [31]. Furthermore, ORF3a affects the lysosomal pathway, causing an impaired autophagocytic process [32]. Thus, the main interactions of ORF3a with cells reported in apoptosis, inflammation such as IL1 secretion, and NLRP3 function [8,32]. ORF3a is a key modulator of IFNs I receptor expression in SARS-CoV and SARS-CoV-2, promoting ubiquitination and lysosomal degradation. SARS-CoV-2 ORF3a has interacted with E3 antiviral regulatory ligases, which can neutralize the antiviral response. NSP14 regulates IFNAR1 expression. NSP14 significantly decreased IFNAR1, which impairs STAT1 phosphorylation after IFN regulation. It has been proposed that Bafilomycin A1 treatment can help prevent this pathway [33,34]. STAT1, STAT2, IRF9, and NFKB1 are cytokine-signaling transcription factors that are activated by ORF3a, ORF7a, MAPK8, MAPK14, and MAP3K7. Furthermore, the ORF3a of SARS-CoV-2 disrupts heme oxygenase-1 (HMOX1), an anti-inflammatory system and heme catabolism component. ORF3a inhibits nuclear factor-kB signaling by preventing nuclear p65 accumulation. Since children are resistant to SARS-CoV-2 infection, attention to children's long-lasting antibody responses against SARS-CoV-2 ORF3a, ORF3b, ORF7a, and ORF8 accessory proteins is necessary. [4,35,36].

3. ORF3b

SARS-CoV-2 ORF3b accessory protein is an IFN inhibitor. This protein of SARS-CoV-2 is an interferon antagonist and suppressor of IFNs I [37]. ORF3b-modified interferon antagonism is associated with the protein's C terminus length subcellular location. Also, ORF3b disrupts IRF3 nuclear translocation. Finally, The SARS-CoV-2 ORF3b has critical activity against type I interferon. The ORF3b protein has been found to interact with Stomatin-like 2 (a mitochondrial protein)[6]. The SARS-CoV-2 ORF3b strongly inhibited IFN responses. Because

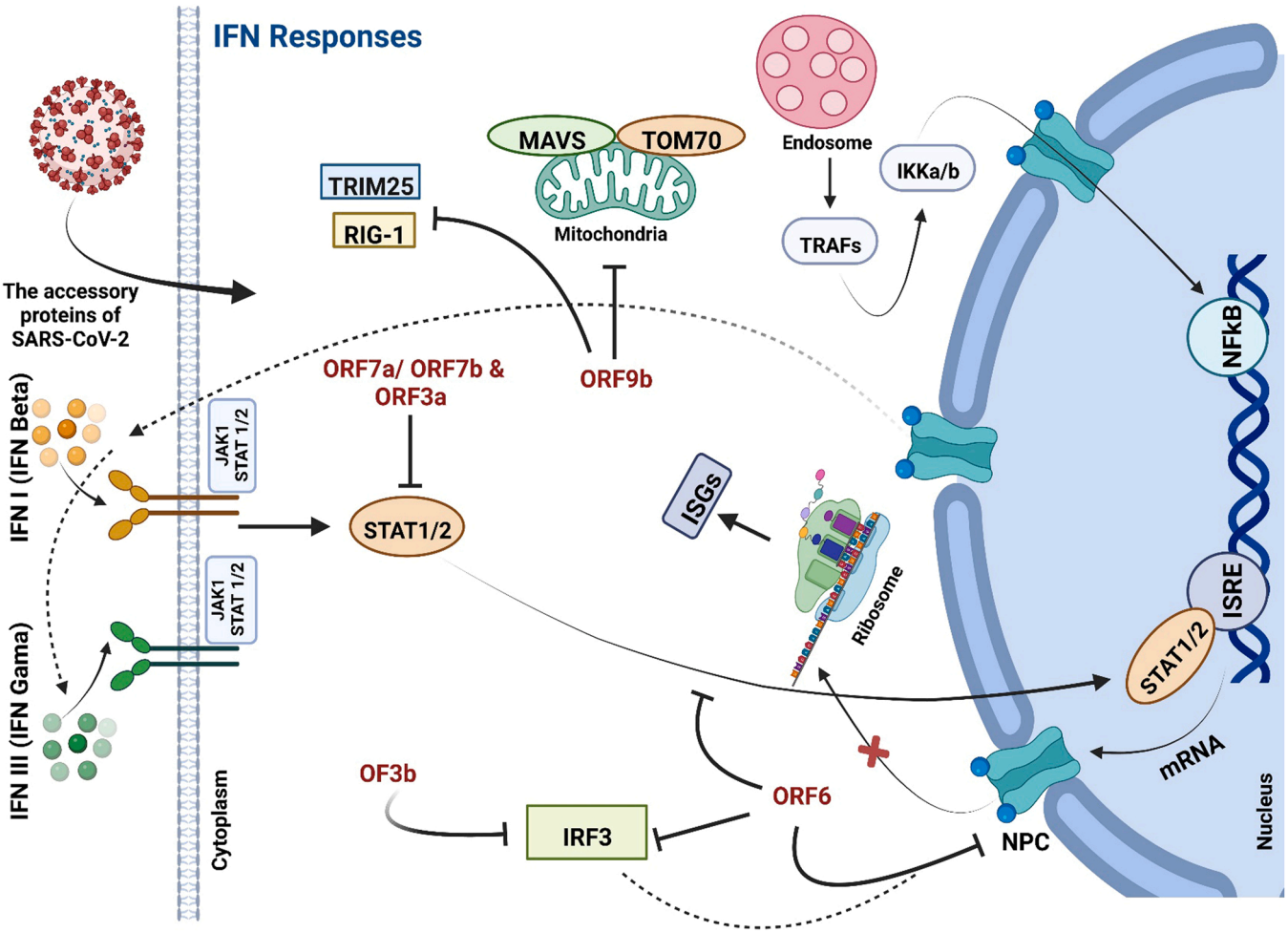

Fig. 1. A diagram illustrates the mechanisms the SARS-CoV-2 virus uses to evade the innate immune response. SARS-CoV-2 has employed various strategies to block innate immune responses and promote effective replication. To develop new vaccines and therapeutic strategies against recently emerging variants, it is necessary to comprehend how SARS-CoV-2 influences innate immunity. [NOTE: IFN: Interferon; STAT1/ 2: Signal transducer and activator of transcription 1/ 2; JAK 1: Janus kinase 1; ISG: interferon-stimulated gene; NPC: nuclear pore complex; ISRE: Interferon-sensitive response element; MAVS: Mitochondrial antiviral signaling; TOM70: Translocase of outer mitochondrial membrane 70; IKK: inhibitor of nuclear factor kappa B kinase)-related kinase; TRAFs: tripartite-motif protein 25 (type I and type II IFN-inducible E3 ligase); RIG-1: retinoic acid-inducible gene I (Type-1 Interferon Pathway); TRIM 25: Tripartite Motif Containing 25; IRF3: Interferon regulatory factor 3].

SARS-CoV-2 lacks a C-terminal nuclear localization signal (NLS), it can become cytosolic, resulting in IRF3 inactivation. NSP12 (viral RNA-dependent RNA polymerase) is an IRF3 direct regulator that inhibits RIG-I/MDA5 by inducing IFN promoter activity. In response to IFN stimulation, NSP12 inhibits nuclear translocation to IRF3 [37,38].

4. ORF6

SARS-CoV-2 encodes the ORF6 protein. 57-aa protein found in the ER and the membranes of vesicles such as lysosomes and autophagosomes [39]. The ORF6 protein functions as an interferon antagonist. [8]. ORF6 has been shown to inhibit IFN activation by blocking STAT [40]. Following IFN therapy, the SARS-CoV-2 ORF6 protein regulates an interferon-sensitive response element (ISRE) promoter-reporter. ORF6 cannot inhibit the phosphorylation of STAT1/Y701 or STAT2/Y689 in response to IFN, but SARS-CoV-2 ORF6 inhibits treatment-free survival/TF translocation. ORF6 inhibits IFN production and STAT1/2 nuclear translocation, both of which are required for ISG transcription activation. It proposed a different mechanism to limit nuclear trafficking [10]. ORF6 interacts with the Nup98 nucleopore complex via its C-terminus, preventing mRNA export. As a result, IFN mRNA nuclear export and transcription factor nuclear import are disrupted. The interaction of the ORF6 nucleopore complex, which has been proposed to block the nuclear pore, has been linked to the inhibition of STAT1 and IRF3 nuclear translocations. Because of ORF6's interaction with the mRNA export receptor, the nuclear pore complex is suppressed, and mRNA accumulates in the nucleus (NPC). Immature poly-A tails disrupt the translation of nascent transcripts, lowering the translation rate. ORF6 selectively disrupts MHC-I function, preventing immune recognition [41]. The primary requirements for IFN-I transcriptional activation are the suppression of phosphorylation of IFN regulatory factor 3 (IRF3) and nuclear translocation. ORF3b and ORF6 have been shown to inhibit IRF3 nuclear translocation [18,37]. ORF6 interacts with importin karyopherin alpha 2. (KPNA2). The inhibition of IRF3 transport in the presence of ORF6 has been attributed to its interaction with KPNA2, an importin involved in IRF3 import [10].

5. ORF7a

The SARS-CoV-2 ORF7b is a transmembrane accessory protein type-I and, in length, has 121 aa residues. This protein deregulated the IFN-I. ORF7a can be ubiquitinated; polyubiquitination of ORF7a occurs at position Lys 119, which may suppress the IFN-I response by inhibiting STAT2. Its reported ectodomain of ORF7 attaches to the CD14 + marker

of monocytes, reducing APC process and producing proinflammatory cytokine overexpression [8]. The interaction between this protein and monocytes suggests that ORF7a may play an essential role in monocyte recruitment to the lung during COVID-19. ORF7a binds to the host ubiquitin system. The host ubiquitin system is altered by forming K63-linked ubiquitin chains, which improves its ability to suppress STAT2 phosphorylation and, as a result, prevents ISGF3 nucleus translocation from inducing ISG [42].

6. ORF7b

ORF7b accessory protein of SARS-CoV-2 has 43 aa long, while SARS-CoV ORF7b protein is one amino acid shorter. ORF7b has 85.4% coverage and 97.2% similarity with SARS-CoV-2 and SARS-CoV [8]. The SARS-CoV-2 ORF7a structure exhibits a distinct binding pattern to specific immune cells [43]. It was found that ORF7a of SARS-CoV-2 initiates antigen-presenting suppression of CD14 + monocytes, and it is involved in the propagation of cytokine storms in patients with COVID-19 [8]. The mechanisms of ORF7b antagonism are unknown, but it appears to inhibit STAT2 activation via ISRE. ORF7b preferentially inhibits STAT2 phosphorylation. STAT2 phosphorylation is inhibited by ORF7b-linked polyubiquitination [17,42]. The phosphorylation of interferon regulatory factor 3 (IRF-3) is reduced due to ORF9b blocking RIG-I and MAVS interaction and ORF7a disrupting TBK1 [44].

7. ORF8

The SARS-CoV-2 ORF8 has the least homology with the other SARS-CoV and SARS-CoV-2 proteins. SARS-CoV-2 ORF8 is a 121-amino acid protein with a signal sequence at the N-terminus. The ORF8 signal sequence is essential for translocation into the endoplasmic reticulum (ER) [45]. Within the ER lumen, ORF8 interacts with several host proteins (involved in ER-associated degradation). IFN-I signaling can be disrupted by ORF8 overexpression. By directly binding to MHC-1, the ORF8 protein, as a SARS-CoV-2 accessory protein, reduces surface and total MHC-1 levels [46]. Furthermore, ORF8 degrades MHC-1 through the autophagy pathway[47]. In addition, ORF8 can prevent antigen presentation and CTL-mediated killing of SARS-CoV-2 infected cells [48]. These findings suggest that ORF8 significantly differs between SARS-CoV and SARS-CoV-2 in terms of structure, sequence variation, function, and critical role in immune evasion [49]. TGF- is just one of the immunological regulatory components with which ORF8 interacts to evade the host's immune system. According to studies, the interaction between ORF8 and the host is caused by two high mutation rates at amino acids 119 and 120. Further investigation revealed that variants with aa120 mutations are more resistant to evasion [50]. Shi demonstrated [51] that ORF8b can form an intracellular insoluble mass and induce cell death, but the mechanism remains unknown. ORF8 is a multifunctional protein that downregulates the class I major histocompatibility complex (MHC-I)-mediated viral antigen presentation, reduces the host innate immune response, and acts as an antagonist of type-I interferon (IFN). Because of this, ORF8 is thought of as a possible antiviral target [52].

8. ORF9b

The ORF9b gene encodes a 97-aa protein found in the mitochondrial membrane. Upregulation of ORF9b results in autophagy and is modified by ATG5. A study revealed that a TOM70 (mitochondrial import receptor) formations a complex beside the ORF9b, which may modulate the host's immune response by interfering with the synthesis of type I IFN [6]. SARS-CoV-2 manipulates organelles in various ways when its RNA enters the host. ORF9b can mediate inflammasome activation to evade immune responses, thereby facilitating viral pathogenesis. ORF9b disrupts the RIG-I-like receptors (RLRs) by binding to the mitochondrial outer membrane protein TOM70. TOM70 recruits IRF3 (transcription factor) to the mitochondria via association with the HSP90. MAVS overexpression reduces IFN activity due to the interaction between ORF9b and TOM70, which also reduces the affinity for HSP90 binding [53,54]. ORF9b interacts with the critical NF-B modulator (NEMO) in an additional antiviral function to prevent K63-linked polyubiquitination. This ubiquitylation is required to activate the phosphorylation activity of the IKK/kinase NEMO, allowing IKK to be ubiquitylated and then degraded. Translocation of NF-B into the nucleus is required to activate proinflammatory cytokine production [55].

9. ORF9c

The ORF9c gene of SARS-CoV-2 has 73 aa and interacts with immune responses. ORF9c contains a transmembrane region that inhibits processes against SARS-CoV-2 infection and interacts with cellular member proteins (Fig. 2). It was found that this accessory protein can interact with various cellular factors (sigma receptors) [6]. SARS-CoV-2 ORF9c may target the NF-Kb signaling pathway, and ERAD/proteasome inhibition may partially reverse the cellular changes caused by ORF9c expression. SARS-CoV-2 uses this accessory protein to cause immune evasion [6,56].

10. ORF10

SARS-CoV-2 ORF10 overexpression blocks the expression of IFNs I and interferon-induced genes in vitro. ORF10 has been shown to repress the interferon by binding to MAVS [56,57]. Furthermore, overexpression of ORF10 can induce mitophagy by increasing LC3 accumulation in mitochondria. ORF10 interacts with antiviral protein activity degradation via the Cul2 ubiquitin ligase [35].

11. Interferon antagonists and accessory proteins' role

Cytokines are primarily responsible for the innate immune response. When immune receptors identify a pathogen, signaling cascades are started, and they lead to the release of IFNs. They activate these cells in an autocrine or paracrine way, resulting in the cells entering an antiviral state, through the activation of hundreds of interferon-stimulated genes (ISGs). Some of the auxiliary proteins are ORF3a, ORF3b, ORF6, ORF7a, ORF7b, ORF8, ORF9b, and ORF10. Historically, viruses have blocked innate immune effectors or activation by using their accessory proteins. The SARS-CoV-2, however, uses its accessory to prevent IFN production and signaling, according to recent research [10,34]. Overall, it is clear that the accessory proteins play a critical role in inhibiting the activation of the IFN system. However, it seems that many of them perform other tasks in addition to hindering innate immunity, such as assisting in virion assembly and viral escape [58].

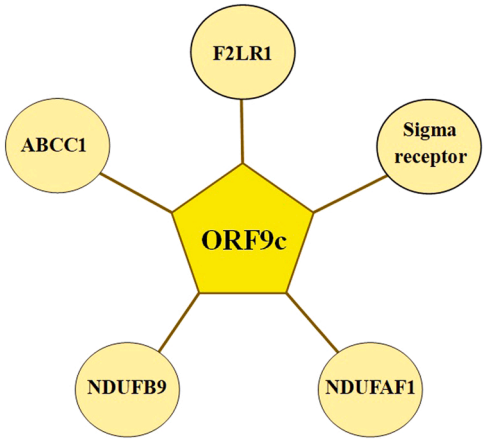

Fig. 2. Interaction of ORF9c with cellular proteins.

Many proteins are encoded at the ORF3 locus, the longest of which is ORF3a. Shorter proteins, ORF3b and ORF3c, are produced from downstream start codons. Increasing Suppressor of Cytokine Signaling 1 (SOCS1) and decreasing IFN signaling are thought to be the mechanisms through which ORF3a prevents JAK activation [59]. IFN synthesis is prevented by ORF3b's C-terminus [37]. Type I and/or III IFN induction and signaling have frequently been shown to be strongly inhibited by ORF6 [17,18,34]. This is because ORF6 prevents STATs from being efficiently imported into the nucleus, which lowers type I IFN induction and signaling [40,60]. According to recent research, ORF7a and ORF7b both inhibit STAT2 phosphorylation, which in turn inhibits type I IFN signaling. ORF7a is modified by covalently linked ubiquitin in a way that improves its capacity to function as an antagonist of type I IFN responses [42]. To reduce type I IFN induction, it has been suggested that ORF9b targets the MAVS signalosome [54].

12. Conclusion

Studies have shown that all of the SARS-CoV-2 accessory proteins play a substantial role in the processes of viral particle replication and immune evasion, suggesting these proteins candidates for the development of new antiviral drugs. Although there have been significant improvements, the development of antiviral drugs based on the SARS-CoV-2 protein structures remains a challenge for this research. The SARS-CoV-2 accessory proteins ORF3a, ORF6, ORF7a, ORF7b, ORF8, and ORF10 may be able to affect the host immune system. They are extensively involved in the host immune response and frequently contribute to pathogenesis. As a result, interest in the field of research into the function of auxiliary proteins has increased. Accessory proteins usually collaborate rather than function independently. They coordinate the various viral replication cycle activities and foster an environment that is favorable for viral reproduction both within and outside of cells. They also regulate host immunity, including stress response, autophagy, apoptosis, and innate immunity. The particular molecular routes used by accessory proteins are yet fully unknown, especially in the infection. We know very little about IFN antagonists produced by viral gene overexpression mechanisms. To design innovative vaccines and potent antivirals, it is imperative to have a thorough understanding of viral evolution. This understanding must include viral transmission, entrance, replication, and immune escape.

Ethics approval

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CRediT authorship contribution statement

Milad Zandi: Writing – review & editing, Conceptualization. **Maryam Shafaati:** Writing – original draft, Visualization. **Davood Kalantar-Neyestanaki:** Data curation, Validation. **Hossein Pourghadamyari:** Data curation, Validation. **Hassan Kaleji:** Investigation. **Mona Fani** and **Saber soltanti**: revised the manuscript. **Samaneh Abbasi:** Writing – review & editing.

Conflict of interest statement

The authors have no relevant financial or non-financial interests to disclose.

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Consent to participate

Not applicable.

Consent to publish

Not applicable.

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