



Review Article

Roles of Human Respiratory Syncytial Virus Proteins in Immune Responses

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Abstract

Human respiratory syncytial virus (hRSV) has evaded active vaccination or effective antiviral therapy for decades and continues to be the leading cause of morbidity and hospitalization in infants, the elderly, and the immunocompromised worldwide. Inadequate understanding of the antigenic intricacies of its viral proteins and the immune responses they generate in the host is the greatest obstacle to the progress of hRSV prevention and treatment. Currently, the prefusion F protein is considered the most effective antigen for inducing protective immunity. Other molecular

components of hRSV, such as the G or N proteins, have also been explored as potential targets for disease control. However, important knowledge gaps remain about the role played by various hRSV proteins in immunobiology and pathology. This review summarizes the unique immunomodulatory aspects of hRSV infection, the viral proteins involved in intracellular immune signaling, and the viral interactions in play with the host's immune system.

Keywords: Human respiratory syncytial virus; Virus proteins; Immunobiology; Pathology; Immune signaling; Immune system

1. Introduction

Human respiratory syncytial virus (hRSV), the leading cause of acute lower respiratory tract infections (LRTIs) in infants and young children worldwide, is also responsible for severe respiratory disease in the elderly and the immunocompromised. It is estimated that hRSV infections cause the hospitalization of about 3.2 million young children with LRTIs, and about 59,000 children younger than 5 years died from them in 2015 [1, 2]. hRSV infections can cause bronchiolitis and result in chronic lung diseases such as wheezing and asthma in later life [3, 4]. Accumulating data now supports a causal role for hRSV LRTIs in the development of hypertension caused by inflammation around the small vessels in the airways [5, 6]. Although hRSV is a major public health burden, effective prevention and therapeutic measures are lacking. Passive prophylaxis with palivizumab, a humanized anti-F monoclonal antibody (mAb), is restricted to premature infants. Vaccines face multiple issues, such as the low activity of neutralizing antibodies after immunization, or the aggravation of pathological reactions from the imbalance of Th1/Th2 cytokine secretion after formalin inactivated hRSV is administered. The biggest barrier to hRSV prevention and treatment is the multiple gaps in our understanding of the pathogenesis and protective immunity mechanisms underlying hRSV infection. This review summarizes the unique immunomodulatory aspects of hRSV infection that are associated with the roles performed by viral proteins in intracellular immune signaling, their interactions with the host immune system, and their possible implications for disease pathology.

2. hRSV

Based on the distinct phylogeny of the polymerase (L) protein and the presence of a conserved M2 gene, both of which are involved in transcriptional regulation and virus morphology, hRSV was renamed human *Orthopneumovirus* to fit with its taxonomical classification as a member of the *Orthopneumovirus* genus (*Pneumoviridae* family) [7]. hRSV is an enveloped virus with a negative-stranded, non-segmented RNA genome containing 10 genes distributed along 15.2 kilobases in the order 3'-NS1-NS2-N-P-M-SH-G-F-M2-L-5' and encoding 11 proteins [8]. The lipid envelope surrounding the nucleocapsid is derived from the host cell plasma membrane during the budding process. This envelope contains three virally encoded surface transmembrane glycoproteins: G, F, and SH. It has been confirmed that G and F surface glycoproteins can induce protective immunity in animal models and are believed to be the main targets for neutralizing hRSV antibodies. Investigations on the mechanism of hRSV-induced immunopathology have increasingly focused on the G and F proteins, and new therapeutic strategies targeting them have gradually arisen. Based on the antigenic differences in F and G, hRSVs fall into subtypes A and B (hRSV-A and hRSV-B), but the F gene shows sequence conservation between hRSV-A and hRSV-B. HVR1 and HVR2, two highly variable regions present in the extracellular region of the highly variable G glycoprotein, are separated by a highly conserved region of 13 amino acids (positions 163–189). Sequence analysis of the HVRs indicates that there is only 67% (nucleotide level) and 53% similarity (deduced amino acid level) between hRSV-A and hRSV-B, respectively [9]. hRSV A and B subtypes can

be divided into a variety of genotypes based on the nucleotide sequences of HVR2.

The virion matrix proteins M1 and M2 are non-glycosylated, and N, P, and L associate with genomic RNA to form the nucleocapsid, whereas two non-structural proteins, NS1 and NS2, are expressed only during cellular infections and are not packaged into the virion [10]. Internal hRSV proteins (e.g., N, M, and M2-1), can induce T cell immunity alone or in combination with surface proteins, and these processes may be important therapeutic targets for regulating immune responses; however, knowledge gaps in this area highlight the need for further research. Nevertheless, the roles played by hRSV proteins in pathogenesis and immunopathology in the host provide clues to their potential contributions to a vaccine.

3. Immune responses and pathology in hRSV infections

The immature infant immune system, the low lymphocyte levels in young children, and the low levels of hRSV-specific neutralizing antibodies in the blood of the elderly and the immunocompromised are the main reasons for the high susceptibility of these people to hRSV. It is important, therefore, to determine how to induce protective antibodies, elicit supportive T helper responses, and invoke sufficient CD8⁺ T cells to clear the virus in hRSV-susceptible populations. However, the unique immunobiology of infection with hRSV means that obstacles to specific treatments or a safe and effective vaccine remain. The host immune responses to hRSV lack immunological prowess during the primary exposure, and this may leave a suboptimal imprint on the adaptive immune response [11]. Adding to that, neonates tend to have an inherent Th2-biased

response that impairs the ability to induce helper T cells and optimal CD8⁺ T cell responses [12].

3.1 Innate immune responses

hRSV first invades respiratory epithelial cells through its G and F viral membrane proteins, thereby internalizing the virions and enabling subsequent replication, transcription, and translation of progeny viruses in cells, which can then infect other cells. It has been suggested that hRSV is anchored to respiratory epithelial cells by pattern recognition receptors (PRRs), including toll-like receptors (TLRs), retinoic-acid-inducible gene-I-like receptors or nucleotide binding-oligomerization domain-like receptors [13], leading to the downstream activation of NF- κ B and interferon regulatory factors to regulate the expression of type I and III IFNs, IFN stimulated genes, and pro-inflammatory mediators [14, 15]. The production of these factors can promote the formation and activation of the inflammasome and cell death in some cases, as well as controlling the virus and directing the development of adaptive immune responses [16].

TLRs are the main extracellular receptors for hRSV recognition. Through its interaction with the F protein present on the viral envelope, the TLR4/CD14 complex provokes an NF- κ B-mediated cytokine response, which includes the secretion of interleukin (IL)-8, IL-10, and IL-6 [17, 18]. TLR2 and TLR4 receptor complexes are able to traffic into the endolysosome, from where they can activate the innate immune response. However, TLR2 alone does not contribute to the production of IFN- α and IFN- β [19]. Instead, TLR3 induction activates the innate immune response through the TRIF-mediated pathway to promote the expression of CCL-5, IFN- α , and IFN- β [20]. In addition, TLR3 and TLR7 recognize viral RNA, and their deficiency can change

the inflammatory environment in the lungs during hRSV infection, leading to enhanced mucus production and worsened lung epithelial cell hyperplasia, but this does not affect viral clearance [21, 22].

Innate PRR signaling also induces a spectrum of chemokine and cytokine expression (e.g., CXCL3, CXCL5, CXCL8, CXCL9, CXCL10, IL-4, IL-6, IL-11, IL-15 and TNF- α), which is crucial to the initiation of anti-hRSV immune responses by regulating leukocyte infiltration and localization in the lungs, further affecting the balance between pathogenesis and virus elimination [23-25]. In addition to their direct cellular effects on the infection sites, these chemokines and cytokines act as potent chemo-attractants, activating and recruiting circulating immune cells, such as neutrophils, NK cells, and cytotoxic T cells, to the airway mucosa. Otherwise, hRSV infection elicits a strong systemic response, especially in the innate immune cells of the respiratory tract (e.g., neutrophils, natural killer cells, dendritic cells, macrophages, monocytes and eosinophils) to form a critical link between recognition and transportation of the viral stimuli to the draining lymph nodes and activation of cell-mediated antiviral responses, while dendritic cells also provide a critical link between the innate immune response and adaptive immunity induction [26]. Tissue-resident macrophages and intraepithelial dendritic cells continually capture viral particles in the airway lumen and internalize them by phagocytosis and macropinocytosis, thereby activating PRRs and initiating immune responses [27]. These innate immune processes work synergistically to immediately and effectively identify the invading pathogens and orchestrate adaptive immune responses.

3.2 Adaptive immune responses

In the later stage of hRSV infections, virions induce specific adaptive immune responses in hosts, including the production of IgA secretory antibodies by respiratory mucosa cells, IgE by eosinophils, serum IgM and IgG antibodies by B cells and cellular immunity by T cells, all which endow antigenic specificity and diversity of antigen recognition. Evidence shows that the production of hRSV-specific antibodies regulates the T-cell response, indicating that responses mediated by antibodies and T cells are interdependent [28]. Antibody responses in infants mainly targeting F and G glycoproteins show a gradually increasing trend from the acute phase to the convalescent phase during natural primary hRSV infections [29]. With circulating CD5⁺ and CD19⁺ B and CD20⁺ B-cell accumulation, there are higher quantities of IgM, IgG, and IgA plasma cells in the lungs of infants with hRSV bronchiolitis [30]. In children with hRSV bronchiolitis or pneumonia, higher serum IgE levels on hospital admission are reportedly associated with prolonged fever and more serious symptoms [31]. The existing natural hRSV-specific nasal IgA and serum IgG with high neutralizing titers in adults are correlated with protection against naturally acquired reinfection with hRSV [32].

The T cell response plays a critical role in host defenses against hRSV infection. An appropriate cellular immune response is essential for generating protective antibodies and clearing primary hRSV infection. However, an unbalanced and dysregulated T cell response can cause pathology. In a murine model of hRSV infection, CD4⁺ T cell production of IL-9, IL-13, and IL-17 contribute to hRSV-induced disease [33]. The types of improper CD4⁺ T cell responses (e.g., Th2-, Th9-, and Th17-related cytokine induction) observed in hRSV-infected infants, which also induce disease in

the mouse model of primary hRSV infection, are also seen in various vaccination settings [34]. CD8⁺ T cells can control acute viral infections by secreting cytokines and lysing infected host cells, but also can result in marked disease enhancement [35]. In early life, CD8⁺ T cell depletion leads to enhanced Th-2 responses during hRSV infection, and virion spreading to the mediastinal lymph nodes results in severe and potentially fatal hRSV infections [36]. CD8⁺ and CD4⁺ T cells both play roles in terminating hRSV replication, while the Th1 and Th2 balance manages the progression of the response. During the course of hRSV infection, the expansion of CD8⁺ T cells is greater than that of CD4⁺ T cells and is accompanied by the release of a series of cytokines and chemokines, imbalanced Th1/Th2-type cytokines, and aggregation of various inflammatory cells, eventually leading to multi-system pathological damage.

4. Roles Played by Viral Proteins in hRSV Infection-Associated Immune Responses and Pathology

4.1 F protein

The F protein, a type I transmembrane glycoprotein, mediates fusion of the viral envelope to cellular membranes and syncytium formation. Its deletion results in a drastic reduction in viral infectivity. It is relatively conserved across all hRSV strains and varies only slightly in its expression pattern. The F protein is highly conserved, and most candidate vaccines and monoclonal antibody drugs are specifically designed to target it. Synthesized as a single inactive polypeptide (F0), its two furin cleavage sites undergo proteolytic cleavage, a process required for membrane fusion activation [37], which produces two disulfide-linked polypeptides, C-terminal F1 and N-terminal F2. The mature F glycoprotein is a homotrimer of F1 and F2

subunits [38]. The F1 region is immunogenic in that it contains the main epitope of the F protein. In addition to promoting viral-cell membrane fusion, studies have shown that the F protein is also involved in viral attachment via its interaction with several cell surface proteins, such as ICAM-1, TLR4, nucleolin, and the epidermal growth factor receptor (EGFR) [39-42]. When F binds to EGFR, mucin production and the secretion of cytokines such as IL-13 occur; therefore, mutations in key sites of the F protein in a live attenuated hRSV vaccine that blocks F binding to the EGFR should weaken the ability of hRSV to generate a successful infection [43]. In murine, the F protein has been shown to interact with, and subsequently signal through, components of the lipopolysaccharide receptor system [44]. The latest research shows that the interaction between the F glycoprotein and the insulin-like growth factor-1 receptor is the mechanism used for viral entry, a finding that may provide new opportunities for the treatment of hRSV infection [45].

It has been shown that the hRSV F glycoprotein stimulates innate immunity by activating the parts of the receptor complexes involved in the innate immune response to hRSV; that is, CD14 and TLR4. Activated TLR4 triggers a signaling cascade by activating the NF- κ B transcription factor and by activating multiple mediators of innate immunity to secrete cytokines, such as IL-8, IL-10, and IL-6, and by sensitizing airway epithelial cells to endotoxin [46-48]. The F protein is involved in both humoral and cellular immunity, and chemokine release mediated by whole viral particles or the F protein has been shown to initiate neutrophil, CD4⁺ T cell and eosinophil chemotaxis. Activated Th1 CD4⁺ T cells can cause changes in the T cell response to infection as well as creating Th1/Th2 cytokine secretion imbalance, which further leads to delayed

hypersensitivity in the host and abnormalities in pulmonary function, such as asthma [49].

The F protein is a major target of the neutralizing antibodies induced by natural infection with hRSV, making it an attractive target for monoclonal antibody development and vaccines. Anti-F antibodies can mediate antibody dependent cell-mediated cytotoxicity (ADCC) *in vitro* [50], and this may offer some potential for treating hRSV. Neutralizing antibodies against the F protein can protect against infection by hRSV subtypes A and B. Most neutralizing epitopes are on the prefusion F (pre-F) protein structure, and the stabilized prefusion protein is more immunogenic and protective than the postfusion protein [51]. To date, at least six neutralizing antigenic sites on F (sites I, II, III, IV, V, and Ø) have been identified and antibodies with major neutralizing activities were found to be specific for the pre-F anti-antigenic Ø site [52]. MEDI8897, a highly potent recombinant hRSV mAb optimized from D25 that recognizes the antigenic Ø site on the apex of the pre-F molecule, has opened up new treatment possibilities for hRSV infection in infants [53].

4.2 G protein

The G membrane protein, a type II putative attachment transmembrane protein embedded in the viral lipid envelope, plays an adhesion function between virions and host cells. In infected cells, hRSV G, which is involved in immune evasion/antagonism, is produced as a full-length membrane-anchored form and a secreted form encoded by an alternative initiation codon in the transmembrane domain [54]. The G protein has highly variable mucin domains at both of its ends, along with a high proline content and extensive O-linked glycosylation surrounding a central conserved domain. Glycosylation changes in G may contribute to hRSV

escape from the host's humoral immune response. The central region of hRSV G contains a CX3C motif that can bind to CX3CR1, which induces leukocyte chemotaxis and may be significant to the biology of hRSV infection and viral pathogenesis.

Accumulating evidence shows that the hRSV G protein has immune modulatory activities. hRSV replication in airway epithelial cells leads to the release of the soluble form of the G-protein, which is involved in the immune modulation process of innate immunity and the T-cell response. During the innate immune response, the G-CX3CR1 interaction influences lymphocyte chemotaxis, and modulates the suppressors of cytokine signaling expression to dampen the production of type I IFN and interferon-stimulated gene (ISG)-15 expression by innate immune cells and the type I cytokine responses of memory T cells [55, 56]. Infections with one hRSV strain lacking the CX3C motif or treated with anti-G monoclonal antibodies to block binding of the CX3C motif to CX3CR1 resulted in increased levels of type I and III IFN [56]. Additionally, through its interaction with CX3CR1, the hRSV G protein induces IL-10 in neonatal regulatory B cells, which downregulates Th1 cell responses. Furthermore, the G protein impedes inflammatory cytokine release by interacting with CX3CR1 and impairs the immune response to hRSV [57]. However, stimulation of naive spleen cells by hRSV or by the G protein induce substantial increases in IL-4 and CX3CL1, suggesting that the expression of G protein during hRSV infection may directly influence the cytokine or chemokine profile. Recent studies have shown that the G-protein can modulate the innate immune system by suppressing TLR4 signaling to baseline levels. Downstream of TLR signaling is a suppressor of the cytokine signaling family of proteins that negatively regulates the type of cytokine and

chemokine expression that is inducible via TLR activation. The hRSV G protein also contributes to immune evasion by modifying host cytokine and chemokine responses whose expression is negatively regulated by suppressor of cytokine signaling (SOCS) proteins [58]. The G protein can also interfere with the roles played by the host's chemokines by reducing their homology to prevent the influx of natural killer cells and CD4⁺ and CD8⁺ T cells, suggesting that G protein expression during hRSV infection may have the adjunct property of reducing the antiviral response to promote hRSV infection or replication [59].

The membrane-bound version of hRSV G is possibly a target of neutralizing antibodies that bind to epitopes in its central conserved domain. Antibodies induced by the G protein are mainly involved in inhibiting viral replication and in immune modulation by interfering with the antibody-mediated neutralization response [60]. It has been suggested that the G-specific antibodies that block G binding to airway epithelial cells or antigen-presenting cells reduce the disease severity, a finding supported by the fact that vaccine-induced anti-G antibodies can block G-induced disease and essentially have an anti-inflammatory effect that results in decreased disease severity. Recently, anti-G murine mAbs 2B11 and 3D3 have shown high efficacy in reducing viral lung titers and ameliorating virus-induced immune dysfunction by blocking G protein binding to CX3CR1 [57].

4.3 NS1 and NS2 proteins

NS1 and NS2 nonstructural proteins are the earliest and most abundantly expressed proteins upon infection with hRSV. They are postulated to perform various roles in hRSV pathogenesis, and they both participate in virus replication and suppress a major component of the

host's innate defenses [61, 62]. For example, hRSV infections provoke considerably weaker innate immune responses compared with other respiratory viruses. This characteristic is largely a consequence of strong interference from NS1 and NS2, which induce type I and III IFN signaling in human epithelial cells, macrophages, and dendritic cells by interrupting the Janus kinase-signal transducers and activators of the transcription 1 (JAK-STAT) pathway [63-65]. NS1 and NS2 impair the activation and effector functions of the interferon regulatory factor (IRF) of transcription factors that are critically involved in the induction of IFN, as well as in the proteasome-mediated degradation of signal transducer and activator of transcription 2 (STAT2) [66, 67]. It has been proposed that NS1 and NS2 impair the activation and effector functions of type I and III IFNs by targeting multiple proteins in the signaling cascade (e.g., RIG-I, MAVS, TRAF3, IKK ϵ , TBK1, and IRFs) [64], as well as delaying the apoptosis of hRSV-infected cells, facilitating prolonged hRSV replication for increased viral yields, and mediating hRSV suppression of the anti-inflammatory activity of the glucocorticoid receptor glucocorticoid receptor transactivation [68]. In the adaptive immune response, NS1 and NS2 inhibit dendritic cell maturation [69], possibly leading to reduced antigen presentation and T-lymphocyte activation, which in turn results in an incomplete immune response that contributes to hRSV reinfection. NS1 and NS2 were also found to induce abnormal signal transduction in bronchial epithelial cells through their enzymatic activities, with both of them negatively modulating the proliferation and activation of CD103⁺ CD8⁺ T cells and driving the abnormal differentiation of CD4⁺ T lymphocytes [70]. These features can enhance disease severity and may be associated with asthma susceptibility after hRSV infection.

4.4 N protein

The nucleocapsid (N) protein polymerizes genomic and anti-genomic RNAs to form separate RNase-resistant nucleocapsids, thereby providing templates for the transcription and replication of the hRSV genome. The N protein is expressed on the surface of a variety of hRSV-infected cells, including DCs. In the hRSV immune response, the N protein, which acts as a major virulence factor, is the recognized major target for helper T cells and the memory cytotoxic T-lymphocyte (CTL) response in humans. By inhibiting T-cell activation, it impairs the acquired immune response and enhances hRSV reinfection susceptibility [71, 72]. Although the N protein does not induce neutralizing antibodies, the ring-nanostructures formed by the recombinant N protein make it a mucosal vaccine candidate because it affords some protection against hRSV to neonatal mice [73]. In terms of vaccine development, N codon optimization may attenuate the viral virulence.

4.5 P protein

The phosphoprotein (P) forms part of the ribonucleoprotein complex by interacting with N and L proteins. It works as a multifunctional adaptor by regulating the connections among components of the nucleocapsid-polymerase complex, and is necessary and sufficient to direct RNA replication [74, 75]. During transcription and replication the P protein, a chaperone protein of N, helps to keep the newly synthesized N polypeptide in a soluble form. It is an essential cofactor for the large polymerase (L), in that it assists with translocation of the polymerase complex along the helical nucleocapsid. The P protein is thought to interact with a transcription antiterminator, the M2-1 protein. The unique P-M2-1 interface present in hRSV

may provide a valuable antiviral target against this pathogen [76]. P protein can subvert the extrinsic apoptosis process in a macrophage-like cell line persistently infected with hRSV, something that may be crucial for establishing persistent infections [77].

4.6 M protein

The matrix (M) protein, a non-glycosylated phosphorylated protein located in the inner surface of the viral envelope, acts as a bridge between the lipid bilayer envelope and the nucleocapsid and helps organize virion components at the plasma membrane before budding [78, 79]. M has been suggested to transport virions in virus-infected cells by interacting with the actin cytoskeleton [80]. The M protein can also recruit and interact with new host factors, including proteins the involved in the innate immune response, cellular trafficking, and host transcription regulation, which is of great significance for antiviral strategies that target M to improve the efficacy of subunit vaccines [81].

4.7 SH protein

The small hydrophobic (SH) protein is a short transmembrane protein containing 64 or 65 amino acids. During infection it primarily localizes to the lipid-raft structures of the Golgi complex, and only very small amounts are associated with the viral envelope. Its function is less clear. Studies show that the SH protein is not involved in binding or infectivity; nor is it essential for viral replication in cell cultures, but it is involved in *in vivo* hRSV survival, at least to some degree [82]. The SH protein is involved in the formation of pentameric ion channels and is not only a target for antibodies with Fc-mediated effector functions, but it can also activate the NLRP3 inflammasome leading to the expression of IL-1 β [83]. It was reported that the SH protein also inhibits apoptosis in host cells during

infection by blocking the TNF- α mediated signaling pathway, which might be advantageous to *in vivo* viral replication [84]. Antibodies to the SH protein can affect *in vivo* viral replication via ADCC [85]. Finally, the live-attenuated hRSV vaccine containing the SH non-coding region has high immunogenicity in children.

4.8 M2 protein

The M2 gene is transcribed as two separate open reading frames (ORFs) that overlap slightly to encode two proteins, M2-1 and M2-2. M2-1, a multifunctional protein, acts as an antitermination factor for the transcriptional process to prevent the synthesis of shortened mRNA, which is a potential target for the development of virus replication inhibitors and contributes to RNA synthesis and mRNA stability by binding to viral RNA and the P protein [86]. The small M2-2 protein is transcribed and translated by the ORF downstream of the M2 gene and is responsible for regulating RNA synthesis during virion assembly. Deletion of M2-2 can cause RNA replication delay, and its overexpression can prevent viral RNA synthesis. As the major targets of CTLs, M2 proteins induce robust T cell responses. A recent study showed that the recombinant hRSV G1F/M2 candidate vaccine, which includes the hRSV G protein domain and a CTL epitope in the M2 protein, can prevent vaccine-associated lung inflammation by regulating the level of CD4⁺/CD8⁺ central memory and Th1-type effector memory [87]. Although the M2-2 deletion appears to restrict viral replication, it induces substantial neutralizing serum antibody responses [88], suggesting that the M2-2 mutant may prove important for the selection of candidate vaccines.

4.9 L protein

The L protein is the largest hRSV protein and, with its function as an RNA-dependent RNA polymerase, it is involved in viral RNA genome replication and mRNA transcription. The L protein is also associated with reduced efficiency of termination at gene-end motifs [89]. Notably, the L protein can interact with the P protein, which is essential for its catalytic activity. There are few studies on the role of the L protein in hRSV immunity. Attenuating mutations in L may reduce virus activity, providing ideas for the development of hRSV attenuated vaccines.

5. Conclusion

hRSV commonly causes LRTIs with substantial morbidity and mortality in infants and the elderly. Advances in recent years have improved our understanding of both the innate and adaptive immune responses contributing to the control of hRSV infections. Protective immunity against hRSV re-infection is usually suboptimal, however, and recurrent infections are common throughout life. Better understanding of the characteristics of hRSV proteins, host immune responses against them, and the pathology caused by them, should identify new ways to develop more effective preventive and therapeutic tools or vaccines. This reviewed has placed emphasis on the F and G glycoproteins to provide helpful information for determining if, or how, these individual proteins contribute to protective immunity and vaccine antigen design, or could be targeted for antiviral drug development.

Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

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