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Molecular Characteristics, Functions, and Related Pathogenicity of MERS-CoV Proteins

Yan-Hua Li [a,b], Chen-Yu Hu [a,b], Nan-Ping Wu [a,b], Hang-Ping Yao [a,b,*], Lan-Juan Li [a,b,*]

[a] State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, Hangzhou 310003, China
[b] Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 31003, China

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ABSTRACT

Middle East respiratory syndrome (MERS) is a viral respiratory disease caused by a *de novo* coronavirus—MERS-CoV—that is associated with high mortality. However, the mechanism by which MERS-CoV infects humans remains unclear. To date, there is no effective vaccine or antibody for human immunity and treatment, other than the safety and tolerability of the fully human polyclonal Immunoglobulin G (IgG) antibody (SAB-301) as a putative therapeutic agent specific for MERS. Although rapid diagnostic and public health measures are currently being implemented, new cases of MERS-CoV infection are still being reported. Therefore, various effective measures should be taken to prevent the serious impact of similar epidemics in the future. Further investigation of the epidemiology and pathogenesis of the virus, as well as the development of effective therapeutic and prophylactic anti-MERS-CoV infections, is necessary. For this purpose, detailed information on MERS-CoV proteins is needed. In this review, we describe the major structural and nonstructural proteins of MERS-CoV and summarize different potential strategies for limiting the outbreak of MERS-CoV. The combination of computational biology and virology can accelerate the advanced design and development of effective peptide therapeutics against MERS-CoV. In summary, this review provides important information about the progress of the elimination of MERS, from prevention to treatment.

1. Introduction

Coronavirus (CoV) is an enveloped, positive, single-stranded RNA virus that causes mild upper respiratory infections in humans. Middle East respiratory syndrome (MERS) is a viral respiratory disease caused by a *de novo* coronavirus—MERS-CoV. MERS-CoV was originally isolated from an Arab patient who died of respiratory failure and renal failure in 2012, and was classified as a member of the CoV family, which is lineage C of the genus *Betacoronavirus*. From April 2012 to 22 May 2019, the total global number of laboratory-confirmed MERS cases reported to the World Health Organization (WHO) was 2428, with 838 associated deaths, or a fatality of about 35% (40.43% in Saudi Arabia) [1]. Aside from MERS-CoV, only a few other CoVs are known to infect humans; these include human coronavirus (HCoV)-229E, HCoV-NL63, HKU1, and OC43, which usually result in relatively mild respiratory disease [2,3]. However, severe acute respiratory syndrome (SARS)-CoV and MERS-CoV are highly pathogenic and lead to high mortality, especially in the elderly and in immunocompromised patients [4,5]. MERS has broken out in 27 countries around the world, with major outbreaks occurring in Saudi Arabia, the United Arab Emirates, and Korea. The dromedary camel is the largest intermediate host for MERS-CoV, and bats are considered to be animal sources of MERS-CoV. However, the specific role of dromedaries in the transmission of the virus is not yet clear. To date, in addition to the safety and tolerability of the fully human polyclonal Immunoglobulin G (IgG) antibody (SAB-301), which is described as a putative therapeutic agent specific for MERS, no effective vaccines or antibodies have been reported for human immunization and therapy [6]. Therefore, different intervention methods are need to treat MERS patients, including control of transmission. In this review, we describe the structure and potential functions of the reported MERS-CoV proteins, and discuss how their respective intervention strategies prevent virus transmission.

* Corresponding authors.
E-mail addresses: yaohangping@zju.edu.cn (H.-P. Yao), ljli@zju.edu.cn (L.-J. Li).

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2. Structural proteins

Many CoVs, including MERS-CoV, have a very large RNA genome of about 30 kb with at least ten predicted open reading frames (ORFs) [3,7]. The ORFs for the structural proteins are arranged as 5′-ORF1a/b-S-E-M-N-poly(A)-3′, which is a similar arrangement to that of other CoVs in lineage C of the genus *Betacoronavirus* [8]. Research has targeted these genes and their encoded proteins for use as vaccines, or for diagnostic or therapeutic purposes (Table 1) [9,10].

Like other CoVs, MERS-CoV carries genes encoding accessory proteins (APs; 3, 4a, 4b, and 5), which antagonize host antiviral responses—typically type I interferon (IFN) responses—and contribute to virulence and pathogenesis. When expressed alone, CoV APs appear to interfere with the innate antiviral signaling pathway [11].

2.1. Spike protein

The spike (S) protein of MERS-CoV is a type I transmembrane glycoprotein that is expressed on the surface of the envelope of the virus and forms spikes from the virus body. The S protein comprises 1353 amino acids, is heavily glycosylated, and has a large extracellular domain and a short cytoplasmic terminal. S protein can be subdivided into S1 and S2, which have important effects in virus binding, fusion, and entry.

2.1.1. Binding

The S1 subunit has an N-terminal domain (NTD) and a receptor-binding domain (RBD), and comprises about 240 residues. MERS–RBD contains internal and external subdomains. The internal subdomains are relatively conserved among CoVs [12], whereas the structure of the external subdomains in CoVs varies greatly; the external subdomains mainly participate in receptor binding, resulting in the use of different receptors between CoVs. The RBD is responsible for the binding of dipeptidyl peptidase IV (DPP4, also known as human CD26). Interestingly, MERS-RBD can induce strong production of antibodies *in vivo*, which indicates that the S protein has a good immunogenicity for the induction of neutralizing antibodies (nAbs) [13–16]. The immunogenicity and protective potential of the NTD have been studied, and the recombinant N-terminal domain (rNTD) is a candidate vaccine against MERS [17].

2.1.2. Viral fusion

There are two regions in the S2 subunit that participate in viral fusion—namely, heptapeptide repeats 1 and 2 (HR1 and HR2), which assemble into a typical six-helix bundle (also called the fusion core). Three HR2 chains in the HR1 side groove core emit three HR1 spirals, forming a central coiled spiral core that is a key membrane fusion structure [18,19]. Upon binding of S1 and DPP4, HR1 binds HR2 to form a temporary intermediate structure, causing the viral and cell membranes to move in close proximity to fuse the membranes.

2.1.3. Virus entry

The S protein plays a key role in CoV tropism and morbidity, with proteolysis between S1 and S2 [20]. There are many kinds of proteases (reviewed in Ref. [21]) in hosts that can digest the S protein of MERS-CoV. For example, furin can be used in two steps by MERS-CoV in the activation process of cleavage during infection. First, furin recognizes the R751/S752 position and cleaves the S protein during biosynthesis. Next, S2 is further digested to S2′ at position R887/S888, which is adjacent to the fusion peptide after viral entry [20].

Table 1
Structural proteins, genes, and their potential functions in MERS-CoV.

Proteins	Coding genes	Functions and/or effect on the cellular response of the host
Spike (S) protein	ORF2	Viral entry, receptor binding, membrane fusion
Envelope (E) protein	ORF6	Virion assembly; putative ion channel activity and is involved in viral budding and release; potential B cell epitopes
Membrane (M) protein	ORF7	Virion assembly—the formation of the viral envelope and viral core by interacting with the N protein; IFN antagonism
Nucleocapsid (N) protein	ORF8a	Main component of the nucleocapsid structure—essential for viral replication and assembly, and post-translational modification Modulating the host's initial innate immune response
AP 3	ORF3	Viral replication and pathogenesis
AP 4a	ORF4a	Viral replication, IFN antagonism, protein kinase R (PKR) antagonism
AP 4b	ORF4b	IFN antagonism, nuclear factor kappa B (NF-κB) inhibition
AP 5	ORF5	IFN antagonist, modulation of NF-κB-mediated inflammation
Nsp1-coding region	nsp1	Specific recognition of viral RNA that is required for efficient viral replication; possibly interacts with cyclophilins and is thought to be a major virulence factor because it suppresses protein synthesis through the degradation of host mRNA
Papain-like protease (PLpro)	nsp3	PLpro is responsible for the cleavage at positions 1–3 to develop three nonstructural proteins (nsps); two selected sites (G720 and R911) were detected in the protease domain; viral replication; membrane proliferation; IFN antagonist; deubiquitination; putative dephosphorylation of Appr-1″-p, a side product of cellular tRNA splicing, to ADP-ribose
Transmembrane domain	nsp4	Viral replication; membrane proliferation
Main, chymotrypsin-like, or 3C-like protease (3CLpro)	nsp5	Viral survival—proteolytic processing of the replicative polyprotein at specific sites and 3CLpro cleaves the remaining positions 4–16 key functional enzymes, such as replicase and helicase
Transmembrane domain	nsp6	Membrane proliferation; interaction with nsp3 and nsp4
Primase	nsp8	Primase activity
Unknown	nsp9	Nsp9 is an essential protein dimer with RNA/DNA binding activity in SARS-CoV
Unknown	nsp10	Membrane proliferation—regulating 2′-*O*-MTase activity
RNA-dependent RNA polymerase	nsp12	Viral replication and transcription
Superfamily 1 helicase	nsp13	Viral replication; affects tropism and virulence
3′-to-5′exonuclease	nsp14	Viral replication—exoribonuclease activity
N7-methyltransferase Nidoviral endoribonuclease specific for U	nsp15	Viral replication—exoribonuclease activity
S-adenosylmethionine-dependent ribose 2′-*O*-methyltransferase	nsp16	Methyltransferase inhibition; viral replication; IFN antagonism

2.2. Envelope protein

The envelope (E) protein is an inner membrane protein that is also a small structural protein of MERS-CoV; it is 82 amino acids in length and is predicted to contain at least one transmembrane helix [22]. The E protein of CoVs plays an important role in intracellular trafficking, host recognition, viral assembly, and virus budding [22]. However, the exact function of the CoV E protein during infection remains unclear.

2.2.1. Virion assembly

Researchers have used infectious bronchitis virus (IBV) infection to prove that the E protein is required for virion assembly, and that its other role is disturbing the secretory pathway [23]. The MERS-CoV E protein has a single hydrophobic domain (HD) that targets the Golgi composite membrane and has cationic channel activity *in vitro*. The purified MERS-CoV E proteins form pentameric ion channels in the lipid bilayer. Lack of the E protein may lead to elimination of channel activity. Based on the proposed channel activity as a virulence factor, the MERS-CoV E protein is a potential antiviral therapeutic target [22].

2.2.2. B cell epitopes

Researchers have used software or programs such as LaserGene (DNASTAR, Inc., USA), PHYRE 2 (Structural Bioinformatics Group, Imperial College London, UK), and PyMOL (Schrödinger, LLC, USA) to identify a potential B epitope with high antigenicity at positions 58–82 of the E protein [24].

2.3. Membrane protein

The membrane (M) protein is important in viral assembly, the formation of the viral envelope, and the formation of the viral core by interacting with the nucleocapsid (N) protein [25,26]. Therefore, the development of fusion peptides and IFN antagonists may be an important treatment option.

2.4. Nucleocapsid protein

The N protein of MERS-CoV is a phosphorylated basic protein and is the second largest structural protein, containing 413 amino acid residues. The N protein binds to the RNA genome to form a nucleocapsid, which is important in virus replication and assembly [27].

2.4.1. Virion replication and assembly

The N-terminus of the N protein (residues 39–165) may be the RNA-binding region, and the C-terminus may be a self-binding oligomer-forming region [28]. A comparison of homology between MERS-CoV and other CoVs shows that although the N protein has low homology over the entire amino acid sequence, certain local amino acid sequences—especially the N-terminal amino acid sequence—are highly conserved. The SARS-CoV N protein can increase replication by overexpression [29]. The nucleocapsid structure not only requires the recognition of the characteristic sequences of the viral RNA, but also must recognize binding to other structural viral proteins. After the N protein forms a complex with the viral genomic RNA, the RNA is protected from destruction by nucleases in the host cell [30]. X-ray diffraction measurement at very high resolution (2.4 Å) was used to discover an intrinsically disordered region (IDR) and an NTD at the N-terminus of the N protein. This model shows that the NTDs in CoVs are relatively conserved [31].

2.4.2. Post-translational modification

The N proteins of SARS-CoV and MERS-CoV are ADP-ribosylated, which is a common post-translational modification; however, it is unclear how this regulates RNA virus infection. In a study that screened for ADP-ribosylating proteins after infection with CoV, researchers identified an ADP-ribosylated protein of about 55 kDa *in vivo*, which was subsequently verified as a CoV N protein. Therefore, CoV N proteins may modulate the post-translational modification of certain important proteins [30].

2.4.3. Modulation of the innate immune response

The N protein of CoV may inhibit type I IFN production by interfering with the interaction between the triple motif protein 25 (TRIM25) and retinoic acid-inducible gene I (RIG-I), and by binding to the E3 ubiquitin ligase of TRIM25. Thus, inhibiting the ubiquitination and activation of RIG-I mediated by TRIM25 ultimately leads to the inhibition of IFN production, suggesting that the N protein of a CoV regulates the host's immune response against the virus [29].

The MERS-CoV N protein is thought to interact with mannose-associated serine protease 2 (MASP2) [32] and translation elongation factor 1 (EF-1A) [33] in the host. Once the mannose-binding lectin recognizes the mannan on the surface of the virus-infected cell, it recruits MASP2 and then activates its enzymatic activity, eventually causing a local or systemic inflammatory response. EF-1A acts as a translation elongation factor, and catalyzes the aminoacyl-tRNA to the A site in the ribosome. EF-1A can play a regulatory role in the assembly of the cytoskeleton, microfilaments, and cytokinesis rings. The interaction between the MERS-CoV N protein and EF-1A disrupts the formation of cytokinesis loops and inhibits lymphocyte proliferation, which may explain the reduction in the number of lymphocytes in patients with MERS.

3. Accessory proteins

MERS-CoV has a large number of genomes encoding a variety of APs (dORF: ORF3, ORF4a, ORF4b, ORF5). Even among closely related CoVs, their number and sequence are different, and lead to host shift and HCoV emergence [34].

3.1. Viral replication

The MERS-CoV AP ORF is important for the replication of the virus *in vivo* and *in vitro*, which allows these ORFs to be used as targets of vaccine production, and emphasizes that MERS-CoV APs are potential targets for monitoring and therapeutic treatment. In addition, parallel disruption of the AP ORF provides a rapid response platform for new mutations of SARS or MERS-CoV AP ORFs [35].

3.2. Dysregulating host responses

The APs of MERS-CoV may invoke a hostile response through different modes, including IFN antagonism and the protein kinase R (PKR) and nuclear factor kappa B (NF-κB) pathways.

3.2.1. IFN antagonism

The attenuated function of dORF3-5 mutants is mainly caused by host-response disorders, including disrupted cellular processes and strong inflammation. ORF4-5 proteins increase IFN pathway activation and are potent IFN antagonists. They can inhibit IFN production (e.g., IRF-3/7) and interferon stimulated response element (ISRE) signaling pathways. The MERS-CoV ORF4a protein most likely interferes with the anti-antiviral effects of IFNs [36].

IFN antagonism is a key element of viral pathogenesis. The IFN-inducible oligoadenylate synthase (OAS)-RNase L pathway is activated after the sensing of viral double-stranded RNA (dsRNA); thus, the virus and single-stranded RNA (ssRNA) of the host are cleaved, which hinders the replication and spread of the virus. ORF4b, which is mainly located in the nucleus (while all the other proteins are located in the cytoplasm), has phosphodiesterase (PDE) activity and activates RNase L. Treatment with NS4b interferes with the pathogenesis of MERS-CoV in *in vivo* models [37].

3.2.2. PKR antagonism

The p4a of MERS-CoV is essential for PKR antagonistic activity. To establish infection and spread, MERS-CoV must regulate a complex antiviral host-response network (type I IFN (IFN-α/β) responses). Phosphorylation of eIF2α by PKR leads to inhibition of the translation of cells and viruses, and the formation of stress granules (SGs), which provide platforms for antiviral signaling pathways [38].

3.2.3. NF-κB inhibition

MERS-CoV 4b needs to prevent a strong host-dependent reaction during infection. During viral infection, 4b acts to interfere with NF-κB-mediated innate immune responses by binding NF-κB to nuclear transcription factor-α4 (KPNA4) and translocating it to the nucleus to play a competitive role [20]. ORF5 also has some effect on the regulation of NF-κB-mediated inflammation [35].

4. Nonstructural proteins

ORF1a and ORF1b at the 5′-terminus of the CoV genome encode polyprotein 1a and polyprotein 1b, which may cleaved into 16 nonstructural proteins (nsps). These proteins are essential for viral replication and transcription [39].

4.1. Proteolysis

MERS-CoV 3C-like protease (3CLpro) and papain-like protease (PLpro) are affected by the different genetic and evolutionary effects of protein sequence formation, codon usage patterns, and codon usage bias. The cleavage points of PLpro are positions 1–3, which produces three nsps, while 3CLpro cleavage occur at the remaining positions 4–16. Nsp3 (~200 kDa) is a multifunctional protein that contains up to 16 different domains and regions, and is associated with viral RNA, nucleocapsid proteins, and other viral proteins involved in polyprotein processing. The PLpro activity of nsp3 is the established target of new antiviral drugs [40]. PLpro is controlled by a wide range of components, mutations, and other effects. Antiviral drugs, such as PLpro inhibitors and 3CLpro inhibitors (Table 2), have been studied for their innate immunosuppression profiles [41,42]. Nsp5 is widely involved in virus-encoded polyproteins (ppla, pplab) and 16 nsps processing; therefore, nsp5 is essential and indispensable for virus survival. In addition, the lack of cell homologs of human nsp5 makes it an ideal target for antiviral drug design [43]. The adaptive evolution of nsp3 in MERS-CoV strains is ongoing. CoV nsp3 can be used as a primary screening target, and nsp3 sequencing should be considered for monitoring and site-specific investigations [44].

4.2. Viral replication

CoV RNA synthesis is associated with replication organelles (ROs) composed of a modified endoplasmic reticulum (ER) membrane. These are converted to double-membrane vesicles (DMVs) containing viral dsRNA and then converted to other crimped membranes, which together form a reticulovesicular network. Nsp3, 4, and 6 of SARS-CoV contain transmembrane domains and are all responsible for the formation of DMVs [45]. In addition, the cis-acting element at the 5′ end of the nsp1 coding region facilitates the specific recognition of viral RNA, which is necessary for efficient virus replication [46].

MERS-CoV helicase (nsp13) is one of the most important viral replication enzymes and can profoundly affect tropism and virulence [47]. Nsp13 unfolds DNA and RNA in the 5′ to 3′ direction and is a kinematic protein that catalyzes the progressive separation of double-stranded nucleic acids into two single-stranded nucleic acids, using energy generated by ATP hydrolysis. Therefore, the nsp13 helicase can be used as a therapeutic target to inhibit the replication of MERS-CoV [48]. In addition, nsp8 has major activity in viral replication; nsp12 (RNA-dependent RNA polymerase (RdRp)) plays a role in virus replication and transcription and can be used as a diagnostic (RdRpSeq assay) and as an antiviral target (polymerase inhibitor); nsp14 has exoribonuclease activity; and nsp15 has endoribonuclease activity, and can effectively prevent the activation of dsRNA sensor (including melanoma differentiation-related protein 5 (Mda5), OAS, and PKR) in host cells [9,49,50].

4.3. Methyltransferase inhibition

Nsp16 is an *S*-adenosine-*L*-methionine (SAM)-dependent 2′-*O*-methyltransferase (2′-*O*-MTase), and nsp10 plays an important role in regulating 2′-*O*-MTase activity. SAM binding can promote the assembly of the nsp10/nsp16 complex, which enzymatically converts 7mGpppG (cap-0) to 7mGpppG2′Om (cap-1) RNA in a SAM-dependent manner through 2′-OH methylation of SAM. Alanine mutagenesis has been used to identify the nsp16 residues that affect RNA recognition. The balance of SAM/*S*-adenosyl-*L*-homocysteine (SAH) can regulate 2′-*O*-methyltransferase activity, which increases SAH hydrolase inhibition and can interfere with CoV replication cycles [51].

4.4. Membrane proliferation

Working together, nsp3 and nsp4 exert their effects in pairing membranes. Nsp6 can promote membrane proliferation by forming perinuclear vesicles, which is thought to require full-length nsp3, because no DMVs are seen in cells co-expressing C-terminally truncated nsp3 with nsp4 and nsp6 [52].

4.5. Interaction with the host

4.5.1. IFN antagonism

Most adaptive events occur through nsp3, which inhibits the IFN response via its deubiquitination and de-esterification activity [41]. Nsp3 is currently undergoing selection, based on MERS-CoV that has been isolated from humans and camels [44]. Positive selection changes in nsp3 (R911C) were observed in human-only viruses, suggesting that virus adaptation in humans represents a potential selective pressure [44]. Nsp16 helps the virus to escape from cellular innate immunity and uses IFN-induced protein with tetratricopeptide repeats 1 (IFIT-1) protein to inhibit viral translation [34]. Using reverse genetics, dnsp16-mutated MERS-CoV showed attenuation based on type I IFN; the mutation was partially recovered in the absence of IFIT-1 [53].

4.5.2. Deubiquitination

PLpro's ubiquitin-like domain (Ubl) becomes fully flexible after binding to ubiquitin, and its increased flexibility is important for its interaction with other downstream proteins and for inhibition of innate immunity [54]. The four major residues involved in deubiquitination-L106, R168, P163, and F265 are conserved in all

Table 2
Summary of potential products targeting MERS-CoV proteins.

Therapeutic products	Targets	*In vivo*/*in vitro*	Safety/advantages
Mersmab	S1 RBD	*In vitro*	—
m336, m337, m338	S1 RBD	*In vitro*/*in vivo* (mouse, rabbit-m336)	—
MERS-4, MERS-27	S1 RBD	*In vitro*	—
4C2	S1 RBD	*In vitro*/*in vivo* (mouse)	Prophylactic and therapeutic
hMS-1	S1 RBD	*In vitro*/*in vivo* (mouse)	—
LCA60	S1 RBD	*In vitro*/*in vivo* (mouse)	Targets both NTD and RBD, stable Chinese hamster ovary (CHO) cell line, prophylactic and therapeutic
3B11-N	S1 RBD	*In vitro*/*in vivo* (rhesus monkeys)	Prophylactic
2F9, 1F7, YS110	Human-anti-DPP4	*In vitro*	—
1E9, IF8, 3A1	S1 RBD	*In vitro*	—
3B12, 3C12, 3B11, M14D3	S1 RBD	*In vitro*	—
RBD s377-588-Fc IgG fusion	RBD-IgG fusion	*In vitro*/*in vivo* (mouse)	Humoral response in mice; potential intranasal administration; improved by adjuvant MF59; divergent strains/escape mutants
G4	S1 RBD	*In vitro*	—
Full-length S protein proprietary nanoparticles	S protein	*In vitro*/*in vivo* (mouse)	Use of adjuvants improves humoral response
MVA expressing full-length S protein	S protein	*In vitro*/*in vivo* (mouse, camel)	T cell and humoral response; entering human clinical trials; potential for veterinary use with camels
Ad5 or ad41 adenovirus expressing full-length S protein	S protein	*In vitro*/*in vivo* (mouse)	T cell and neutralizing antibody responses
Measles virus expressing full-length S protein (vaccine candidate)	S protein	*In vitro*/*in vivo* (mouse)	T cell and neutralizing antibody responses
GLS-5300 plasmid vaccine	S protein	*In vitro*/*in vivo* (mouse, camels, and macaques), human clinical trials	T cell and neutralizing antibody responses; in a phase I clinical trial
MERS-GD27 and MERS-GD33	S protein	*In vitro*	Receptor-binding site and the effect of synergism in neutralizing MERS-CoV
HR2P	Anti-HR2	*In vitro*	—
HR2P-M2	Anti-HR2	*In vitro*/*in vivo* (mouse)	Blocks 6 HB bundle formation; enhances IFN-β effect; potential intranasal treatments
IFNs	IFN antagonists	*In vitro*/*in vivo*	Combination therapy allows reduced amounts of each IFN
Camostat	TMPRSS2 inhibitor	*In vivo* (mouse), SARS-CoV	Already in clinical use (chronic pancreatitis)
Nafamostat	TMPRSS2 inhibitor	Split-protein-based cell–cell fusion assay	Already in clinical use (anti-coagulant)
Teicoplanin dalbavancin oritavancin telavancin	Cathepsin L inhibitor	High-throughput screening	Already in clinical use (antibiotic Gram-positive bacterial infections)
6-mercaptopurine (6MP) 6-thioguanine (6TG)	PLpro inhibitor	*In vitro*	Potential for more MERS-specific agents
F2124-0890	PLpro inhibitor	*In vitro*	—
Lopinaivor	Mpro	*In vitro*/*in vivo* (marmosets)	High activity in low micromolar range *in vitro*; better outcomes, reduced mortality in marmosets

MERS-CoVs, but differ from other betacoronaviruses. Thus, down-regulation of the deubiquitination of nsp3 may lead to a weakened interaction between MERS-CoV and the host immune system [54,55].

4.5.3. Inhibition of the expression and translation of host mRNA

Nsp1 inhibits the host innate immune response by binding to dsRNA and cell double-stranded DNA; therefore, it is considered to be the best checkpoint of viral invasion [56]. At the same time, the C-terminus of nsp1 targets TNF receptor-associated factor 3 (TRAF-3) to inhibit the production of IFN-β [57]. In addition, activation of interferon-regulated transcription factor 3 (IRF3) [58] and PKR can be inhibited by other nsp1 domains [59]. Nsp1 of CoV may also interact with cyclophilin, and is considered to be a major virulence factor because it degrades host mRNA. The mRNA degradation activity of MERS-CoV nsp1 is separate from its translational inhibitory function. Furthermore, certain residues of MERS-CoV nsp1 are crucial for evading translational shutdown [46]. The escape of MERS-CoV mRNAs from inhibition by MERS-CoV nsp1 is promoted by their cytoplasmic origin [60].

5. Potential strategies to treat MERS

There is no completely effective treatment for MERS-CoV. Current treatment methods mainly imitate the treatment of SARS-CoV, and comprise the following: symptomatic treatment, including biological therapy such as monoclonal antibodies (mAbs), blockers, protease inhibitors, corticosteroids, and IFN; chemical drugs, such as Acyclovir [61], chlorpromazine hydrochloride [62], loperamide, and lopinavir [63]; broad-spectrum antibiotics, such as ribavirin, lopinavir-ritonavir, or mycophenolate mofetil [64]; and nanoparticle vaccines [65]. However, well-organized clinical trials have not yet been carried out. Therefore, we analyzed potential prevention and treatment methods from the perspective of their molecular characteristics, which provides a reference for the development of effective and targeted prevention and treatment, and especially of biological strategies.

5.1. Strategies related to the S protein

5.1.1. Antibodies toward S protein

The mAbs are regarded as a potential intervention method and have been used to diagnose many diseases. The main products are shown in Table 2. LCA60 is the only antibody isolated from recovered MERS patients. Preclinical development of this product has been completed, and good manufacturing practices (GMP)-approved cell lines that express high concentrations of purified and highly efficient antibodies have been established [66]. Studies of human nAbs [67] and mAbs [68,69] have been performed. These antibody-based drugs are specific and effective inhibitors of RBD,

and show strong resistance to MERS-CoV-neutralizing activity. Table 2 describes other studies on potential antibodies and mAbs. Potential antibodies REGN3051 and REGN3048 have demonstrated effective prophylaxis in MERS-CoV-infected animal models [70,71], as have the following treatments: Mersmab [72]; 1E9, IF8, 3A1, 2F9, 1F7, and YS110 [73]; hMs-1 [74]; 4C2 [75]; and RBD s377-588-Fc IgG fusion [76]. G4 recognizes the variable glycosylation loops in CoVs that define the four conformational states of the trimer. These studies may contribute to our understanding of fusion initiation and provide a basis for the design of structure-based CoV vaccines [5,77].

5.1.2. Inhibitors

MERS-CoV SP is essential for the virus to enter cells; therefore, other therapeutic agents that blockade viral cell membrane fusion should be exploited, such as feline protein inhibitors [78], TMPRSS2 inhibitors [79], PLpro inhibitors [41], furin inhibitors [80], kinase inhibitors [81], and IFN-induced transmembrane (IFITM) proteins [82].

5.1.3. Peptides

HR1P at position 998–1038 and HR2P at positions 1251–1286 have been identified by Lu et al. [19] and Liu et al. [83] as being responsible for the formation of the fusion core. The HR2P peptide can inhibit viral replication of MERS-CoV in cell lines, such as Calu-3 and HFL [19]. HR2P plays a role in the inhibition of the replication of MERS-CoV and its stimulatory fusion. Although there is little knowledge concerning assembly and release, inhibiting these viral processes may be an important goal in the future [64,84]

5.1.4. Potential therapeutic components

Low-temperature electron microscopy recently revealed a global pre-fusional structure of the full-length S-exodomain, indicating that the NTD and C terminal domain (CTD) form a "V" shape that helps the overall S trimer's triangular appearance [85–87]. The S2 subunit is linked to the viral membrane and is characterized by the presence of a long alpha helix. This structural information could form the basis for the structure-based immunogen design of vaccines against betacoronaviruses. The combination of computational biology and virology could accelerate advanced designs of agents acting against MERS-CoV, resulting in higher efficacy [64].

5.2. Strategies related to the N protein

The N protein of MERS-CoV is abundantly produced during infection and shows strong immunogenicity, indicating that it is an ideal antigen for virus antibody detection. Anti-N IgG can be used as an indicator of susceptibility to human CoV infection [88]. Reports have described new methods to generate mAbs directed against N proteins. Using the wheat germ cell protein synthesis system, a large number of MERS-CoV N protein antigens were successfully prepared in a highly soluble and intact immune state [89].

5.3. Potential strategies to treat MERS-CoV based on its characteristics

Given the biological characteristics of MERS-CoV, infection prevention and control measures are critical to prevent the possible spread of this virus. For patients with MERS, effective early diagnosis and early treatment are difficult to achieve, mainly because of atypical early symptoms, the limitations of detection methods, and airborne transmission. Therefore, regardless of the diagnosis, healthcare providers should always take consistent standard precautions for all patients. IgM-type antibodies against MERS-CoV proteins need to be detected more effectively, necessitating the development of more effective antibodies.

6. Conclusion

Explosive epidemics of MERS-CoV pose a serious threat to global public health and emphasize the need to further investigate the epidemiology and pathogenesis of this virus, in addition to developing effective therapeutic and preventive drugs against MERS-CoV infection. Despite rapid advances in diagnostics and public health measures, new MERS-CoV cases are still being reported. This suggests that multiple interventions targeting different affected populations may be necessary to stop these outbreaks. As described herein, the structural information associated with the MERS-CoV proteins may be used to develop different potential interventions to limit the outbreak of MERS-CoV. There is a need to better understand the pathogenesis of MERS-CoV in order to identify viral and host factors that play an important role in the development of MERS in humans; this may provide potentially novel therapeutic and intervention options.

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Compliance with ethics guidelines

Yan-Hua Li, Chen-Yu Hu, Nan-Ping Wu, Hang-Ping Yao, and Lan-Juan Li declare that they have no conflict of interest or financial conflicts to disclose.

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