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Review

T-cell immunity of SARS-CoV: Implications for vaccine development against MERS-CoV

William J. Liu [a, b, c, **], Min Zhao [d, e], Kefang Liu [a, b], Kun Xu [d, e, f], Gary Wong [c, d], Wenjie Tan [a, b], George F. Gao [a, c, d, e, f, *]

[a] National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention (China CDC), Beijing, 100052, China
[b] College of Laboratory Medicine and Life Sciences, Wenzhou Medical University, Wenzhou, 325035, China
[c] Shenzhen Key Laboratory of Pathogen and Immunity, Shenzhen Third People's Hospital, Shenzhen, 518112, China
[d] CAS Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences (CAS), Beijing, 100101, China
[e] University of Chinese Academy of Sciences, Beijing, 100049, China
[f] Research Network of Immunity and Health (RNIH), Beijing Institutes of Life Science, Chinese Academy of Sciences, Beijing, 100101, China

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ABSTRACT

Over 12 years have elapsed since severe acute respiratory syndrome (SARS) triggered the first global alert for coronavirus infections. Virus transmission in humans was quickly halted by public health measures and human infections of SARS coronavirus (SARS-CoV) have not been observed since. However, other coronaviruses still pose a continuous threat to human health, as exemplified by the recent emergence of Middle East respiratory syndrome (MERS) in humans. The work on SARS-CoV widens our knowledge on the epidemiology, pathophysiology and immunology of coronaviruses and may shed light on MERS coronavirus (MERS-CoV). It has been confirmed that T-cell immunity plays an important role in recovery from SARS-CoV infection. Herein, we summarize T-cell immunological studies of SARS-CoV and discuss the potential cross-reactivity of the SARS-CoV-specific immunity against MERS-CoV, which may provide useful recommendations for the development of broad-spectrum vaccines against coronavirus infections.
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* Corresponding author. National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, #100 Yingxin Road, Xicheng District, Beijing, 100052, China.
** Corresponding author. National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, #100 Yingxin Road, Xicheng District, Beijing, 100052, China.
E-mail addresses: liujun@ivdc.chinacdc.cn (W.J. Liu), gaofu@chinacdc.cn (G.F. Gao).

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1. Introduction

The successful control of the spread of severe acute respiratory syndrome coronavirus (SARS-CoV) in 2003 facilitated the establishment of an international collaboration system for infectious disease prevention and control (Ksiazek et al., 2003; Rota et al., 2003). During this process, a large number of studies were performed that focused on epidemiology, pathophysiology, immunology, vaccine development and structural studies of SARS-CoV. These studies helped widen knowledge of both SARS-CoV and the whole coronavirus family, as well as promoted methodologies for studying viruses.

Almost ten years after the emergence of SARS, a novel coronavirus termed Middle East respiratory syndrome coronavirus (MERS-CoV) was first reported in the Middle East (Zaki et al., 2012), and this virus was found to be responsible for an outbreak of acute respiratory illness in a public hospital in Zarqa, Jordan during April 2012 (Hijawi et al., 2013). Imported cases have since been reported in France, Germany, Italy, Tunisia, the United States and the United Kingdom (Su et al., 2016; Wong et al., 2015). During 2015, an outbreak of MERS-CoV was reported in South Korea, with 36 deaths in 186 confirmed cases (http://www.who.int/emergencies/mers-cov/en/), in which one patient traveled from South Korea to China, representing the first case of MERS-CoV in China (Su et al., 2015; Wang et al., 2015).

The clinical features of patients infected by MERS-CoV are very similar to SARS, *i.e.*, severe pneumonia (Feng and Gao, 2007; Zaki et al., 2012). SARS-CoV-like viruses have been discovered in insectivorous *Rhinolophid* bats, and viruses genetically related to MERS-CoV have also been detected in *Neoromicia capensis* bats from Africa (Corman et al., 2014; Lau et al., 2013; Li et al., 2005b; To et al., 2013). Recently, the bat origins of MERS-CoV were further supported by evidence that bat coronavirus HKU4 also uses the human MERS-CoV receptor CD26 for virus entry (Wang et al., 2014). Additionally, MERS-CoV-like viruses are also widespread in dromedary camels, with sero-epidemiological studies indicating 90% seroprevalence in adult animals (Reusken et al., 2014).

A pivotal role for virus-specific memory T-cells in broad and long-term protection against SARS-CoV infection has been elucidated (Channappanavar et al., 2014; Zhao et al., 2010a). Indeed, the crucial protective role of T-cell immune responses in coronavirus infections has been clearly documented in several animal models, *e.g.*, feline infectious peritonitis virus (FIPV), mouse hepatitis virus (MHV), and avian infectious bronchitis virus (IBV) (Li et al., 2016; Takano et al., 2014; Trujillo et al., 2014). These animal models provide useful references for the study of human infection by SARS-CoV or MERS-CoV. Infection of mice with MHV, a member of the same betacoronavirus group as SARS-CoV and MERS-CoV, was used as an early experimental model to elucidate the role of T-cells in viral clearance and cytotoxicity (Le Prevost et al., 1975) and in T-cell oriented vaccine development (MacNamara et al., 2008; Zust et al., 2007). A primary role for cytotoxic T lymphocytes (CTL) has been demonstrated in protection from MHV and virus clearance (Stohlman et al., 1995).

In this review, we focus on lessons learned from T-cell immunological studies of SARS-CoV, including the immunogenicity of SARS-CoV structural proteins, T-cell epitopes identified, T-cell-oriented vaccine development, and the structural immunology of SARS-CoV based on human leucocyte antigen class I (HLA I)/peptide structures (Hilgenfeld and Peiris, 2013). Based on the knowledge of the T-cell immunity of SARS and recent studies on MERS, the immune correlation and potential T-cell cross-reactivity between SARS-CoV and MERS-CoV are evaluated, which may have implications for vaccine development against human coronavirus infections.

2. Immunogenicity of SARS-CoV and T-cell epitopes

Natural infection with SARS-CoV gives rise to dominant responses against the structural antigens of SARS-CoV in humans and animals (Channappanavar et al., 2014). The spike (S) protein is responsible for both receptor binding and membrane fusion of the virus (Lu et al., 2015), but also acts as a major antigen for both humoral and cellular immunity (Du et al., 2009a). Xu and colleagues detected SARS-CoV-specific T-cell responses from peripheral blood mononuclear cells (PBMCs) of convalescent SARS patients using overlapping peptides covering the whole SARS-CoV proteome (Li et al., 2008). They found that most of the antigenic peptides are located in the structural proteins (especially S protein) rather than non-structural proteins. The biased distribution of T-cell epitopes in the viral proteins may correlate with the different protein synthesis phases during the infection and the diverse abundance of the viral proteins in infected cells or antigen-presenting cells.

The nucleocapsid (N) protein of SARS-CoV can also trigger antibody and T-cell responses in humans, though the neutralizing/protective activities of these antibodies/T-cells still need to be defined (Leung et al., 2004; Lin et al., 2003; Woo et al., 2004). The membrane (M) protein, which is the most abundant protein in the SARS-CoV virion, also acts as a dominant immunogen as revealed by both clustering regions of B-lymphocyte and CTL epitopes (He et al., 2005; Liu et al., 2010b).

Dozens of T-cell specific epitopes derived from SARS-CoV structural proteins have been identified through diverse strategies in the past years (Table 1). We reviewed the reports concerning T-cell specific immunology of SARS-CoV and collected the $CD8^+$ and $CD4^+$ T-cell epitopes defined since the initial SARS outbreak (Fig. 1). Among the $CD8^+$ T-cell epitopes (Table 1), most peptides identified thus far are derived from the S protein with HLA-A2 restriction, which is the consequence of the immunodominance of the SARS-CoV S protein and the high coverage of HLA-$A2^+$ populations in different ethnic groups all over the world. HLA-A*1101-, HLA-A*2402-, HLA-B*15-, and HLA-B*4001-restricted T-cell epitopes have also been defined, which can be used to detect T-cell immune responses among populations other than HLA-A2 (Liu et al., 2010b; Oh et al., 2011). Also, several H-2^d-, H-2^k-, and H-2^b-restricted $CD4^+$ T-cell and $CD8^+$ T-cell epitopes have been identified using different

Table 1
SARS-CoV-derived T-cell epitopes and their conservation in human coronaviruses. [a]The position information is based on the SARS-CoV (strain TJF; GenBank accession no. AY654624) [b]The sequences of the corresponding peptides in SARS-CoV, HCoV-OC43 (strain HK04-01; GenBank accession no. JN129834), and HKU1 (strain BJ01-p3; GenBank accession no. KT779555). The variable residues in the peptides compared to MERS-CoV are underlined in bold. [c]The references that identified the peptides. [d]The references that used the peptides as vaccines or used the peptides to evaluate SARS-related vaccines. [e]The positions of HLA-restricted peptides with three or less variable residues between MERS-CoV and any of the three coronaviruses SARS-CoV, HCoV-OC43 and HKU1 are shown in bold.

Position[a]	MERS-CoV	SARS-CoV[b]	HCoV-OC43	HKU1	MHC restriction	Identification[c]	Vaccine evaluation[d]
S411-420	KQSFSNPTCL	K**LPDDFMGCV**	**RIDTTATSCQ**	K**IDTTSSSCQ**	HLA-A*0201	(Zhou et al., 2006)	(Zhou et al., 2006)
S787-795	LEPVSISTG	**ILPDPLKPT**	**INFSPVLGC**	**INFKSLVGC**	HLA-A*0201	(Tsao et al., 2006)	(Tsao et al., 2006)
S1042-1050	LYFMHVGYY	**VVFLHVTYV**	LYF**IHFNYV**	L**L**FMH**FSYK**	HLA-A2		
S958-966	SIGDIIQRL	**VLNDILSRL**	S**LQEILSRL**	S**LQEILSRL**	HLA-A*0201	(Lv et al., 2009)	(Lv et al., 2009)
S978-986[e]	LINGRLTTL	LI**T**GRL**QS**L	LINGRLT**A**L	LINGRLT**A**L	HLA-A2	(Wang et al., 2004b)	(Zhou et al., 2006), (Kohyama et al., 2009)
S1203-1211	FIAGLVALA	FIAGL**IAIV**	**ICLAGVAML**	**ISFSFIIFL**	HLA-A2		
S1167-1175	SLQQVVKAL	R**L**NE**V**AK**N**L	R**L**QE**AI**K**V**L	L**I**QE**S**IK**S**L	HLA-A*0201	(Wang et al., 2004a)	(Wang et al., 2004a), (Zhou et al., 2006)
S1174-1182	ALNESYIDL	**N**LNES**L**IDL	**V**LN**H**SYI**N**L	**S**LN**N**SYI**N**L	HLA-A*0201		(Doytchinova and Flower, 2003)
S365-374	TCSQISPAAI	**K**C**YGV**S**ATKL**	TC**NN**I**DA**A**K**I	S**C**N**NFDESK**I	H-2[d]	(Huang et al., 2007)	(Huang et al., 2007), (Du et al., 2008),
S436-443	LKYSYINK	**YNYKYRYL**	**WNKRFGFI**	**WNRRYGFN**	H-2[b], H-2[b]	(Zhi et al., 2005)	(Zhao et al., 2010a)
S525-532	VEYSLYGV	V**NFNFNGL**	V**NYD**LYG**I**	V**DYD**LYG**I**	H-2[d]		
S366-374	CSQISPAAI	C**YGVSATKL**	C**NN**I**DA**A**K**I	C**NNFDESK**I	H-2[d]		
S884-891	IFYRLNGV	**MA**YR**F**NG**I**	**VQ**YR**I**NG**L**	**VQ**YR**I**NG**L**	H-2K[b]	(Poh et al., 2009)	(Poh et al., 2009)
S1116-1123	STNLPPPL	**NNTVYD**PL	**PYVMLNTS**	**PLVYLNHS**	H-2K[b]		
N216-224	GAVGGDLLY	G**ETALALL**L	**VTPDMADQI**	**VKPDMADEI**	HLA-B*4001	(Rivino et al., 2013)	
N323-332	DDHGNPVYFL	**MEVTPSGTW**L	D**EPQKD**VYEL	D**SPVKD**VFEL	HLA-B*4001		
N223-231	LYLDLLNRL	L**L**LD**R**LN**Q**L	**QIAS**L**VLAK**	**EIAN**L**VLAK**	HLA-A*0201	(Tsao et al., 2006)	(Tsao et al., 2006), (Ohno et al., 2009)
N227-235	LLNRLQALE	**R**LN**QLESKV**	L**V**L**AKLGKD**	L**V**L**AKLGKE**	HLA-A*0201		
N317-325	GMSQFKLTH	GMS**RIGMEV**	**FGSKLELAK**	**FGSKLDLVK**	HLA-A*0201		
N220-228	GDLLYLDLL	**LA**LL**L**LD**R**L	**MADQIASLV**	**MADEIANLV**	HLA-A*0201	(Cheung et al., 2007)	(Cheung et al., 2007)
N216-225	GAVGGDLLYL	G**ETALA**LL**L**L	**VTPDMADQIA**	**VKPDMADEIA**	HLA-B*4001	(Oh et al., 2011)	(Oh et al., 2011)
N222-231	LLYLDLLNRL	LL**L**LD**R**LN**Q**L	**DQIASLVLAK**	**DEIANLVLAK**	HLA-A*0201	(Ohno et al., 2009)	(Ohno et al., 2009)
N266-275	TKSFNMVQAF	TK**QYNVTQ**AF	N**K**Q**C**T**V**QQ**C**F	N**K**H**C**N**V**QQ**C**F	HLA-B*1525	(Ng et al., 2016)	
N346-354	NYNKWLELL	**QFKDNVI**LL	**GFETIMKV**L	**GFETIMKV**L	HLA-A*2402	(Liu et al., 2010b)	
N362-370	KTFPKKEKK	KTFP**PTEP**K	**QQQDGMMNM**	**VNSNQNTDS**	HLA-A*1101	(Blicher et al., 2005)	
M60-69	SMALSIFSAV	**TLACFVLA**AV	**TII**L**TTF**N**C**V	**TIT**L**T**IF**NCF**	HLA-A*0201	(Liu et al., 2010a)	(Liu et al., 2010a)
M88-96	AMMWISYFV	**GL**MW**L**SYFV	**II**MW**I**VYFV	**IVI**W**IL**YFV	HLA-A*0201		
M147-155	HLKMAGMHF	HL**R**MAG**HSL**	HL**YIQ**G**IKL**	HL**YIQ**G**VKL**	HLA-B*1502	(Ng et al., 2016)	
PP1a3709-3717	AYLVFVTTL	**SMWALVISV**	**LLMLASLFG**	**LLFITAFLG**	HLA-A*0201	(Kohyama et al., 2009)	(Kohyama et al., 2009)
PP1a1775-1787	VEHTTPWLL	V**QQESSFVM**	V**RFDV**P**FL**I	**TKLNV**P**FL**I	HLA-B*1501	(Roder et al., 2008)	
N353-365	LLEQNIDAYKTFP	LL**NKH**IDAYKTFP	**VLSE**N**LN**AY**QQQD**	**V**LE**EN**L**N**AY**VNSN**	H-2[d],HLA-DR2,DR3	(Zhao et al., 2010a)	(Zhao et al., 2016)

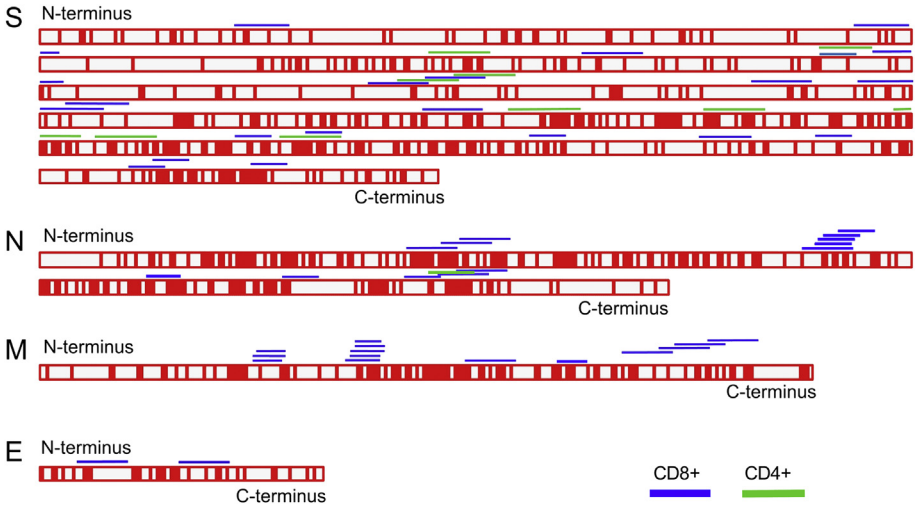

Fig. 1. T-cell antigenic peptides in SARS-CoV structural proteins and the conservation of the corresponding sequences in MERS-CoV. The sequences of the four major structural proteins of SARS-CoV were aligned with that of MERS-CoV. Identical residues are denoted in red and variable residues in white. Peptides with T-cell antigenicity are shown along the protein sequence. Peptides with $CD8^+$ T-cell-specific antigenicity and $CD4^+$ T-cell antigenicity are shown in blue and green, respectively.

mouse strains, which are helpful for SARS-CoV-specific T-cell studies among these animal models and the development of vaccines (Zhao et al., 2007, 2010a; Zhi et al., 2005). Given that all the $CD4^+$ T cell epitopes identified thus far are derived from the SARS-CoV S protein, further epitope discovery is still needed, especially in other structural proteins.

Based on an efficient T-cell epitope screening platform, with a combination of bioinformatics, cell biology, mouse models and

structural biology (Liu et al., 2011b), several HLA-A2-restricted $CD8^+$ T-cell-specific epitopes derived from the SARS-CoV S and M proteins were identified by our group (Zhou et al., 2006). Interestingly, we also defined a clustering region of CTL epitopes within the transmembrane region of the M protein (Fig. 1) (Liu et al., 2011a), which may correlate with the sequence preference by the peptide-processing related proteins, such as transporter associated with antigen processing (TAP), immunoproteasome and TAP binding protein (tapasin) (Huang et al., 2016). The protein transmembrane regions are usually rich in aliphatic amino acids (*e.g.* Leu, Ile and Val), which are preferred for the primary anchor residues of HLA-A2 peptides (Sharpe et al., 2010). Further work is needed to elucidate whether the M protein is a good candidate antigen for a prophylactic vaccine inducing both cellular and humoral immunogenicity. We also identified the first HLA-A24-restricted immunodominant T-cell epitope from the SARS-CoV N protein (Liu et al., 2010b). Recently, several MERS-CoV-derived H-$2K^d$-restricted T-cell epitopes have been identified (Liu et al., 2016). T-cell responses to one of these newly defined peptides (peptide 37-1) have a protective effect against MERS-CoV challenge. The protective efficiency of the peptide is depended on an uncommon interaction of Ile5 in peptide 37-1 with Trp73 of the host MHC I H-$2K^d$. These T-cell epitopes with different HLA-I-restrictions and also the T-cell epitope screening platform may help to understand the T-cell immunogenicity of different proteins from coronaviruses and provide potential candidates for vaccine development.

3. T-cell-oriented vaccine development for SARS-CoV

Well-defined antigenic peptides act as useful agents in the studies of SARS-specific immunity and immunopathogenesis and, more importantly, as candidates for vaccine development. The major antigens in these T-cell-targeting vaccines are focused on the S or N proteins of SARS-CoV.

3.1. General strategies for T-cell based vaccine development

Due to the low immunogenicity of single peptide vaccinations, many different strategies have been developed to elicit effective T-cell responses and efficient protection by T-cell-based vaccines. DNA vaccines encoding N or S gene segments that cover the immunodominant T-cell epitopes have been developed in mouse models. Cheung et al. produced a potential DNA vaccine candidate expressing an antigenic peptide from the SARS-CoV N protein with a single-chain-trimer (peptide-β_2m-MHC I) approach that induces SARS-CoV-specific T-cells with cytotoxicity to N protein-expressing cells (Cheung et al., 2007). Recombinant adeno-associated virus is also an ideal carrier for T-cell immunogens of SARS-CoV, inducing strong mucosal immune responses and protective CTL responses (Du et al., 2008). Mammalian CHO cells-expressing segments of the SARS-CoV S protein also induce potent T-cell immune responses and protection against the virus (Du et al., 2009b, 2010). Zhao and colleagues observed that enhanced virus-specific $CD8^+$ T-cells in mice by immunization with SARS-CoV peptide-pulsed dendritic cells also result in earlier virus clearance and increased survival (Zhao et al., 2010a). In a Phase I clinical trial, a single-plasmid DNA vaccine encoding the S protein was well tolerated and induced SARS-CoV-specific $CD4^+$ T-cell responses in all vaccinees, as well as $CD8^+$ T-cell responses in ~20% of individuals (Martin et al., 2008). MHC II-related antigen presentation in professional antigen-presenting cells may be involved in the dominant $CD4^+$ T-cell response of DNA vaccines, which is required for neutralizing antibodies, though cross-priming to stimulate $CD8^+$ T-cells also occurs (Akbari et al., 1999).

3.2. Adjuvants and their beneficial effects

Different adjuvants have been investigated to increase the efficacy of peptide vaccines. Surface-linked liposomal peptide (Kohyama et al., 2009; Ohno et al., 2009), muramyl dipeptide derivative adjuvant (Chen et al., 2010b), and CpG oligodeoxynucleotide (CpG ODN) (Zhao et al., 2010b, 2011) can augment peptide-specific T-cell responses, some of the which are protective (Du et al., 2010, 2009b, 2008; Ohno et al., 2009; Zhao et al., 2010a) and durable (Du et al., 2008; Kohyama et al., 2009; Yang et al., 2009). Virus-like particle (VLP) vaccines containing S protein as the dominant immunogen and adjuvanted with aluminum provided protection against mice challenged with SARS-CoV (Liu et al., 2011c; Lokugamage et al., 2008).

These studies demonstrate that T cells play a crucial role in SARS-CoV clearance and elucidate the avenues for vaccine development against other human coronaviruses, including MERS-CoV (Du et al., 2016; Wang et al., 2016a). However, some SARS-CoV vaccines led to the occurrence of Th2-type immunopathology in spite of antibody and protection against infection with SARS-CoV in mice (Tseng et al., 2012). Therefore, the application of a SARS-CoV vaccine in humans should proceed with caution.

4. Human immune responses against SARS-CoV and memory responses

Since the outbreak of SARS-CoV, the kinetics of the primary and memory immune responses after SARS-CoV infection have been investigated in patients and survivors. The milestones of these studies are presented in Fig. 2.

4.1. Humoral immunity to SARS-CoV

IgG against N protein can be detected in the sera as early as 4 days after illness onset, based on immunofluorescence assays and ELISA, with most patients seroconverting by day 14 (Hsueh et al., 2004). IgG and neutralizing antibodies peak at 4 months and then progressively decrease over time (Liu et al., 2006). Eighty-nine percent of the recovered patients have detectable IgG antibodies to SARS-CoV at 24 months post-infection as shown by ELISA, while neutralizing antibodies are detectable in an even higher percentage of donors (Liu et al., 2006). Nevertheless, disease severity may also impact the appearance time and magnitude of the antibody responses. Cameron and colleagues reported that SARS-CoV-infected patients with fatal outcomes display deficient antibody production against the S protein compared to non-severe patients (Cameron et al., 2007). During the long term follow-up of SARS survivors, IgG is only detectable in 2 of 23 recovered donors at 6 years after illness onset (Tang et al., 2011), suggesting diminishing levels of memory B-cells against SARS-CoV.

4.2. T-cell immunity to SARS-CoV

Laboratory investigation of clinical patients demonstrated that SARS-CoV-specific T-cells are important for the recognition and clearance of infected cells, particularly in the lungs of infected individuals (Gu et al., 2005). Although whether the memory T-cell response is sufficient to protect from reinfection requires further investigation, it has been suggested that a more robust CTL response contributes to protection against SARS-CoV in mice (Channappanavar et al., 2014; Chen et al., 2010a; Zhao et al., 2010a). Based on a series of T-cell epitopes identified thus far, as well as the overlapping peptide pools covering full-length antigens of SARS-CoV, durable memory T-cell responses against SARS-CoV have been evaluated in recovered patients (Chen et al., 2005; Fan et al.,

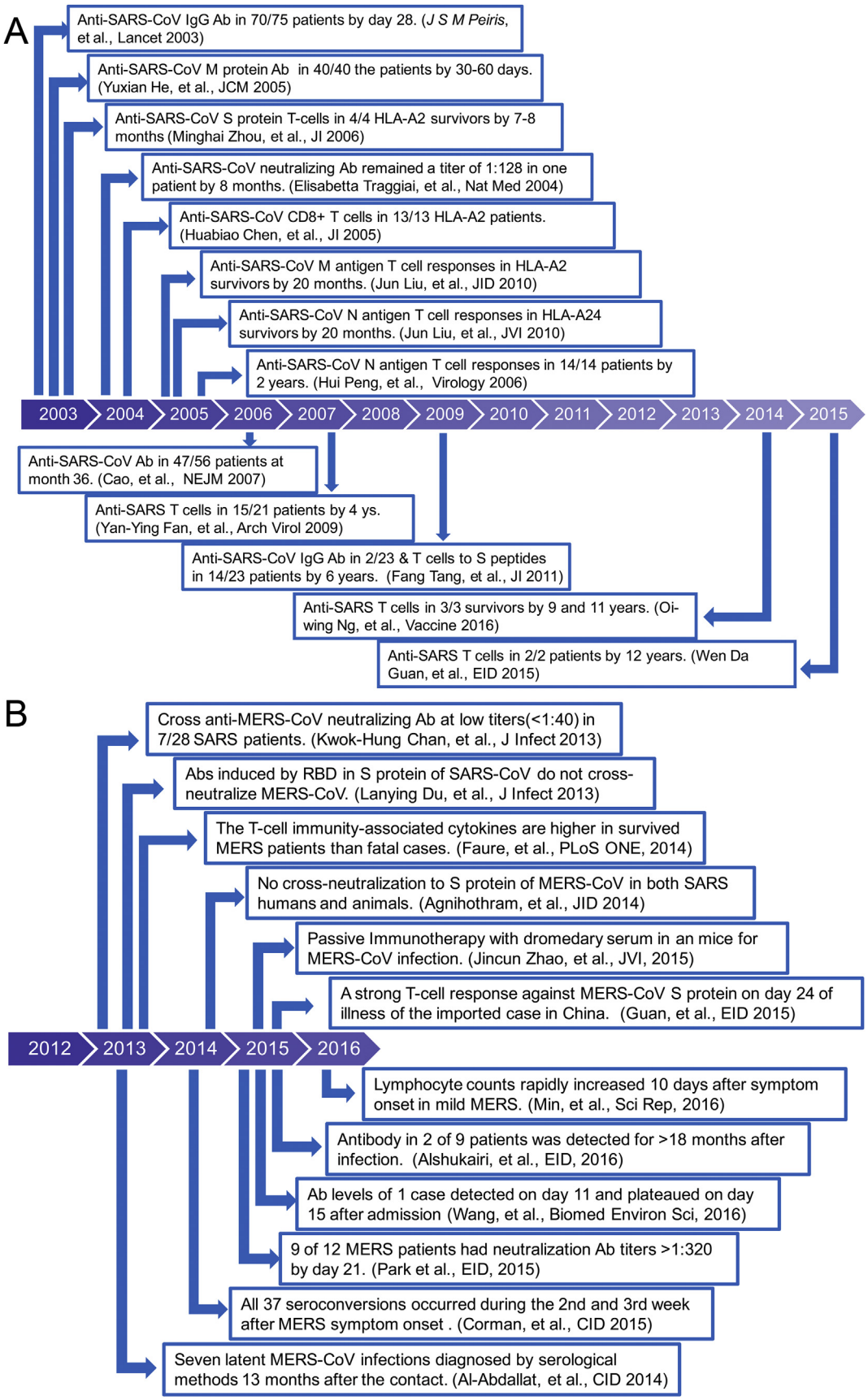

Fig. 2. Milestones of research on humoral and T-cell immunity against SARS-CoV and MERS-CoV. A. Studies that evaluate SARS-CoV-specific memory antibody and cellular responses in SARS patients or recovered donors are summarized along the 12-year time axis after the outbreak of the SARS epidemic. Arrowheads point to the time of sampling. B. Studies on human immunity to MERS-CoV and the cross-reactivity of MERS-CoV and SARS-CoV are listed.

2009; Peng et al., 2006; Yang et al., 2006, 2007). Unlike waning serum antibody levels in patients, CTL responses against the S and N proteins can still be detected from the PBMCs of recovered SARS patients 1, 2, 4, 6, and even >10 years post-infection (Da Guan et al., 2015; Ng et al., 2016; Oh et al., 2011; Tang et al., 2011). These findings are expected to have implications for disease surveillance and potential cross-reactivity to other coronaviruses.

4.3. Current knowledge of adaptive immune responses to MERS-CoV

Knowledge of adaptive immunity to MERS-CoV is also based on the most recent clinical data and laboratory investigation of specimens from MERS patients in South Korea (Min et al., 2016; Park et al., 2015), China (Da Guan et al., 2015; Wang et al., 2016b), the United States (Kapoor et al., 2014) and the Kingdom of Saudi Arabia (Corman et al., 2016). Seroconversion for most patients occurs during the second and third week after symptom onset (Corman et al., 2016; Park et al., 2015; Wang et al., 2016b). Similar to SARS patients, weak and delayed antibody responses are associated with more severe disease or fatal outcome in patients infected by MERS-CoV (Corman et al., 2016; Min et al., 2016; Park et al., 2015). However, Corman and colleagues (Corman et al., 2016) discovered that the levels of IgG and neutralizing antibodies are weakly and inversely correlated with viral loads in the lower respiratory tract, suggesting that the presence of antibodies does not lead to the elimination of the virus. For MERS-CoV-specific T-cell responses, PBMCs obtained on day 24 after illness onset show a strong specific T-cell response against the MERS-CoV S protein (Da Guan et al., 2015). Interestingly, persistent and gradual increases of lymphocyte responses after symptom onset in MERS patients with or without mild pneumonia may be required for effective immune responses against MERS-CoV, whereas all of the deceased patients displayed rapid drops in their lymphocyte counts (Min et al., 2016). T-cell immunity-associated cytokines IL-12 and IFN-γ levels were lower in a fatal case than in a patient who survived the infection (Faure et al., 2014).

Seven previously unconfirmed individuals tested positive for MERS-CoV-specific antibodies 13 months after a MERS outbreak in a Jordanian hospital, indicating that the antibodies persisted for at least a year in these asymptomatic cases (Al-Abdallat et al., 2014). However, in a longitudinal study of MERS survivors in Jeddah, Saudi Arabia, only two out of nine survivors remained positive for MERS-specific antibodies when tested 18 months after illness onset (Alshukairi et al., 2016). Furthermore, Arabi and colleagues (Arabi et al., 2016) reported that only 4 (36.7%) of 11 healthcare workers who had a history of laboratory-confirmed MERS-CoV infection had detectable MERS-CoV-antibody levels by ELISA a median of 381 days after infection. These data indicate that MERS-CoV-antibody levels decline more rapidly, compared to SARS-CoV survivors. Thus, it is valuable to investigate the features of T-cell immunity against MERS-CoV in survivors, which may shed light on MERS-CoV vaccine development with long-term protection in the future.

5. The structural immunology of SARS-CoV based on the HLA I/peptide structures

Structural proteome studies of viruses such as SARS-CoV, HIV, and influenza virus have revealed a series of important antibody-recognized epitopes of the major surface antigens and indicate their B-cell-specific antigenic features (Julien et al., 2012; Li et al., 2005a; Zhou et al., 2010). Herein, we summarize the structural characteristics of SARS-CoV-specific T-cell epitopes presented by HLA I molecules and recognized by T-cell receptors, including the structures of three HLA-A*0201, one HLA-A*1101, one HLA-A*2402, and one HLA-B*1501 molecule (Blicher et al., 2005; Liu et al., 2011a, 2010a, 2010b; Roder et al., 2008).

5.1. Structural evidence for T-cell epitope identification

The structural determination of SARS-CoV-derived peptide-HLA I complexes provides corroborative evidence for T-cell epitope identification. Based on the unambiguous electron density of the anchoring residues of the HLA I-bound peptides, we can conclude whether the peptide in question is a typical HLA I-restricted epitope. Similar to other HLA I molecules, the major anchoring residues are in the second (from the N-terminus) and last positions of the peptides in all structures of HLA I complexed with SARS-CoV peptides.

Interestingly, we previously identified two HLA-A2-restricted epitopes, Md3 (TLACFVLAAV) and its C-terminal truncated peptide Md3-C9 (LACFVLAAV) (Liu et al., 2010a, 2011a). Based on the structural determination of the two peptides complexed with HLA-A*0201, we found that they use different residues for the primary anchor at P2 position, though their C-terminal anchors are the same. The Leu2 of peptide Md3 inserts into the B pocket of HLA-A*0201, while the P2 anchor of peptide Md3-C9 is Ala2, which corresponds to Ala3 in Md3. The solvent-exposed conformations of the two peptides are completely distinct, which indicates that these two peptides may function as independent epitopes and correspond to different specific T-cell repertoires (Liu et al., 2014).

5.2. Implications of SARS-CoV epitope-HLA I structure interaction

In the structure of a T-cell epitope from the SARS-CoV N protein complexed with HLA-A*2402, we determined a novel immunodominant peptide presentation strategy (Liu et al., 2010b). In the structure of HLA-A*2402 complexed to peptide N1, the main chain of the central region of N1 exists in a moderately bulged conformation that is stabilized by two intra-chain hydrogen bonds and two water molecules in the peptide binding groove. The featured “A”-shaped conformation of N1 causes the three residues (Asp4, Asn5, and Val6) of the central region to bulge out of the HLA-A*2402 surface for potential T-cell receptor (TCR) docking (Fig. 3).

The HLA I-peptide complex structures hold the information necessary for a direct approach to peptide-based vaccine design (Sun et al., 2014). For example, in the structure of the HLA-A*1101-SARS-CoV peptide (Blicher et al., 2005), Thr6 does not make efficient use of the E pocket of the HLA-A*1101 groove, and is thus a potential target for optimization when developing peptide vaccines against SARS-CoV.

The residues in different positions of a peptide play distinct roles in HLA I-binding and TCR docking. Thus, structural studies of HLA I-peptide complexes can have direct visible implications for the T-cell antigenic variability of closely related peptides within different viruses. Based on the structures of T-cell epitopes derived from SARS-CoV, the antigenic variability of MERS-CoV compared to SARS-CoV can be rationally analyzed.

6. Immune correlation of SARS and MERS

6.1. Potential cross-immune reactivity between SARS-CoV and MERS-CoV

Since MERS-CoV belongs to the genus betacoronavirus with SARS-CoV, it is necessary to investigate whether the immune responses induced by the SARS-CoV have cross-reactivity against MERS-CoV. Chan and colleagues conducted a seroprevalence study on archived sera by using indirect immunofluorescence (IF) screening and confirmatory neutralizing antibody tests from 28

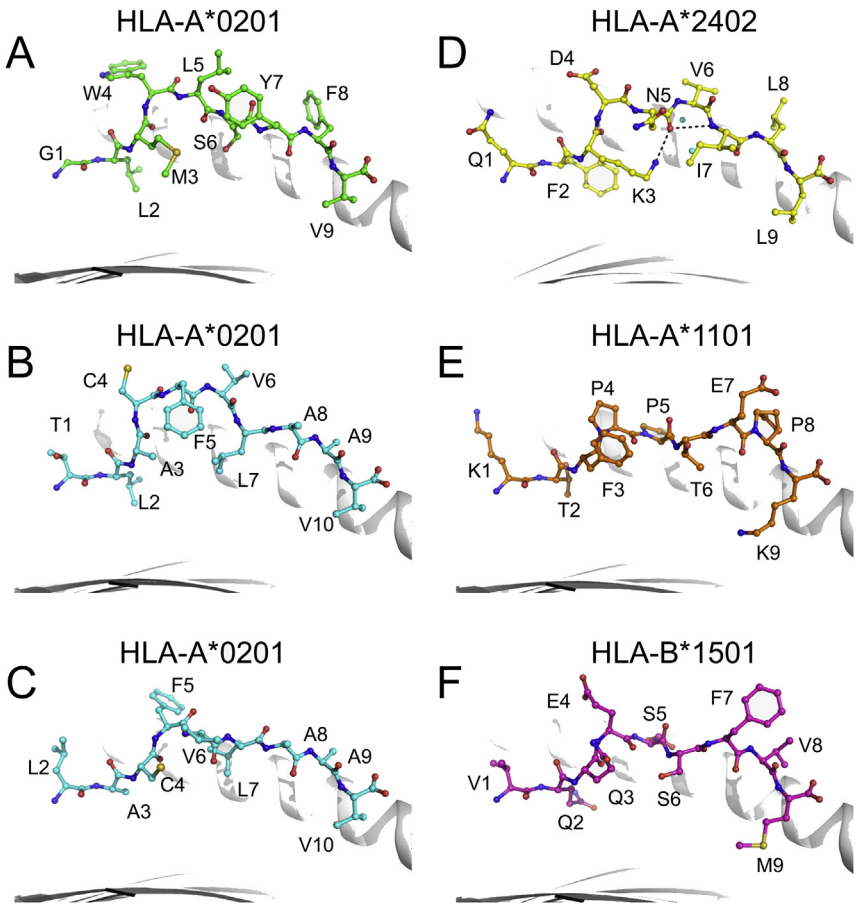

Fig. 3. Structures of SARS-CoV-derived T-cell peptides presented by HLA I molecules. Conformations of T-cell epitope peptides from SARS-CoV presented by HLA-A*0201 (A-C, PDB codes: 3I6G, 3I6K and 3TO2), HLA-A*2402 (D, PDB code: 3I6L), HLA-A*1101 (E, PDB code: 1X7Q) and HLA-B*1501 (F, PDB code: 3C9N). The peptides are shown as sticks and spheres in different colors. The names and the positions of residues in the peptides are denoted in black letters and numbers. Peptide Md3 (B) and its overlapping peptide Md3-C9 (C) are shown in the similar cyan color, and their residue positions are shown in the corresponding numbers. The peptide N1 (D) forms a bulged conformation that is stabilized by intra-chain hydrogen bonds (dashed black lines) and two water molecules (cyan).

SARS patients in Southern China. Anti-SARS-CoV IF and neutralizing antibodies against SARS-CoV were found in the majority (96.4%) of the SARS patients, as expected. Seventeen (60.7%) SARS patients tested positive for MERS-CoV-specific IgG detected by indirect IF, with titers ranging from 1:20 to 1:320, while seven (25%) had low titers (1:20 or less) of MERS-CoV neutralizing antibody (Chan et al., 2013). However, subsequent studies showed the absence of cross-reactivity between SARS-CoV and MERS-CoV (Agnihothram et al., 2014; Du et al., 2013; Meyer et al., 2014; Reusken et al., 2013). Du and colleagues discovered that a series of SARS-CoV receptor binding domain (RBD)-specific monoclonal antibodies (mAbs) or serum polyclonal antibodies against SARS-CoV S-RBD in vaccinated individuals do not cross-react with or neutralize MERS-CoV (Du et al., 2013).

Based on our sequence analysis (Fig. 1), the amino acid identity of the S protein and RBD of MERS-CoV and SARS-CoV is 29.08% and 18.6%, respectively, which may explain the observed low cross-neutralizing activity. However, the protein sequence identity of the S2 region of the S proteins from MERS-CoV and SARS-CoV, which is responsible for virus fusion with cells, is 40.98%, whereas the identities of the N, M and E proteins are 45.03, 41.63 and 37.80%, respectively. This suggests there may be potential cross-T-cell reactivity between MERS-CoV and SARS-CoV.

Analysis of the known $CD8^+$ T-cell epitopes derived from SARS-CoV showed that 14 of the 21 HLA allele-restricted epitopes are located in the S2 region of the S protein, or N and M proteins, which are more conserved (Fig. 1, Table 1). Furthermore, three peptides from the S2 region of the S protein, two from the N protein, and one from the M protein of MERS-CoV only have two or three variable residues when aligned to the corresponding sequence of SARS-CoV. Among these six peptides, Mn2 (GLMWLSYFV) in the M protein (amino acids 88–96) of SARS-CoV was previously identified as an immunodominant T-cell epitope based on a combination of functional and structural strategies (Liu et al., 2010a). The corresponding peptide (AMMWISYFV) in the M protein of MERS-CoV has three different residues: Gly88Ala, Leu89Met, and Leu92Ile. The structure of peptide Mn2 presented by HLA-A*0201 (Fig. 3A) shows that the P1 and P2 residues, Gly88 and Leu89, are located in the A and B pockets of HLA-A*0201, respectively, as the anchor residues. The corresponding residues Ala88 and Met89 in the MERS-CoV-derived peptide can also act as the anchor residues, which may not influence the conformation of the peptide when binding to HLA-A*0201. The solvent-exposed residue Leu92 in peptide Mn2 of SARS-CoV corresponds to an Ile in MERS-CoV. Considering the similar characteristics of Leu and Ile, this change may only have a limited influence on the TCR binding of the peptide. Thus, the peptides such as Mn2 in SARS-CoV and its parallel peptide in MERS-CoV may have a cross-TCR repertoire, which should be experimentally validated. In a recent study, a relatively conserved $CD4^+$ T cell epitope (Fig. 1) was also recognized in SARS-CoV- and MERS-CoV-infected HLA-DR2 and -DR3 transgenic mice, indicating vaccine strategies targeting conserved epitopes may be broadly applicable with different

coronaviruses (Zhao et al., 2016).

The conservation of the corresponding peptides among other human coronaviruses, such as (HCoVs) OC43 and HKU1 is shown in Table 1. Some conserved T-cell epitopes exist among different HCoVs. For an instance, peptide S978-986 from SARS-CoV (LITGRLQSL) is an HLA-A2 restricted $CD8^+$ T-cell epitope. The corresponding peptides among MERS-CoV (LINGRLTTL), HCoV OC43 (LINGRLTTL) and HKU1 (LINGRLTTL) have only one variable residue at position 8. For Mn2 (GLMWLSYFV) of SARS-CoV, the corresponding peptides from HCoV OC43 (IIMWIVYFV) and HKU1 (IVIWILYFV) still have the typical HLA-A2-restricted anchoring residues. These data may also indicate that prior infections with OC43 and HKU1 may also cross-protect against MERS-CoV or SARS-CoV, considering the common existence of cross-reactivity between sequence-related virus epitopes (Zhang et al., 2015), and would be an important topic for experimental investigation.

Da Guan and colleagues (Da Guan et al., 2015) have found that healthcare workers who were infected with SARS-CoV 12 years ago still have detectable responses to SARS-CoV S protein, with a low-level of cross-T-cell responses against MERS-CoV S protein. Interestingly, based on PBMCs obtained from a traveler to Guangdong, China, who acquired this disease during the 2015 MERS outbreak in South Korea, a strong T-cell-specific response against full-length MERS-CoV S protein was detected, but not cross-reactivity against the S1 subunit of the SARS-CoV S protein. This may be due to the low levels of conservation in the S1 subunit, as discussed above, and may also correlate with the low efficacy of stimulating T-cell responses by recombinant proteins.

6.2. Influence of SARS-CoV and MERS-CoV on immunopathogenesis through interaction with their receptors

As a functional SARS-CoV receptor, ACE2 plays a critical role in SARS-CoV-induced lung injury (Kuba et al., 2005). The injection of SARS-CoV S protein into wild-type mice worsens acute lung failure *in vivo* by both blocking the renin-angiotensin pathway and down-regulation of ACE2 expression. Further, significant pathological features appear in the mice, such as changes in the lung parenchyma and increased lung edema. In contrast, treatment with S protein did not affect the severity of lung failure in *Ace2* knockout mice, which indicates that the effect of S protein on acute lung injury is ACE2-specific.

The MERS-CoV receptor is dipeptidyl peptidase 4 (DPP4), also named CD26 (Raj et al., 2013). The interaction of CD26 with its *in vivo* natural ligand adenosine deaminase (ADA) plays a crucial role in glucose metabolism and likely in T-cell activation, chemotaxis modulation, cell adhesion, and apoptosis (Morimoto and Schlossman, 1998). Our recent structural study of the RBD of MERS-CoV S protein complexed with CD26 indicates potential competitive binding of MERS-CoV S protein to CD26 instead of ADA (Lu et al., 2013), which implies the influence of MERS-CoV infection on the pathogenesis via its receptor CD26, similar to ACE2 in SARS-CoV infection.

Recently, Chu and colleagues demonstrated that MERS-CoV efficiently infects T-cells from the peripheral blood and from human lymphoid organs, including the spleen and tonsils, and induces apoptosis in T-cells (Chu et al., 2016). However, whether the interaction of MERS-CoV S protein and its cellular receptor CD26 is involved in this process requires further exploration. Indeed, the efficacy of antiviral therapies based on mAbs against MERS-CoV (Corti et al., 2015; Li et al., 2015; Tang et al., 2014) may be related to both the direct neutralization of the virions and the blockage of the intervention of functional CD26 by the S protein of the virus (Li et al., 2015).

6.3. Similar immune antagonism strategies of SARS-CoV and MERS-CoV

Pathogenesis studies of SARS-CoV have elucidated diverse strategies used by the virus to evade and block host immune responses, facilitating infection and transmission. A SARS mouse model demonstrates that lethal pneumonia is contributed by dysregulated type I interferon and inflammatory monocyte-macrophage responses (Channappanavar et al., 2016). Recent studies indicate that MERS-CoV has also evolved specific interferon antagonists to counteract the innate immune response (Niemeyer et al., 2013; Yang et al., 2015; Zhou et al., 2014). Serum IFN-α levels were not elevated in a fatal case as compared to a patient who recovered from the infection (Faure et al., 2014).

Similar strategies were developed to evade/inhibit host innate immune functions in both SARS-CoV and MERS-CoV (Vijay and Perlman, 2016). Nsp1 inhibits interferon signaling in SARS-CoV-infected cells by inhibiting phosphorylation of STAT1 (Jauregui et al., 2013) and inhibiting host gene expression by inactivating the translation activity of the ribosomes. Nsp3 interacts with IRF3, inhibiting its phosphorylation, dimerization, and nuclear translocation (Chen et al., 2014). Nsp16 renders viral RNA indistinguishable from host T-cell RNA by effecting 2′-O methylation (Frieman et al., 2010; Menachery et al., 2014). N protein inhibits NFκB signaling and PKR function (Kopecky-Bromberg et al., 2007). The similarity of the immune evasion strategies shared by SARS-CoV and MERS-CoV may indicate common therapeutic targets for drug development.

7. Perspective

Thus far, MERS-CoV vaccines have been shown to provide efficacious protection in animal models, though none of the vaccines developed has been tested in human clinical trials. Major strategies for vaccine development are focused on the elicitation of serum antibodies against the major antigen (S protein) of MERS-CoV (Zhang et al., 2014). Further, passive immunotherapy using convalescent phase human plasma is being considered in MERS patients after its success in animal models (Zhao et al., 2015). However, studies also demonstrate that MERS-CoV S protein-derived vaccines induce specific $CD8^+$ T-cell and virus-neutralizing antibodies, which could contribute to complete protection against MERS-CoV in animal models (Lan et al., 2014; Volz et al., 2015). Based on the investigations of immune memory against SARS-CoV in follow-up studies of recovered patients (discussed above), T-cell responses can provide robust long-term memory and possess a considerable potential for cross-reactivity with heterotypic coronaviruses. Thus, vaccines combining both cellular and humoral responses should be considered for coronavirus prevention. The similarities between the immunopathogenesis of SARS-CoV and MERS-CoV through interaction with cell receptors and blockage of host innate immune responses also point to potential therapeutic targets in patients infected with other pathogenic coronaviruses.

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Conflict of interest

No conflicts of interest.

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