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

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.

© 2016 Elsevier B.V. All rights reserved.
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 (Li 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-2d-, H-2b-, and H-2k-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. *The position information is based on the SARS-CoV (strain TGF, GenBank accession no. AY054624).†The sequences of the corresponding peptides in SARS-CoV, HCoV-OC43 (strain HKU4-01; GenBank accession no. JN129834), and HKU1 (strain BJ01-p3; GenBank accession no. KF779055). The variable residues in the peptides compared to MERS-CoV are underlined in bold. "The references that identified the peptides. ‡The sequences of the corresponding peptides in SARS-CoV, HCoV-OC43 and HKU1 are shown in bold.

| Position | MERS-CoV | SARS-CoV | HCoV-OC43 | HKU1 | MHC restriction | Identification | Vaccine evaluation |
|----------|----------|----------|-----------|------|----------------|----------------|-------------------|
| S411-420 | KQSFNSPTCL | KLDDPFMNGYV | RBDITATIQQ | KFTDTSQQ | HLA-A*0201 | (Zhou et al., 2006) | (Zhou et al., 2006) |
| S787-795 | LEVPISITG | IFDPLKPT | INFSPLGC | INFSLVGC | HLA-A*0201 | (Tao et al., 2006) | (Tao et al., 2006) |
| S1042-1050 | LYMIVHVVY | WLYHIVVY | LYMIVHVVY | LYMIVHVVY | HLA-A2 | | |
| S598-966 | SIGDQRLL | VLNLISLR | SLQSLTSL | SLQSLTSL | HLA-A*0201 | (Lv et al., 2009) | (Lv et al., 2009) |
| S978-986 | LINGRTTL | LINGRTTL | LINGRTTL | LINGRTTL | HLA-A2 | (Wang et al., 2004b) | (Zhou et al., 2006), (Kohyama et al., 2009) |
| S1203-1211 | FIAGLVALA | FIAGLIAV | ICLAVAM | IFSSHFI | HLA-A2 | | |
| S1167-1175 | SLQQVVKAL | RNCIAMNL | RNCIAMNL | RNCIAMNL | HLA-A*0201 | (Wang et al., 2004a) | (Wang et al., 2004a), (Zhou et al., 2006) |
| S1174-1182 | ALNESYDVL | NINLISQDL | NVYQHSDL | NVYQHSDL | HLA-A*0201 | (Doytchinova and Flower, 2003) | |
| S365-374 | TCSQISPAAI | RLYIATRL | TNNDARAI | SNNFDESI | H-2d, H-2k | (Huang et al., 2007) | (Huang et al., 2007), (Dyck et al., 2007), (Dyck et al., 2008), (Dyck et al., 2009) |
| S436-443 | LKYSYINIK | VVYYTL | NKKRIFGL | WNPQGE | H-2d, H-2k | (Zhi et al., 2005) | (Zhou et al., 2010a) |
| S525-532 | VEYSLVG | VNENL | VNENL | VNENL | H-2d | (Poh et al., 2009) | (Poh et al., 2009) |
| S536-374 | CSGISPAAI | CYGVIAK | CNNDIADAI | CNNDIADAI | H-2d | | |
| S884-891 | IFYRLNCPV | MAYVRGFL | MEVRGFL | MEVRGFL | H-2k | | |
| S1166-1173 | STNEPPFLL | NTVVEPDL | PTVVNAQ | PTVVNAQ | H-2k | | |
| N216-224 | GAVGGIDTYL | GETAALLL | VIPDMADQA | VIPDMADQA | HLA-B*4001 | (Rivino et al., 2013) | |
| N233-322 | DDHGNYYVFL | MEGVITISL | DSGKDVIYEL | DSGKDVIYEL | HLA-B*4001 | | |
| N223-231 | LYLDNLRLR | ILLDRLQ | QIASVLAK | FIAYLIAK | HLA-A*0201 | (Tao et al., 2006), (Tao et al., 2006), (Ohno et al., 2009) | |
| N227-235 | LLNRLQALE | RNCIENQKL | LVYKGKDL | LVLKAGKLE | HLA-A*0201 | | |
| N317-325 | CMSQKLTHL | CMSQKLTHL | CMSQKLTHL | CMSQKLTHL | HLA-A*0201 | | |
| N220-228 | GILLYLDDL | LLYLDLL | LLYLDLL | LLYLDLL | HLA-A*0201 | | |
| N226-275 | TSKFNMQVF | TKQNYOAT | TKQNYOAT | TKQNYOAT | HLA-B*1525 | (Ng et al., 2016) | |
| N346-354 | NYKWWLLEL | QKRENTVM | QKRENTVM | QKRENTVM | HLA-B*2402 | (Liu et al., 2010b) | |
| N362-370 | KIPFKEKIK | KIPFKEKIK | KIPFKEKIK | KIPFKEKIK | HLA-A1101 | (Blicher et al., 2005) | |
| M60-69 | SMALSISAVV | SLAQSAV | HQEIEVQ | HQEIEVQ | HLA-B*1202 | (Liu et al., 2010a), (Liu et al., 2010a) | |
| M88-96 | AMMWWISYFYV | GMMWWISFYV | GMMWWISFYV | GMMWWISFYV | HLA-A*0201 | | |
| M147-155 | HMKMAGMHI | HMKMAGMHI | HMKMAGMHI | HMKMAGMHI | HLA-B*1502 | (Ng et al., 2016) | |
| M371-377 | AILYFVPVVTL | SMVALWTVL | LILIAFLPG | LILIAFLPG | HLA-B*1502 | (Kohyama et al., 2009) | (Kohyama et al., 2009) |
| M377-387 | VEHTIPWLL | VAVVPSSFM | VAVVPSSFM | VAVVPSSFM | HLA-B*1502 | (Rodr et al., 2008) | |
| N353-365 | LLEQNDAYKTFP | LLNKHIKDAYKTFP | VIYELNAVQOD | VIYELNAVQOD | H-2d, HLA-DR2, DR3 | (Zhou et al., 2010a) | (Zhou et al., 2010a) |

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 those 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 antigenity 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 (Zhou 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 identified 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 M protein (Liu et al., 2010b). Recently, several MERS-CoV-derived H-2Kd-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 dependent on an uncommon interaction of Ile5 in peptide 37–1 with Trp73 of the host MHC I H-2Kd. 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-β2m-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 vaccinated, 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 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 infection in mice 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.
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. 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-A*-restricted epitopes, Md3 (TLACFVLAAV) and its C-terminal truncated peptide Md3-C9 (LACFVLAV) (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
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 (AMMWLSYFV) 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 (LITGLRLQL) is an HLA-A2 restricted CD8 T-cell epitope. The corresponding peptides among MERS-CoV (LINGRITTL), HCoV OC43 (LINGRITTL) and HKU1 (LINGRITTL) have only one variable residue at position 8. For Mn2 (GLMVLVSYFV) of SARS-CoV, the corresponding peptides from HCoV OC43 (IMMVWFFVFV) and HKU1 (IVIWLFFVFV) 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 lower 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 no 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 (Momomoto 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 retained to both the direct neutralization of the virions and the blockade 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 interferes 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 (Zhao 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 blockade of host innate immune responses also point to potential therapeutic targets in patients infected with other pathogenic coronaviruses.

Financial supports

This work was supported by grants from the China Mega-Project on Infectious Disease Prevention (Grants 2016ZX10004222-003, 2014ZX10004001-006 and 2013ZX10004608-002), the National 973 Project of the China Ministry of Science and Technology (Grant 2015CB910500 and 2011CB910700), and the National Natural Science Foundation of China (Grants 81401312, 31370432 and 81373141). The funders had no role in the study design, data collection and interpretation, or the decision to submit the work for publication. G.F.G. is a leading principal investigator of the National...
Conflict of interest

No conflicts of interest.

Acknowledgements

We are grateful to Ms. Xiaoying Liang for her kind and professional assistance with the preparation of the figures.

References

Agnihotrim, S., Gopal, R., Yount Jr., B.L., Donaldson, E.F., Menachery, V.D., Graham, R.L., Scobey, T.D., Griswold, L.E., Denison, M.R., Zambon, M., Baric, R.S., 2014. Evaluation of serologic and antigenic relationships between Middle Eastern respiratory syndrome coronavirus and other coronaviruses to develop vaccine platforms for the rapid response to emerging coronaviruses. J. Infect. Dis. 209, 995–1006.

Alabbad, M.M., Payne, D.C., Alqasrawi, S., Bha, B., Tohme, R.A., Abedi, G.R., Al Sus, M., Bin Ali, J., Al-Jour, N., Falegh, H., Al-Sani, T., Al Am, S., Al-Hasan, A., Al-Harbi, N., Al-Khalaf, M., Al-Arifi, K., Al-Saadi, A., 2013. Feasibility of using convalescent plasma immunotherapy for MERS-CoV infection. Saudi Arabia. Emerg. Infect. Dis. 22, 1544–1561.

Alshukairi, A.N., Khalid, I., Ahmed, W.A., Dada, A.M., Bayumi, D.T., Malic, L.S., Agnihotram, S., Gopal, R., Yount Jr., B.L., Donaldson, E.F., Menachery, V.D., 2013. Dominant epitopes on the membrane protein of the severe acute respiratory syndrome coronavirus (SARS-CoV) spike protein induce strong mucosal immune responses and provide long-term protection against SARS-CoV infection. J. Immunol. 188, 948–956.

Fan, Y.Y., Huang, Z.T., Li, L., Wu, M.H., Yu, T., Koup, R.A., Bai, R., Wu, C.Y., 2009. Characterization of SARS-CoV-specific memory T cells from recovered individuals 4 years after infection. Arch. Virol. 154, 1093–1099.

Fau, E., Poissy, J., Goffard, A., Fournier, C., Kipnis, E., Titrebat, M., Bortolotti, P., Martinez, L., Dubucquoi, S., Desen, R., Gasser, P., Mathieu, D., Guery, B., 2014. Dendritic immune response in recovered MERS-CoV-infected patients: can we go from bed to bench? PloS One 9, e88176.

Feng, Y., Gao, G.F., 2007. Towards our understanding of SARS-CoV, an emerging and devastating but quickly conquered virus. Comp. Immunol. Microbiol. Infect. Dis. 30, 309–327.

Friedman, M.B., Chen, J., Morrison, T.E., Whitney, D., Fink, R., Wu, C., Chen, M., Martire, E., Huang, D., Paterson, J., Lai, T., 2013. Transgenic mice expressing the SARS-CoV spike protein do not cross-neutralize the novel human coronavirus CoV-EMC. J. Virol. 87, 219–236.

Gottstein, B., Oberste, M.S., Beigel, J.H., Zaki, S.R., 2016. Middle East respiratory syndrome coronavirus efficiently infects human primary T lymphocytes and activates the extrinsic and intrinsic apoptotic pathways. J. Infect. Dis. 213, 904–914.

Gottstein, B., Albarak, A.M., Al-Kahtani, A.F., Al-Qahtani, A.F., Al-Omari, A., Al-Hameed, F., Hayden, F., Fowler, R., Du, L., Tai, W., Zhou, Y., Wang, M., Yuen, K.Y., 2013. Cross-reactive antibodies in convalescent SARS patients’ sera against the emerging novel human coronavirus (EMC-2012) by both immunofluorescent and neutralizing antibody capture. J. Infect. Dis. 207, 1037–1040.

Gottstein, B., Channappanavar, R., Fehr, A.R., Vijay, R., Mack, M., Zhao, J., Meyerholz, D.K., Alshukairi, A.N., Khalid, I., Ahmed, W.A., Dada, A.M., Bayumi, D.T., Malic, L.S., Agnihotram, S., Gopal, R., Yount Jr., B.L., Donaldson, E.F., Menachery, V.D., 2013. Intranasal vaccination of recombinant adeno-associated virus encoding receptor-binding domain of severe acute respiratory syndrome coronavirus (SARS-CoV) spike protein induces strong mucosal immune responses and provides long-term protection against SARS-CoV infection. J. Immunol. 188, 948–956.

Harcourt, J.L., Kuhar, D.T., Swerdlow, D.L., Erdman, D.D., Pallansch, M.A., Harcourt, J.L., Kuhar, D.T., Swerdlow, D.L., Erdman, D.D., Pallansch, M.A., 2015. Prophylactic and postexposure efficacy of a potent human monoclonal antibody against MERS coronavirus. Proc. Natl. Acad. Sci. U. S. A. 112, 10473–10478.

Lau, W., Wang, M., Koc, Z., Chen, Z.L., Feng, L.Q., Li, Z.T., Huang, J.C., Ke, C.W., Deng, X., Ling, Y., Wu, S.G., Ni, X.F., Perera, R.A., Dav, X., Zhao, J., Zhang, L.Q., Li, Y.M., Chen, R.C., Peiris, M., Chen, L., Zhong, N.S., 2015. Characteristics of traveler with Middle East respiratory syndrome coronavirus, China, 2015. Emerg. Infect. Dis. 21, 2278–2280.

Doychinova, I., Flower, D., 2003. The HLA-A2-supermaretif: a QSR definition. Organ. Biomol. Chem. 1, 2648–2654.

Du, L., He, Y., Zhou, Y., Liu, S., Zheng, B.J., Jiang, S., 2009a. The spike protein of SARS-CoV as a target for vaccine and therapeutic development. Nat. Rev. Microbiol. 7, 226–236.

Du, L., Ma, C., Jiang, S., 2013. Antibodies induced by receptor-binding domain in spike protein of SARS-CoV do not cross-neutralize the novel human coronavirus hCoV-EMC. J. Infect. Dis. 67, 348–350.

Du, L., Tai, W., Zhou, Y., Jiang, S., 2016. Vaccines for the prevention against the threat of MERS-CoV. Expert Rev. Vacc. 15, 1123–1134.

Du, L., Zhao, G., Cai, C.C., Li, L., He, Y., Zhou, Y., Zheng, B.J., Jiang, S., 2010. A 219-mer CHO-expressing receptor-binding domain of SARS-CoV’S protein induces potent immune responses and protective immunity. Viral Immunol. 23, 211–219.

Du, L., Zhao, G., Li, L., He, Y., Zhou, Y., Zheng, B.J., Jiang, S., 2009b. Antigenicity and immunogenicity of SARS-CoV S protein receptor-binding domain stably expressed in CHO cells. J. Virolog. Methods. 158, 486–490.

Du, L., Zhao, G., Lin, Y., Sui, H., Chan, C.A., Ma, S., He, Y., Jiang, S., Wu, C., Yuen, K.Y., Jin, D.Y., Zhou, Y., Zheng, B.J., 2008. Intranasal vaccination of recombinant adeno-associated virus encoding receptor-binding domain of severe acute respiratory syndrome coronavirus (SARS-CoV) spike protein induces strong mucosal immune responses and provides long-term protection against SARS-CoV infection. J. Immunol. 180, 948–956.

Fau, E., Poissy, J., Goffard, A., Fournier, C., Kipnis, E., Titrebat, M., Bortolotti, P., Martinez, L., Dubucquoi, S., Desen, R., Gasser, P., Mathieu, D., Guery, B., 2014. Dendritic immune response in recovered MERS-CoV-infected patients: can we go from bed to bench? PloS One 9, e88176.

Feng, Y., Gao, G.F., 2007. Towards our understanding of SARS-CoV, an emerging and devastating but quickly conquered virus. Comp. Immunol. Microbiol. Infect. Dis. 30, 309–327.

Friedman, M.B., Chen, J., Morrison, T.E., Whitney, D., Funck, H., Ward, J.M., Lamirande, E.W., Roberts, A., Heise, M., Subbarao, K., Baric, R.S., 2010. SARS-CoV pathogenesis is regulated by a STAT1 dependent but a type I, II and III interferon receptor independent mechanism. PLoS Pathog. 6, e1000848.

Gu, J., Gong, E., Zhang, B., Zheng, J., Gao, Z., Zhong, Y., Zou, W., Zhan, J., Wang, S., Xie, Z., Zhuang, H., Wu, B., Zhong, H., Shao, H., Fang, G., Dai, P., Li, X., He, Z., Ma, D., Shi, X., Anderson, M.Y., Leong, A., 2015. Multiple organ infection and the pathogenesis of the SARS. J. Exp. Med. 202, 415–424.

He, Y., Zhou, Y., Siddiqui, P., Ni, J., Jiang, S., 2005. Identification of immunodominant epitopes on the membrane protein of the severe acute respiratory syndrome-associated coronavirus. J. Virol. 80, 3726–3726.

Hijawi, B., Abdallat, M., Sayaydeh, A., Alqasrawi, S., Haddadin, A., Jaarour, N., Alsheikh, S., Alsanouri, T., 2013. Novel coronavirus infections in Jordan, April 2012: epidemiological findings from a retrospective investigation. East Mediterr. Health J 19 (Suppl. 1), S12

Huang, M., Zhang, W., Guo, J., Wei, X., Phiwkan, Z., Zhang, J., Zou, X., 2016. Improved transgenic mouse model for studying HLA class I antigen presentation. Sci. Rep. 6, 33302.

Jauregui, A.R., Savalia, D., Lowry, V.K., Farrell, C.M., Wattelet, M.G., 2013. Current developments in vaccine platforms for the rapid response to emerging coronaviruses. J. Infect. Dis. 211 (Suppl. 1), S12–S18.
Identification of residues of SARS-CoV ns1 that differentially affect inhibition of gene expression and antiviral signaling. PloS One 8, e62416.

Julien, J.-P., Lee, P.S., Zheng, G., Pena, I., Kilian, M., 2012. Structural insights into key sites of vulnerability on HIV-1 Env and influenza HA. Immunol. Rev. 250, 180–198.

Kapoor, M., Pringle, K., Kumar, A., Dearth, S., Liu, L., Lovchik, J., Perez, O., Pontones, P., Richards, S., Yeadon-Fahboukn, J., Breakwell, L., Chea, N., Cohen, N., Schachter, J., Elbirt, D., Haynes, L., Pallansch, M., Tan, Y., Tong, S., Gerber, S., Sverdlow, D., Feikin, D.R., 2014. Clinical and laboratory findings of the first imported case of Middle East respiratory syndrome coronavirus to the United States. Clin. Infect. Dis. 59, 1511–1518.

Kojima, Y., Tanisho, S., Yokoyama, S., Morii, M., Kobayashi, A., Hayashi, H., Uchida, T., Matsuji, M., 2009. Efficient induction of cytotoxic T lymphocytes specific for severe acute respiratory syndrome (SARS)-associated coronavirus by immunization with surface-linked liposomal peptides derived from a SARS-coronavirus 1a leader protease. Vaccine 27, 168–177.

Kopecky-Bromberg, S.A., Martinez-Sobrido, L., Frieman, M., Baric, R.A., Palese, P., Kapoor, M., Pringle, K., Kumar, A., Dearth, S., Liu, L., Lovchik, J., Peng, O., Pontones, P., 2007. Severe acute respiratory syndrome coronavirus-like particles carrying severe acute respiratory syndrome coronavirus (SARS-CoV) S glycoprotein protect mice against challenge with SARS-CoV. Vaccine 26, 339–348.

Lai, G., Hu, Y., Wang, Q., Qi, J., Gao, F., Li, Y., Zhang, Y., Zhang, W., Yuan, Y., Gao, B., Wang, Z., Shi, Y., Yan, J., Gao, J.F., 2013. Molecular basis of binding between novel human coronavirus MERS-CoV and its receptor CD26. Nature 500, 223–231.

Lau, T., Gao, G.F., 2010b. Novel immunodominant peptide presentation strategy: a new paradigm in vaccine development. Exp. Biol. Med. 235, 253–267.

Liu, W., Fontanet, A., Zhang, P.H., Zhan, L., Xion, Z.T., Baril, L., Tang, F., Yu, H., Gao, W.C., 2006. Two-year prospective study of the humoral immune response of patients with severe acute respiratory syndrome. J. Infect. Dis. 193, 792–795.

Liu, W., Lan, J., Liu, K., Deng, Y., Yao, Y., Wu, S., Chen, H., Bao, L., Zhang, H., Zhao, M., Wang, H., Han, L., Chan, P.K., Cao, S., Tan, W., 2016. Protective T-cell responses featured by concordant recognition of MERS-CoV-derived CD8+ T-cell epitopes and host MHC. J. Immunol. (in press).

Lv, Y.Y., Massare, M.J., Barnard, D.L., Kort, T., Nathan, M., Wang, L., Smith, C.G., 2011c. Characterizing the severe acute respiratory syndrome coronavirus (SARS-CoV) S glycoprotein and influenza matrix 1 efficiently force virus-like particles (VLPs) that protect mice against challenge with SARS-CoV. Vaccine 29, 6606–6613.

Lokugamage, K.G., Yoshikawa-Iwata, N., Ito, N., Watts, D.M., Wyde, P.R., Wang, N., Nicholson, P., Kent, J.O., Oh, Y., Li, Y., Meng, C., Qin, C., Gao, G.F., Tan, W., 2008. Chimeric coronavirus-like particles carrying severe acute respiratory syndrome coronavirus (Scov) S glycoprotein protect mice against challenge with Scov. Vaccine 26, 3305–3308.

May, C.K., Cheon, S., Ba, N.Y., Sohn, K.M., Kim, Y., Aigerim, A., Shin, H.M., Choi, J.Y., In, K.S., Kim, J.H., Moon, J.Y., Choi, M.S., Cho, N.H., Kim, Y.S., 2016. Comparative and kinetic analysis of viral shedding and immunological responses in MERS patients representing a broad spectrum of disease severity. Sci. Rep. 6, 25397.

Morimoto, C., Schlossman, S.F., 1998. The structure and function of CD26 in T-cell immune response. J. Immunol. 160, 3476–3485.

Newman, P., Kent Tseng, C.T., Peters, C.J., Makino, S., 2008. Chimeric severe acute respiratory syndrome coronavirus (SARS-CoV) S glycoprotein and in vivo vaccine against infection in mice. J. Virol. 82, 2231–2240.

Nieto, V., Zhou, X., Gao, G.F., Zhu, B., 2014. Characterization of human alphabeta TCR vaccine development. Exp. Biol. Med. 239, 253–267.

Osterhaus, A.D., Bosch, B.J., Haagmans, B.L., 2013. Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. Nature 495, 795–798.

Poh, W.P., Narasaraju, T., Pereira, N.A., Zhong, F., Phoon, M.C., Macary, P.A., Park, W.B., Perera, R.A., Choe, P.G., Lau, E.H., Choi, S.J., Chun, J.Y., Oh, H.S., Song, K.H., Meyers, B., Drosten, C., Muller, M.A., 2014. Serological assays for emerging coronavirus infection. J. Virol. 88, 12495–12505.

Poh, W.P., Pereira, R.A., Cho, P.C., Lau, E.H., Choi, S.J., Chun, J.Y., Oh, H.S., Song, K.H., Jiang, B.H., Kim, E.S., Kim, H.B., Park, S.W., Kim, N.J., Man Poon, L.L., Peiris, M., Oh, M.D., 2015. Kinetics of serologic responses to MERS coronavirus infection in humans, South Korea. Emerg. Infect. Dis. 21, 2086–2089.

Poh, W.P., Huang, L.T., Wang, L.Y., Li, J., Wang, L., Bao, Y., Gao, G.F., 2015. Long-lived memory T lymphocyte responses against SARS coronavirus nucleocapsid protein in SARS-infected patients. J. Virol. 89, 10464–10471.

Oshino, S., Kojima, S., Taneichi, M., Moriya, O., Hayashi, H., Oda, H., Mori, M., Kobayashi, A., Aikikusa, T., Uchida, T., Matsuji, M., 2009. Synthetic peptides coupled to the surface of liposomes effectively induce SARS-coronavirus-specific cytotoxic T lymphocytes and viral clearance in HLA-A*0201 transgenic mice. Vaccine 27, 3912–3920.

Park, W.B., Pereira, R.A., Cho, P.C., Lau, E.H., Choi, S.J., Chun, J.Y., Oh, H.S., Song, K.H., Jiang, B.H., Kim, E.S., Kim, H.B., Park, S.W., Kim, N.J., Man Poon, L.L., Peiris, M., Oh, M.D., 2015. Kinetics of serologic responses to MERS coronavirus infection in humans, South Korea. Emerg. Infect. Dis. 21, 2086–2089.

Poh, W.P., Huang, L.T., Wang, L.Y., Li, J., Wang, L., Bao, Y., Gao, G.F., 2015. Long-lived memory T lymphocyte responses against SARS coronavirus nucleocapsid protein in SARS-infected patients. J. Virol. 89, 10464–10471.
Trujillo, J.A., Gras, S., Twist, K.A., Croft, N.P., Channappanavar, R., Rossjohn, J., To, K.K., Hung, I.F., Chan, J.F., Yuen, K.Y., 2013. From SARS coronavirus to novel coronavirus infection in cats. Antivir. Ther. 19, 645–652.

Rota, P.A., Oberste, M.S., Monroe, S.S., Nix, W.A., Campagnoli, R., Icenogle, J.P., Rota, P.A., Purcell, A.W., Perlman, S., 2014. Structural and functional correlates of enhanced T-cell responses in recovered patients with severe acute respiratory syndrome coronavirus. J. Immunol. 186, 7264–7273.

Wang, Y., Wu, G., Gao, G.F., 2015. Origin and possible genetic recombination of the Middle East respiratory syndrome coronavirus from the first imported case in China: phylogenetics and coalescence analysis. mBio 6 e001215.

Wang, Y.D., Sin, W.Y., Xu, G.B., Yang, H.H., Wong, T.Y., Pang, W.X., He, X.Y., Zhang, H.G., Ng, J.N., Cheng, C.S., Yu, J., Meng, L., Yang, R.F., Lai, S.T., Guo, Z.H., Xie, Y., Chen, W.F., 2004b. T-cell epitopes in severe acute respiratory syndrome (SARS) coronavirus spike protein: a specific T-cell immune response in patients who recover from SARS. J. Virol. 78, 5612–5618.

Wang, G., Liu, W., Liu, Z., Yu, B., Li, Y., Gao, G.F., 2015. MERS, SARS, and Ebola: the role of super-spreaders in infectious disease. Cell Host Microbe 18, 398–401.

Wool, C., Liu, E.K., Tso, B.H., Tso, W.H., Feng, A.M., Chan, K.H., Tan, V.K., Peiris, J.S., Yuen, K.Y., 2004. Detection of specific antibodies to severe acute respiratory syndrome (SARS) coronavirus nucleocapsid protein for serodiagnosis of SARS coronavirus pneumonia. J. Clin. Microbiol. 42, 2306–2309.

Yang, K., Sun, K., Srirunasan, K.M., Salmon, J., Marques, E.T., Xu, J., August, J., 2009. Immune responses to T-cell epitopes of SARS-CoV-N protein are enhanced by N immunization with a chimeric of lysosome-associated membrane protein. Gene Ther. 16, 1337–1362.

Yang, L., Peng, H., Zhu, Z.J., Li, G., Huang, Z., Zhao, Z., Koup, R.A., Bailer, R.T., Wu, C., 2007. Persistent memory CD4+ and CD8+ T-cell responses in recovered severe acute respiratory syndrome (SARS) patients to SARS coronavirus M antigen. J. Gen. Virol. 88, 2740–2748.

Yang, L.T., Peng, H., Zhu, Z.L., Li, G., Huang, Z.T., Zhao, Z.X., Koup, R.A., Bailer, R.T., Wu, C.Y., 2006. Long-lived effector/memory CD8+ T-cell responses to severe acute respiratory syndrome coronavirus (SARS-CoV) S in recovered SARS patients. Clin. Immunol. 120, 167–178.

Yang, Y., Ye, F., Zhu, N., Wang, D., Yen, Z., Zhao, T., Tan, W., 2015. Middle East respiratory syndrome coronavirus ORF4b protein inhibits type I interferon production through both cytoplasmic and nuclear targets. Sci. Rep. 5, 17554.

Zaki, A.M., van Boheemen, S., Bestebroer, T.M., Osterhaus, A.D., Fouchier, R.A., 2012. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. New Engl. J. Med. 367, 1814–1820.

Zhang, N., Jiang, S., Du, L., 2014. Current advances and potential strategies in the development of MERS-CoV vaccines. Expert Rev. Vacc. 13, 761–774.

Zhang, S., Bakshi, R.K., Suneetha, P.V., Fytili, P., Antunes, D.A., Vieira, G.F., Jacobs, R., Klade, C.S., Manns, M.P., Kraft, A.R., Wedemeyer, H., Schlaphoff, Y., Cormegn, M., 2015. Frequency, specific diversity, and cross-reactivity of preexisting HIV antibodies (HCV-Specific CD8+ T cells in HCV-seronegative individuals: implications for vaccine responses. J. Virol. 89, 8304–8317.

Zhou, J., Huang, Q., Wang, W., Zhang, Y., Li, P., Gao, X.M., 2007. Identification and characterization of dominant helper T-cell epitopes in the nucleocapsid protein of the Middle East respiratory syndrome coronavirus from the first imported case in China. J. Virol. 81, 6117–6120.

Zhou, J., Zhao, J., Mangalam, A.K., Channappanavar, R., Fett, C., Meyerholz, D.K., Agnihotri, S., Baric, R.S., David, C.S., Perlman, S., 2016. Airway memory CD8+ T cells mediate protective immunity against emerging respiratory coronavirus infections. Immunity 44, 1379–1391.

Zhou, J., Zhao, J., Perlman, S., 2010a. T cell responses are required for protection from severe acute respiratory syndrome coronavirus infection in cats. J. Virol. 84, 7191–7200.

Zhou, K., Wang, H., Wu, C., 2011. The immune responses of HLA-A*0201 restricted SARS-CoV-S protein-specific CD8+ T cells are augmented in varying degrees by CpG ODN, PolyI: C and R848. Vaccine 29, 6670–6679.

Zhou, K., Yang, B., Wu, X., Yen, C.M., 2010b. CD8+ T cell response in HLA-A*0101 restricted SARS-CoV-S protein specific CD4+ T cells are augmented in varying degrees by CpG ODN, PolyI: C and R848. Vaccine 29, 6670–6679.

Zhou, J., Chu, H., Li, C., Wong, B.H., Cheng, Z.S., Poon, V.K., Sun, T., Lau, C.C., Wong, K., Chan, J.V., Chan, J.F., To, K.K., Chan, K.H., Zheng, B.J., Yuen, K.Y., 2014. Active replication of Middle East respiratory syndrome coronavirus and aberrant induction of inflammatory cytokines and chemokines in human macrophages: implications for pathogenesis. J. Infect. Dis. 209, 1311–1324.

Zhou, M., Xu, D., Li, X., Li, H., Shun, M., Tang, J., Yang, M., Wang, M., Wang, F.S., Zou, X., Tan, H., He, W., Tien, P., Gao, G.F., 2006. Screening and identification of specific antibody responses to severe acute respiratory syndrome-associated coronavirus-specific CTL epitopes. J. Immunol. 177, 2138–2145.

Zhou, T., Georgiev, I., Wu, X., Yang, Z.Y., Dai, K., Finzi, A., Kwon, Y.D., Scheid, J.F., Shi, X., Wu, L., Yang, Y., Zhu, J., Nussenzweig, M.C., Sodroski, J., Shapiro, L., Nabel, G.J., Mascola, J.R., Kwong, P.D., 2010. Structural basis for broad and potent neutralization of HIV-1 by antibody VRC01. Science 329, 811–817.

Zhu, F., Santos-Barragan, L., Ruit, B., Blakopeck, G., Weber, F., Ludwig, B., Thiel, V., 2007. Coronavirus non-structural protein 1 is a major pathogenicity factor: implications for the rational design of coronavirus vaccines. PLoS Pathog. 3, e109.