Human T-cell immunity against the emerging and re-emerging viruses

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INTRODUCTION

Recently emerging and re-emerging viruses such as avian influenza viruses, Middle East respiratory syndrome coronavirus (MERS-CoV), Zika virus (ZIKV), and Ebola virus (EBOV) represent global public health concerns. Most of these newly emerged viruses, arising from the human-contacted wild or domesticated animals, pose new challenges to the human immune system and induce severe diseases causing high mortalities (Olival et al., 2017). The clinical features of patients infected by MERS-CoV and avian influenza viruses, such as H7N9, were characterized by severe pneumonia but with different fatality ratios (Assiri et al., 2013; de Groot et al., 2013; Gao et al., 2013; Wu and Gao, 2013). The ZIKV infections are generally mild, ranging from asymptomatic infection to a febrile illness, characterized by headache, arthralgia, myalgia, maculopapular rash, conjunctivitis, vomiting, and fatigue. However, severe neurological complications associated with ZIKV infection, such as Guillain-Barré syndrome (GBS) in adults and congenital birth defects, including microcephaly in the developing fetus, have emerged from recent epidemics, making ZIKV an emerging public health emergency (Brasil et al., 2016; de Araújo et al., 2016). Ebola virus, first identified in central...
Africa in 1976, re-emerged at the end of 2013 in West Africa with a high mortality rate of 40% (Baize et al., 2014; Liu, 2016). Though diverse clinical manifestations were observed in these infectious diseases, there were similar immune features that correlated with the disease severities in patients, such as high levels of proinflammatory cytokines and chemokines, termed hypercytokinaemia, lymphopenia, and thrombocytopenia in the early stage of the infections, and also extensive tissue infiltration of immune cells (Assiri et al., 2013; Gao et al., 2013; Tan et al., 2017). However, the magnitude, time phase and longevity of the virus-specific serological and T-cell responses are represented differently, though the data on adaptive immune responses against these emerging viruses in patients remain limited (Liu et al., 2014; Liu et al., 2017b). The demonstration of the immune features of these viruses will provide beneficial recommendations for clinical intervention and vaccine development against these diseases (Mandl et al., 2015).

Although the population is susceptible to new viruses, infection from previously emerged viruses that are genetically correlated with the currently emerging viruses may induce a cross-immune response in humans, which means that a preexisting immune response may be elicited. Evidently, pre-existing T-cell immunity established by previous infections from related viruses would be able to recognize the conserved epitopes and sometimes even cross-recognize the mutated epitopes, thereby helping the host to clear the novel viruses and also shed light on its universal vaccine development (Quiñones-Parra et al., 2016; Wang et al., 2016b). However, such pre-existing cellular immunity could be a double-edged sword. Sometimes, the host could mount cytotoxic T lymphocytes (CTLs) targeting the previous strains with weak responses to other immunogenic variants present, which is the “original antigenic sin” of T-cells (McMichael, 1998). The “original antigenic sin” correlated with preexisting T-cell might result in anergy and enhancement of the disease (Cobey and Hensley, 2017). Cross-reactive dengue-specific T-cells in a cohort of HLA-A*24 individuals showed suboptimal granulation and high cytokine production, likely leading to immunopathology (Mongkolsapaya et al., 2006). Thus, a timely summarization and analysis of the features of T-cell immunity in patients toward the newly emerging and re-emerging viruses and the potential cross-immune reactivity in the population will be important references for both clinical and basic research on these emerging viral diseases.

T-CELL IMMUNITY TO AVIAN INFLUENZA A (H7N9) VIRUS

After H7N9 influenza virus infection, most patients developed severe respiratory illness such as acute respiratory distress syndrome and pneumonia, and showed high T-cell responses and inflammatory cytokines in lungs and peripheral blood (Goraya et al., 2015; Guo et al., 2015; Huang et al., 2014; Wang et al., 2014). T-cells in patients with severe H7N9 infection presented delayed responses in the acute phase. Under non-specific stimulation of Streptococcus antigens, the T-cell status was abnormal in severely affected patients one month post infection compared to that in patients with mild symptoms (Chen et al., 2016; Diao et al., 2014). In the H7N9 patients with different hospitalization periods, it was observed that early robust CD8\(^+\) T-cell responses correlated with rapid recovery from severe H7N9 disease (Wang et al., 2015). These observational studies indicate that T-cells, especially CD8\(^+\) T-cells, may play a pivotal role in protection against severe H7N9 disease.

The envelop proteins of H7N9 virus, such as hemagglutinin (HA) and neuraminidase (NA), get mutated frequently, while the internal proteins share highly conserved sequences; matrix 1 (M1), nucleoprotein (NP) and polymerase basic 1 (PB1) show 92%, 93% and 96% similarity, respectively, when aligned to pH1N1 virus. Among the internal proteins, M1 and NP possess dominant immunogenicity in T-cell immunity (Grant et al., 2016; Lee et al., 2008; Liu et al., 2014). Despite a decrease in T-cell epitope immunogenicity owing to substitutions in H7N9, there was a moderate level of cross-reactive T-cell response between seasonal influenza A viruses and H7N9 (Liu et al., 2016; van de Sandt et al., 2014). Quiñones-Parra et al. showed that immunogenic peptides derived from H7N9 could be recognized by memory CD8\(^+\) T-cells generated from former influenza infection, but the recognition ability varied among different ethnicities (Quiñones-Parra et al., 2014). Van de Sandt et al. found that CD8\(^+\) T-cells in the peripheral blood of healthy population collected before the H7N9 epidemic could cross-react with H7N9 viruses (van de Sandt et al., 2014). The H7N9 variant of the NP\(_{418-426}\) epitope could be recognized by sh3N2-specific CD8\(^+\) T-cells derived from a HLA-B*35 subject. This cross-reactivity between different influenza viruses may be contributed by the immunodominant T-cell epitopes as indicated by our previous study (Figure 1A) (Liu et al., 2012; Liu et al., 2013). Immunoinformatic analyses (Duvvuri et al., 2014) showed that nearly half of the predicted conserved epitopes derived from internal proteins could induce CD4\(^+\) T-cell immunity. Furthermore, it was observed from different studies in mice that the cross-reactive memory T-cells could offer heterosubtypic protection against H7N9 with hierarchies, which might depend on the size of memory T-cell pool (Duan et al., 2015; McMaster et al., 2015). Thus, the pre-existing cross-reactive T-cell immunity established by seasonal influenza virus infection in the population may contribute to the virus clearance and symptom relief in patients (Liu et al., 2014).
Despite the widely existing cross-reactive T-cell immunity between influenza viruses, it has also been noticed that H7N9 virus-specific substitutions in T-cell epitopes could induce immune evasion in subjects naïve to H7N9 (Liu et al., 2016; Quiñones-Parra et al., 2014). The substitutions in human leucocyte antigen (HLA)-A*0101-restricted T-cell epitope NP44–52 from H7N9 virus compared to seasonal influenza viruses, made the peptide-major histocompatibility complex (MHC) less stable and accessible to cytotoxic T-cells (Quiñones-Parra et al., 2014; van de Sandt et al., 2014). Our group identified that the two amino acid substitutions in HLA-A*1101-restricted NP188–198 led to a dramatic antigenic variability of the corresponding T-cell epitope in H7N9 (Figure 2A and B) (Liu et al., 2016). Generally, based on a combination of such structural and functional studies, it was elucidated that the immunogenetic variations are majorly modulated by the substitutions, impacting HLA-binding and/or T-cell receptor (TCR) recognition.

Influenza A virus-specific T-cell immunity could last for a long term. van de Sandt et al. demonstrated that influenza virus-specific CTLs are long-lived through following donors from 1999–2012 (van de Sandt et al., 2015). However, it is noteworthy that among the T-cells established by former influenza infection, there might be T-cells with TCRs targeting subdominant epitopes that barely induce T-cell responses. Previous studies of CTLs in lymphocytic choriomeningitis virus (LCMV) infection showed that the virus could escape the immune system via varied mutants, which could generate helpless CTLs, thus resulting in “original antigenic sin” (Klenerman and Zinkernagel, 1998; McMichael, 1998). The immune responses focusing on the conserved epitopes may not be efficiently activated when new influenza strains develop mutations in these conserved regions, resulting in immune anergy and disease progression.

**T-CELL IMMUNITY TO ZIKA VIRUS**

ZIKV is a mosquito-transmitted flavivirus that was first isolated in 1947 in Uganda from a febrile Rhesus macaque, and recently rocketed into a public health emergency due to its rapid dissemination in the Americas and the southeast Asian countries (Dick et al., 1952; Hazin et al., 2016; Zhang et al., 2016). Several ZIKV cases were imported into China, and the first ZIKV strain from a returned traveller was isolated and analyzed by Deng et al. (Deng et al., 2016). Although ZIKV infection is known to occasionally cause mild illness, there is evidence that ZIKV infection can lead to severe birth defects, including microcephaly and neurological complications, which may develop into Guillain-Barre syndrome (Calvet et al., 2016; Cao-Lormeau et al., 2016). In addition to arthropod borne transmission, sexual transmis-
T-cell-mediated immune responses that reduced infectious ZIKV levels as well as altered the immunodominance pattern in the DENV-immune setting relative to the naive type (Table 1) (Wen et al., 2017b). Similarly, virus-specific CD8+ T-cells induced by previous DENV infection can predominantly mediate cross-protective responses to ZIKV in mice (Wen et al., 2017a). A recent study showed that ZIKV NS1- and E-reactive memory CD4+ T-cells had a low cross-reactivity with DENV (Table 1) (Stettler et al., 2016). However, in humoral immunity, it has been shown that DENV antibodies are cross-reactive to ZIKV and can enhance ZIKV infection mediated by antibody-dependent enhancement (ADE) (Bardina et al., 2017).
Although many studies have explored cross-reactivity between DENV and ZIKV in humoral and cellular immunity, the dual role of cross-reactive T-cells to flaviviruses in protection and pathogenesis remains unknown. Cross-reactive T-cells in ZIKV may also lead to the failure of an appropriate immune response to the other flavivirus serotypes, as “original antigenic sin” occurred between flavivirus and cross-reactive T-cells. Mongkolsapaya et al. have discussed “original antigenic sin” in the context of the suboptimal degranulation of cross-reactive dengue-specific T-cells, besides the high cytokine storm in DENV infection (Mongkolsapaya et al., 2006). Thus, more researchers should focus on vaccine development for ZIKV, especially for application in recipients previously infected by other flaviviruses, such as DENV (Halstead, 2007; Mongkolsapaya et al., 2003). There is an urgency in determining the mechanism for “original T-cell antigenic sin” in flaviviruses, which might benefit future efficacious vaccine formulations and antiviral therapy.

### T-CELL IMMUNITY TO EBOLA VIRUS

Since its first identification as the causative agent of a hemorrhagic fever syndrome in the Democratic Republic of Congo, Ebola virus disease (EVD) has infected thousands of humans with a case fatality rate of 40 % to 90 % (Chowell and Nishiura, 2014). The recent EVD outbreak in West Africa is the largest epidemic in history, with more than 28,000 confirmed and probable cases (Wong et al., 2016). Studies in animal models have provided some insight into the immune responses against EBOV infection (Zeng et al., 2016). However, human immune responses have been poorly investigated, mainly because most outbreaks have occurred in remote areas of Africa.

Currently, studies based on limited blood specimens from EBOV patients indicated that hypercytokinemia was common in the acute phase of human infection (Ruibal et al., 2016). Using the Luminex technology, T-cell-related immune mediator analysis of Ebola patient blood samples, obtained between 1996 and 2003 from Gabon and Republic of Congo, showed an aberrant innate immune reaction characterized by a “cytokine storm” with hypersecretion of numerous proinflammatory cytokines, chemokines, and growth factors (Wauquier et al., 2010). Especially among the pediatric patients younger than 21 years of age, the ones who survived had higher levels of the chemokine regulated on activation, normal T-cell expressed and secreted marker (RANTES), and lower levels of plasminogen activator inhibitor 1 (PAI1), soluble intracellular adhesion molecules (Kam et al., 2017), and soluble vascular cell adhesion molecules (VCAM) compared with pediatric patients who died (McElroy et al., 2014).

T lymphocytes are proposed to be critical for the clearance and protection against EBOV infection. Sequence-based HLA-B typing performed using leukocytes isolated from Sudan virus (SUDV) patients, from an outbreak in northern Uganda in 2000–2001, indicated that alleles B*67 and B*15 were associated with fatal outcomes, whereas B*07 and B*14 were associated with nonfatal outcomes, suggesting a pivotal role of HLA-restricted T-cell responses during the anti-virus process (Sanchez et al., 2007). Immunosuppression in fatal pediatric patients was characterized by very low levels of circulating cytokines produced by T lymphocytes and by massive loss of peripheral CD4⁺ and CD8⁺ lymphocytes (Wauquier et al., 2010). Another study based on the patients from the outbreak in Gabon in 1996 showed that although activation of T-cells, indicated by considerable release of IFN-γ in plasma, could be observed in the early disease stage in fatal cases, these patients were characterized by the disappearance of T-cell-related mRNA (including IFN-γ) in the days preceding death, indicating an important role of T-cell immune responses in the virus clearance (Baize et al., 1999). During the recent EVD outbreak in West Africa, analysis of fatal patients, using a multiparametric flow cytometry established in the field, showed that they were characterized by a high percentage of CD4⁺ and CD8⁺ T-cells expressing the inhibitory molecules CTL-associated antigen 4 (CTLA-4) and programmed death 1 (PD-1), which corre-

### Table 1

| Hosts               | Viruses/vaccines for immunization | Target antigens for test | Cross-reactivities               |
|---------------------|----------------------------------|--------------------------|---------------------------------|
| Humans              | ZIKV                             | DENV                     | +(IFN-γ-based FCScan)           |
| Humans              | DENV                             | ZIKV                     | +(IFN-γ-based ELISPOT)          |
| Humans              | YFV vaccine                      | ZIKV                     | +(IFN-γ-based FACScan)          |
| HLA I-transgenic mice | DENV                            | ZIKV                     | +(IFN-γ/TNF-based FACScan)      |
| Mice                | DENV                             | ZIKV                     | +(IFN-γ-based FACScan)          |
| Mice                | ZIKV                             | DENV                     | +(IFN-γ-based FACScan)          |
| Mice                | DENV                             | ZIKV                     | +(IFN-γ-based FACScan)          |

a) FACSan, FACScan flow cytometry; ELISPOT, enzyme-linked immunospot assay.
lated with elevated inflammatory markers and high virus load (Ruibal et al., 2016). Conversely, surviving individuals showed significantly lower expression of CTLA-4 and PD-1, but still had comparable overall T-cell activation. These studies indicate that robust EBOV-specific T-cell responses might play a pivotal role in virus clearance, which could be helpful for the survival of the patients.

Another unconventional feature of the immune responses to EBOV was the persistent activation and long-term immune memory post infection (McElroy et al., 2015; Sobarzo et al., 2013). In 2014, the T-cell test data from the four Ebola patients in Emory University showed that Ebola-specific CD8^+ T-cells were still activated one month after the recovery of the patients, and remained detectable for up to 144 days after the disease onset (McElroy et al., 2015). However, the magnitude of CD8^+ T-cell response varies along with the time. The mount of activated CD8^+ T cell responses (HLA-DR CD38 CD3^+ CD8^+) went down in about one month post infection, but went up eventually (McElroy et al., 2015). Considering the long-term persistence of viral RNA in the body fluids of the survivors, it is valuable to detect the immune responses post EBOV infection, which may help identify useful immune biomarkers for persistent viral RNA (Thorson et al., 2016; Zeng et al., 2017).

**T-CELL IMMUNITY TO MERS-COV AND THE CROSS-REACTIVITY TO SARS-COV**

In 2012, a novel human infective coronavirus called MERS-CoV was first reported in the Middle East countries (Zaki et al., 2012), and soon imported cases were diagnosed in the European, African, American, and Asian countries (Su et al., 2015; Su et al., 2016; Wong et al., 2015). In 2015, an outbreak of MERS-CoV characterized by local human-to-human transmission occurred in South Korea (Su et al., 2015). The clinical features of patients infected with MERS-CoV are quite similar to those infected with severe acute respiratory syndrome (SARS)-CoV, i.e. severe pneumonia and acute respiratory distress syndrome (ARDS) (Gao et al., 2016; Hong et al., 2017). Moreover, severe MERS-CoV infections are also characterized by renal dysfunction, which is observed in much lower numbers in SARS-CoV infections (Cha et al., 2015; Chu et al., 2005). Recent studies strongly elucidated a pivotal role for virus-associated immunopathological events in causing fatal pneumonia in both MERS and SARS patients, which are often associated with massive inflammatory cell infiltration and elevated proinflammatory cytokine/chemokine responses termed hypercytokinemia in the patients (Channappanavar and Perlman, 2017). Immunopathology-related mediators, such as interleukin 10 (IL-10), were elevated in an imported case of a Chinese individual who returned from South Korea during the acute infection phase (Da Guan et al., 2015). However, the levels of antiviral adaptive Th1 immune response-associated cytokines IL-12 and IFN-γ were lower in a fatal case than in a patient who survived (Faure et al., 2014). These data indicate that dysregulated immune response-associated lung immunopathology in MERS-CoV-infected patients might lead to deleterious clinical manifestations. In addition, recent evidence (Al-Otaibi et al., 2017) showed that the capture of the human CD26 by MERS-CoV S protein suppressed macrophage responses via CD26-mediated induction of Toll-like receptor (TLR) signaling. This discovery verified our previous observation about the potential impact of both MERS-CoV and SARS-CoV on the host immune responses through interaction with their receptors (Liu et al., 2017b).

To study the MERS-CoV-specific T-cell responses in patients, peripheral blood mononuclear cells (PBMCs) were obtained from the imported case in China on day 24 after the illness, and a strong specific T-cell response against the MERS-CoV S protein was observed (Da Guan et al., 2015). Our studies on the follow-up of SARS survivors found that SARS-CoV M and N protein-specific T-cells could exist in the peripheral blood for a long period (Figure 1C) (Liu et al., 2010a; Liu et al., 2010b; Ng et al., 2016). Interestingly, it was found that SARS-CoV S protein-specific T-cells could still be detected in healthcare workers who were infected with SARS-CoV 12 years ago, and a low level of cross-T-cell response against MERS-CoV S protein could also be detected (Da Guan et al., 2015). In contrast, the direct cross-reactivity to SARS-CoV antigens in the MERS patients or survivors was not yet reported. In our recent study, MERS-CoV-specific T-cell responses were detected in animal models after virus infection or vaccine immunization and their pivotal role in anti-virus protection was well-defined (Figure 1D) (Lan et al., 2014; Liu et al., 2017a). The cross-reactivity to SARS-CoV by MERS-CoV was also detected in animal models. Zhao et al. found that a previously identified conserved CD4^+ T-cell epitope can be recognized in HLA-DR2 and -DR3 transgenic mice infected by either SARS-CoV or MERS-CoV, and showed a certain level of protective effect (Zhao et al., 2016). Our previous analysis also indicated that the relatively conserved T-cell epitopes with limited substitutions between SARS-CoV and MERS-CoV may still possess cross-reactive immunogenicity (Liu et al., 2017b). Essentially, the crystal structure of peptide M88–96 from SARS-CoV complexed with HLA-A*0201 and our modeling of the substitution peptide from MERS-CoV complexed with HLA-A*0201 showed quite similar conformations (Figure 2E and F), indicating a potential cross-reactivity. Thus, more investigations are still needed to elucidate a complete landscape of the immunogenic relationship between the highly pathogenic SARS-CoV and MERS-CoV.
PTERSPECTIVES

Although the characteristic evaluation of the T-cell immunity of the emerging or re-emerging viruses provides beneficial references for clinical intervention or vaccine development, many aspects of the general mechanism of cell-mediated immunity in these viral diseases remain poorly understood. There are several techniques commonly used in detecting antigen-specific T-cells, including cell proliferation assay, cytotoxicity assay, intracellular cytokine staining (ICS), enzyme-linked immunosorbent spot (ELISPOT) assay and, peptide-MHC tetramer staining (or pentamer/multimer staining); however, systematic approaches are required to comprehensively understand the virus-specific T-cell immunity. (Liu et al., 2011). Key and dominant immune mediators in the immunopathogenesis-related hypercytokinemia during the acute phase of the viral infection have not been well defined. Although heterosubtypic protection induced by T-cell immune responses between different subtypes of influenza viruses is evident, the possibility to develop a T-cell-based universal vaccine against genetically related viruses is still in debate. This is also a concern for the recently re-emerged ZIKV and other flaviviruses, such as DENV. Furthermore, it is not well determined whether the long-lasting virus-specific T-cells in the survivors can still contribute towards an effective protection against the re-infection of the same virus or even cross-protection against other genetically-related viruses. Long-term follow-up of the survivors and development of new animal models for the pre-clinical trials may be the future orientation for studies focusing on the emerging and re-emerging viruses.

Compliance and ethics

The author(s) declare that they have no conflict of interest.

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