Understanding the T cell immune response in SARS coronavirus infection

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The severe acute respiratory syndrome (SARS) epidemic started in late 2002 and swiftly spread across 5 continents with a mortality rate of around 10%. Although the epidemic was eventually controlled through the implementation of strict quarantine measures, there continues a need to investigate the SARS coronavirus (SARS-CoV) and develop interventions should it re-emerge. Numerous studies have shown that neutralizing antibodies against the virus can be found in patients infected with SARS-CoV within days upon the onset of illness and lasting up to several months. In contrast, there is little data on the kinetics of T cell responses during SARS-CoV infection and little is known about their role in the recovery process. However, recent studies in mice suggest the importance of T cells in viral clearance during SARS-CoV infection. Moreover, a growing number of studies have investigated the memory T cell responses in recovered SARS patients. This review covers the available literature on the emerging importance of T cell responses in SARS-CoV infection, particularly on the mapping of cytotoxic T lymphocyte (CTL) epitopes, longevity, polyfunctionality and human leukocyte antigen (HLA) association as well as their potential implications on treatment and vaccine development.

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neutralizing antibodies can prevent viral entry, the body also requires SARS-CoV specific CD4+ T helper cells for the development of these specific antibodies. Similarly, CD8+ cytotoxic T cells are important for the recognition and killing of infected cells, particularly in the lungs of infected individuals. Despite the increasing number of reports that investigated memory CD4+ and CD8+ T cell responses in recovered SARS patients, there is a lack of data that describes the kinetics of the T cell response during a SARS-CoV infection. This review will focus on the memory T cell studies and its possible implications on treatment and vaccine development. For easy reference, all the T cell epitopes identified are summarized in Table 1.

CHARACTERIZATION OF T CELL EPITOPES IN THE SPIKE (S) GLYCOPROTEIN

Amongst the SARS-CoV structural proteins, the S protein has been found to elicit neutralizing antibodies17,18 with its major immunodominant epitope found between residues 441 to 700. Using an online database and with verification from T2 binding assays, the first two HLA-A*02:01-restricted T cell epitopes (S1203–1211 and S978–986) were identified in the S protein of SARS-CoV.19 They were immunogenic and elicited high IFNγ-specific T cell response in patients who have recovered from SARS. In comparison, the homologous peptide from HCoV229e did not elicit a significant response. A third CTL epitope, S1167–1175 (also known as SSp-1) was reported shortly after HCoV229e infection.20 Interestingly, 5 healthy individuals without contact history with inactivated SARS-CoV particles elicited CTL response to all three S epitopes when compared to the recovered SARS patients.21

Table 1 Summary of T cell epitopes found in the SARS-CoV

| Protein       | Amino acid position | HLA restriction | Identification | References |
|---------------|---------------------|-----------------|----------------|------------|
| Spike         | 1203 to 1211        | HLA-A*02:01     | Human PBMCs    | 19         |
| Spike         | 978 to 986          | HLA-A*02:01     | Human PBMCs    | 19         |
| Spike         | 1167 to 1175        | HLA-A*02:01     | Transgenic mouse and verified in human PBMCs | 20 |
| Spike         | 787 to 795          | HLA-A*02:01     | Human PBMCs    | 22         |
| Spike         | 1042 to 1050        | HLA-A*02:01     | Human PBMCs    | 22         |
| Spike         | 411 to 420          | HLA-A*02:01     | Human PBMCs    | 23         |
| Spike         | 958 to 966          | HLA-A*02:01     | Transgenic mouse and verified in human PBMCs | 24 |
| Nucleocapsid  | 223 to 231          | HLA-A*02:01     | Transgenic mouse | 22         |
| Nucleocapsid  | 227 to 235          | HLA-A*02:01     | Transgenic mouse | 22         |
| Nucleocapsid  | 317 to 325          | HLA-A*02:01     | Transgenic mouse and verified in human PBMCs | 22 |
| Nucleocapsid  | 331 to 347          | HLA-A*02:01     | Human PBMCs    | 28         |
| Nucleocapsid  | 346 to 362          | HLA-A*02:01     | Human PBMCs    | 28         |
| Nucleocapsid  | 211 to 235          | HLA-A*02:01     | Human PBMCs    | 32         |
| Nucleocapsid  | 330 to 354          | HLA-A*02:01     | Human PBMCs    | 32         |
| Nucleocapsid  | 216 to 225          | HLA-B*40:01     | Human PBMCs    | 33, 34     |
| Membrane      | 21 to 44            | ND              | Human PBMCs    | 41         |
| Membrane      | 65 to 91            | ND              | Human PBMCs    | 41         |
| Membrane      | 117 to 140          | ND              | Human PBMCs    | 41         |
| Membrane      | 200 to 220          | ND              | Human PBMCs    | 41         |
| Membrane      | 146 to 160          | ND              | Human PBMCs    | 34         |
| 3a            | 36 to 50            | ND              | Human PBMCs    | 34         |
| 3a            | 6 to 20             | HLA-B*58:01     | Human PBMCs    | Unpublished|

*ND indicates not determined.

Other T cell epitopes identified include S787–795, S1042–1050 (found in the S2 domain) and S411–420 (P15) (found in the S1 domain).22,23 These epitopes were found to be immunogenic and able to induce strong IFNγ production from PBMCs of recovered SARS patients. At the same time, HLA-A*02:01 transgenic mice immunized with DNA vaccines encoding the S protein were able to induce significant peptide-specific response.24 In this study, the HLA-A*02:01 restricted epitope S958–966 (also known as Sp8), first identified based on HLA-A*02:01 binding peptide and proteosomal cleavage prediction systems, was found capable of inducing specific CTLs in the PBMCs of healthy individuals as well as in transgenic mice immunized with S DNA vaccine.24 This suggests that there may already be SARS-CoV-specific CTL precursor cells within the T-cell repertoire of healthy individuals.

Animal studies using mice primed intramuscularly with S DNA vaccine and boosted with subcutaneous HLA-A*02:01 restricted peptides25 or with the S DNA vaccine alone26 elicited antigen-specific CD8+ T cell responses. In fact, one recent study showed that prime-boost immunization of transgenic mice with 5 of the HLA-A*02:01 S peptides together with CpG oligodeoxynucleotide (ODN) could significantly enhance the frequency of peptide-specific CD8+ T cells.27 Taken together, the S protein is not only capable of inducing neutralizing antibodies but also contains several immunogenic T cell epitopes. Some of these epitopes found in either the S1 or S2 domain of the protein should therefore be considered during SARS-CoV vaccine development.

CHARACTERIZATION OF T CELL EPITOPES IN THE NUCLEOCAPSID (N) PROTEIN

Besides the S glycoprotein, persistently high levels of anti-N protein antibodies and T cell responses were also found in the SARS-recovered individuals 2 years post-infection.28,29 For other coronaviruses, some protective effects were found to be conferred through N-specific CD8+ T cells.30,31 Using a similar approach of HLA peptide binding prediction algorithm with validation from T2-cell binding assay, Tsao...
et al. identified several HLA-A*02:01 restricted epitopes in the N protein (peptide N223–231, N227–235 and N317–325) and showed that they could induce specific CTL responses in transgenic mice immunized with N proteins or peptides with CpG ODN. In addition, peptide N317–325 was able to stimulate the recall of CD8+ T cell response in PBMCs of recovered SARS patients.

There had been numerous attempts to screen for CTL epitopes in the N protein through the use of overlapping peptides spanning the entire N protein. One such study that used PBMCs from recovered SARS patients 2 years post-infection has revealed that the major dominant antigenic site of the N protein lies in the C-terminal region (amino acids 331 to 362). At least 2 different T cell epitopes (N331–347 and N346–362) have been found in this region when the PBMCs were stimulated with a pool of 57 overlapping N peptides in vitro, followed by IFN-γ Enzyme-linked immunosorbent spot (ELISPOT) assay. Using the same approach, another group identified 2 potential CTL epitopes at positions N211–235 and N330–354 in the N protein. More recently, we also identified the same dominant response (N216–230) in SARS-recovered patients 6 years post-infection. This response was observed in 19% [3/16] of our cohort of recovered SARS patients. Similarly, a comprehensive study of T cell responses against all the SARS-CoV proteins conducted by Li et al. showed that 11% of their SARS subjects gave positive T cell responses against peptide N211–225, and it was identified as the most recognized epitope in the N protein. Exact epitope mapping by our group further indicated that the CTL epitope was a 10-mer (N216–225) restricted by HLA-B*40:01 and that PBMCs from healthy individuals can be transduced to become N peptide-specific T cells.

In one of the first animal studies conducted in monkeys, adenoviral-based vectors were used to test the efficacy of the S, M and N proteins. The monkeys were injected intramuscularly with adenoviral-based vectors that expressed codon-optimized S1 domain, M and N proteins. The S1 domain of the S protein was found to induce strong humoral response, while the N protein elicited high frequency of IFN-γ-producing T cells as determined using N peptides as the antigen in the ELISPOT assay. This was the first indication that the N protein could be a good vaccine candidate for cell-mediated T cell response. This phenomenon was also found in mice where DNA vaccines encoding the N protein elicited good T cell responses. C3H/He mice intramuscularly immunized with N protein pcDNA-3 vector showed both high antibody titre and CTL activity after 3 injections; and using Balb/c mice, two other groups showed that DNA vaccines encoding N protein alone could elicit T cell proliferation, IFN-γ release, delayed-type hypersensitivity (DTH) and in vivo cytotoxic T cell activity. Further experiments reported enhanced T cell response when calreticulin (CRT)-linked DNA vaccine was used or when DNA vaccination was performed with the addition of a chemical adjuvant levamisole. Synthetic N peptides coupled to the surface of liposomes were also reported to enhance T cell response. These synthetic N peptides not only induced CTL response, but the mice were also able to clear vaccinia virus-expressing SARS-CoV epitopes when challenged.

In summary, several different studies have identified immunogenic regions in amino acids 211 to 362 of the N protein to contain T cell epitopes. However, to date, the only epitope characterized in detail is the 10-mer epitope (N216–225) which is restricted by HLA-B*40:01.

CHARACTERIZATION OF T CELL EPITOPES IN OTHER SARS-COV PROTEINS

There are very few studies of T cell response to other SARS-CoV proteins. Nonetheless, animal studies using DNA vaccines suggest that the M protein may induce T cell response, albeit to a lesser degree than the S and N proteins. Yang et al. demonstrated that it was possible to induce recall T cell response from the PBMCs of SARS patients who have recovered for more than 1 year by using overlapping peptides spanning the entire M protein. In this study, four human T cell immunodominant peptides, M21–44, M65–91, M117–140 and M200–220, were subsequently identified. Similarly, Li et al. also reported that 9% of their SARS subjects had T cell response against the M peptide region, M146–160. The largest accessory protein of SARS-CoV is the 3a protein of 274 amino acids. However, other than Li et al.’s report, there had been no demonstration of T cell responses against this protein. The 3a protein peptide 3a36–50 was one of the three most frequently recognized T cell epitopes identified in their study. Similar to the results reported by Li et al. our data showed that the 3a protein peptide 3a6–20 was able to elicit both CD8+ and CD4+ responses. Interestingly, mice immunized with 3a DNA vaccine were shown to have high levels of humoral response as well as Th1 response. These observations indicated that the accessory 3a protein is immunogenic and able to induce T cell response.

Although T cell response could be observed for the M protein, current studies seem to suggest that the 3a protein is more immunogenic in comparison, and T cell epitopes identified in it may play an important role in recovery from a primary SARS-CoV infection and in vaccine development.

LONGEVITY AND PHENOTYPE OF CD4+ AND CD8+ T CELL RESPONSES

To date, there is only one study that investigated T cell response against whole SARS-CoV in humans. In this study, PBMCs from 1-year post-infected patients showed T cell response to eight (replicate, S, N, E, M, 3a, 3b, and 9b) out of the fourteen SARS-CoV proteins when tested using overlapping peptides spanning the entire SARS-CoV genome. Of the 70% of T cell responses elicited against the structural proteins, the S protein induced the most dominant responses (41%). In fact, the three most commonly recognized T cell epitope were that of one found in 3a, and the other two in the S protein (S435–451 and S633–650). The latter were not reported in the other studies. Although both CD4+ and CD8+ T cell responses were observed in the study, the frequency and magnitude of the CD8+ T cell responses were greater than the CD4+ T cell responses. However, the CD4+ and CD8+ T cells were found to have similar central memory phenotypes (CD45RA−CCR7−CD62L−). A separate study by Peng et al. showed that the N-specific CD4+ T cells had central memory (CD45RA−CCR7−CD62L−), whereas most of the CD8+ T cells had effector memory (CD45RA−CCR7−CD62L−) phenotype. Similar observations were made with the M-specific CD4+ and CD8+ T cells, whilst the S-specific CD8+ T cells were reported to have effector memory (CD45RA+CCR7−CD62L−) phenotype.

Using peptides in the four SARS-CoV structural proteins, an analysis of the memory T cell response in recovered SARS patients four years post-infection revealed that both CD4+ and CD8+ T cells produced IFN-γ. In support of Li et al.’s study, Fan et al. also found that CD4+ memory T cells produce IL-2, TNF-α and IFN-γ, with the exception of one patient. It was also observed that S peptides induced the highest percentages of IFN-γ producing cells. Interestingly, the frequency of these polyfunctional CD4+ T cells (T cells producing multiple cytokines) was higher in the individuals with severe SARS infection than in moderately severe patients. On the other hand, this difference between moderate severe and severe patients was...
not observed with the CD8\(^+\) T cells which produced mainly IFN\(\gamma\). Nonetheless, a proportion of the CD8\(^+\) T cells were found to produce TNF\(\alpha\) and granulocyte-degranulate (with the detection of CD107a).

Since polyfunctional T cells were associated with better control of human immunodeficiency virus (HIV) infection and vaccination efficacy,\(^{44,45}\) we further characterized the cytokine profile of the SARS-specific T cells in our recent study.\(^{54}\) A summary of our findings is shown in Figure 1. We have observed that the majority of CD4\(^+\) T cells produced IFN\(\gamma\), IL-2, and TNF\(\alpha\), with a small percentage of the cells also simultaneously producing inflammatory cytokines such as macrophage inflammatory protein (MIP) 1\(\alpha\), MIP 1\(\beta\) and granulocyte-macrophage colony-stimulating factor (GM-CSF). Of these, the majority of the CD8\(^+\) T cells produced IFN\(\gamma\), TNF\(\alpha\), MIP 1\(\alpha\) or MIP 1\(\beta\) alone or in combination. Only a small percentage produced IFN\(\gamma\), IL-2, and TNF\(\alpha\). Moving forward, we cloned the \(\alpha\) and \(\beta\) T cell receptor (TCR) chains of one immunodominant CTL epitope in the N protein (amino acid 216 to 225) from the SARS-CoV specific CD8\(^+\) T cells and used them to redirect the specificity of lymphocytes of healthy subjects lacking SARS-CoV specific memory T cells. These TCR-redietcted T cells were found to possess a cytokine production profile similar to SARS-CoV specific memory CD8\(^+\) T cells in recovered SARS patients (as mentioned above). Thus we proposed that these T cells may be potential therapeutic treatments for this life threatening infection.

Despite the numerous reports describing the elevation of inflammatory cytokines in primary infected patients (reviewed by Zhu et al.\(^{46}\)), it is not known if these cytokines are beneficial or contribute to the pathogenicity of the infection. Moreover, there is currently no report confirming the protective effect of T cells during a primary SARS-CoV infection in humans. In fact, research in this area is hampered by the lack of systematic sample collection during the 2003 SARS outbreak which lasted for a relatively short period of ~16 weeks. Since there is no second major outbreak of SARS, the protective effect of memory T cell response in recovered SARS patients is not known. Nevertheless, the phenotype and cytokine profile of the T cells in these recovered individuals indicate the possible protective effect of T cell response in the initial infection or during any subsequent infections.\(^{51-53}\)

**HLA ASSOCIATION**

The association of certain HLA genotypes with increased resistance or the ability to clear viral infections have been reported in hepatitis C virus (HCV) and human papillomavirus studies.\(^{47-50}\) Although earlier studies done on SARS patients from Taiwan and Hong Kong suggested that the HLA-B, HLA-Cw and HLA-DR alleles were highly associated with SARS infection and disease development,\(^{51-53}\) further investigation is required. Of these literature, SARS individuals from Hong Kong showed that HLA-B*07:03 and HLA-DR*03:01 conferred factors for susceptibility and resistance to SARS infection, respectively.\(^{54}\) In agreement with this, a study on a Taiwanese cohort of SARS patients found that both HLA-Cw*15:02 and HLA-DR*03:01 were associated with resistance to SARS infection.\(^{55}\) These observations suggested the important role of HLA-DR*03:01 in viral disease progression through enhancing the function of CD4\(^+\) T helper cells. Similarly, we observed that the CD8\(^+\) T cell responses against both the N and S\(\alpha\) proteins were all restricted by HLA-B subtype (unpublished data), thus pointing to the possible role of HLA-B subtypes in viral immunity. Among the HLA class I genes, HLA-B is known to be the most polymorphic,\(^{56}\) and was associated in protective roles against the HIV,\(^{57-58}\) HCV\(^{59}\) and acute influenza infections.\(^{60}\)

**CONCLUSION**

Currently, no antiviral therapy has yet been proven useful for SARS. Attempts to test potential anti-SARS agents using antiviral antibodies, entry inhibitors, proteinase inhibitors, calpain inhibitors, ribavirin (nucleoside analogues), interferons, and short interfering RNAs were riddled with contradictory reports from different laboratories. The lack of clinical trials also prevented the reaching of a conclusive agreement for effective anti-SARS strategies (reviewed by Weiss et al.\(^{61}\)). Nevertheless, human convalescent-phase plasma seemed to shorten hospitalization without adverse effects if it is administered as an immunotherapy to SARS patients early in the course of infection.\(^{62}\) With the finding that recovered SARS patients have higher and more sustainable levels of neutralizing antibodies when compared to those who had succumbed to the disease,\(^{63}\) monoclonal antibodies for passive immunization were also obtained using phase-display antibody libraries and immortalization of B cells from convalescent SARS patients.\(^{64,65}\)

Although it is still not known whether naturally acquired immune responses can confer protection from re-infection of SARS-CoV, vaccines are likely to be the most effective way to provide protection against a future re-emergence of SARS-CoV. Several strategies for vaccine development included DNA vaccines, inactivated whole virus vaccines,\(^{66,67}\) virus-like particles,\(^{68,69}\) recombinant virus vector vaccines,\(^{70}\) and recombinant protein vaccine.\(^{71}\) Most SARS-CoV vaccines that elicited neutralizing antibodies are believed to be protective, but as described, T cells may also play an important role in viral clearance in a primary SARS-CoV infection.\(^{72,73}\) Zhao et al. suggested that inefficient immune activation and a poor virus-specific T cell response underlay severe disease in SARS-CoV infected mice.\(^{74}\) In their recent report, they showed that virus-specific T cells were necessary and sufficient for virus clearance and protection from clinical disease in mouse-adapted SARS-CoV (MA15) virus-infected mice.\(^{75}\) In addition, CD4\(^+\) T cells in a senescent mouse model were found to play an important role in viral clearance in a primary infection with SARS-CoV.\(^{72}\) In humans, SARS-CoV-specific memory T cells were found to persist in the peripheral blood of SARS patients up to 6 years.

**Figure 1** Diagram showing the cytokine profiles of the CD4\(^+\) and CD8\(^+\) T cells from SARS recovered patients. The big and small arrows indicate the major and minor populations of CD4\(^+\) and CD8\(^+\) T cells producing the cytokines indicated respectively.
post-infection despite a lack of specific memory B cell response in these patients. This seems to suggest that SARS-CoV-specific T cell response could persist longer and thus indicating that cell-mediated immune response is important for protecting against re-infection. As all these studies suggest that T cell may play a crucial role in the clearance of SARS-CoV, there is therefore a need for detailed characterization of the T cell response to SARS-CoV for the development of future vaccine candidates.

Finally, it is important to note that T cells can play a protective and/or pathological role during an infection. In the case of mouse-hepatitis virus (MHV), an increase of morbidity and mortality in infected mice has been associated with memory T cells. Although no direct evidence have shown that SARS-CoV-specific T cell responses contribute to immunopathology in SARS, it is a question that needs to be further addressed.

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1 Zhong NS, Zheng BJ, Li YM, Poon, Xie ZH, Chan KH et al. Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People’s Republic of China, in February, 2003. Lancet 2003; 362: 1353–1358.
2 WHO. Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003. 2003. Available at http://www.who.int/csr/sars/country/ table2003_09_23/en/.
3 Peiris JS, Lai ST, Poon LL, Guan Y, Yern YL, Lim W et al. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet 2003; 361: 1319–1325.
4 Drosten C, Günther S, Preiser W, van der Werf S, Brodt HR, Becker S et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med 2003; 348: 1967–1976.
5 Ksiaztek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S et al. Novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med 2003; 348: 1953–1966.
6 Guan Y, Peiris JS, Zheng B, Poon LL, Chan KH, Zeng FY et al. Molecular epidemiology of the novel coronavirus that causes severe acute respiratory syndrome. Lancet 2004; 363: 99–104.
7 Lau SK, Woo PC, Li KS, Huang Y, Tsoi HW, Wong BH et al. Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. Proc Natl Acad Sci USA 2005; 102: 14040–14045.
8 Li W, Shi Z, Yu M, Ren W, Smith C, Epstein JH et al. Bats are natural reservoirs of SARS-like coronaviruses. Science 2005; 310: 676–679.
9 Snijder EJ, Bredepenk PJ, Dobbe JC, Thiel V, Ziebuhr J, Poon LL et al. Unique and conserved features of genome and proteome of SARS-coronavirus, an early split-off from the coronavirus group 2 lineage. J Mol Biol 2003; 331: 991–1004.
10 Rota PA, Oberste MS, Monroe SS, Nix WA, Campagnoli R, ledgele JP et al. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science 2003; 300: 1394–1399.
11 Marra MA, Jones SJ, Astell CR, Holt RA, Brooks-Wilson A, Butlerfield YS et al. The Genome sequence of the SARS-associated coronavirus. Science 2003; 300: 1399–1404.
12 Ziebuhr J, Snijder EJ, Gorbaley AE. Virus-encoded proteinases and proteolytic processing in the Nidovirales. J Gen Virol 2000; 81: 853–879.
13 Cheng VC, Lau SK, Woo PC, Yuen KY. Severe acute respiratory syndrome coronavirus as an agent of emerging and reemerging infection. Clin Microbiol Rev 2007; 20: 660–694.
14 Tan YJ, Lim SG, Hong W. Understanding the accessory viral proteins unique to SARS coronavirus. J Virol 2004; 78: 78–88.
15 Narayanan K, Huang C, Makino S. SARS coronavirus accessory proteins. Virus Res 2008; 133: 113–121.
16 Hsieh PR, Huang LM, Chen PJ, Kao CL, Yang PC. Chronological evolution of IgM, IgG and neutralisation antibodies after infection with SARS-associated coronavirus. Clin Microbiol Infect 2004; 10: 1062–1066.
17 Buchholz UJ, Bukreyev A, Yang L, Lamanide EW, Murphy BR, Subbarao K et al. Contributions of the structural proteins of severe acute respiratory syndrome coronavirus to protective immunity. Proc Natl Acad Sci USA 2004; 101: 9804–9809.
18 Lu L, Manopa I, Leung BP, Ching HH, Ling AE, Chee LL et al. Immunological characterization of the spike protein of the severe acute respiratory syndrome coronavirus. J Clin Microbiol; 42: 1570–1576.
49 Thio CL, Thomas DL, Goedert JJ, Vlahov D, Nelson KE, Hilgarter MW et al. Racial differences in HLA class II associations with hepatitis C virus outcomes. J Infect Dis 2001; 184: 16–21.

50 Hu W, Giri SN, Bunce M, Satsangi J, Collier J, Chapman R et al. Effect of HLA class II genotype on T helper lymphocyte responses and viral control in hepatitis C virus infection. J Viral Hepat 2001; 8: 174–179.

51 Lin M, Tseng HK, Trejaut JA, Lee HL, Loo JH, Chu CC et al. Association of HLA class I with severe acute respiratory syndrome coronavirus infection. BMC Med Genet 2003; 4: 9.

52 Chen YM, Liang SY, Shih YP, Chen CY, Lee YM, Chang L et al. Genetic correlates of severe acute respiratory syndrome coronavirus infection in the hospital with the highest nosocomial infection rate in Taiwan in 2003. J Clin Microbiol 2006; 44: 359–365.

53 Robinson J, Malik A, Parham P, Bodmer JG, Marsh SG. IMGT/HLA database—a sequence database for the human major histocompatibility complex. Tissue Antigens 2000; 55: 280–287.

54 Wang SF, Chen KH, Chen M, Li WY, Chen YJ, Tsao CH et al. Human-Leukocyte Antigen Class I (Cow 1502) and Class II (DRB1*0301) genotypes with susceptibility and resistance to the development of severe acute respiratory syndrome. J Infect Dis 2006; 196: 515–518.

55 Ng MH, Lau KM, Li L, Cheng SH, Chan WY, Hui PK et al. Neutralization of SARS coronavirus. J Med Microbiol 2004; 53: 871–875.

56 Carrington M, O’Brien SJ. The influence of HLA genotype on AIDS. Annu Rev Med 2003; 54: 535–551.

57 Kielpe P, Leslie AJ, Honeyborne I, Ramduth D, Thobakgale C, Chetty S et al. Dominant influence of HLA-B in mediating the potential co-evolution of HIV and HLA. Nature 2004; 432: 769–775.

58 Bhih F, Frahm N, Di Gianmarino L, Sidney J, John M, Yusim K et al. Impact of HLA-B alleles, epitope binding affinity, functional avidity, and viral coinfection on the immunodominance of virus-specific CTL responses. J Immunol 2006; 176: 4094–4101.

59 Neumann-Haefelin C, McKiernan S, Ward S, Viazov S, Spangenberg HC, Kilinger T et al. Dominant influence of an HLA-B27 restricted CD8+ T cell response in mediating HCV clearance and evolution. Hepatology 2006; 43: 563–572.

60 Boon AC, De Mutsert G, Fouchier RA, Sintnicolaas K, Osterhaus AD, Rimmelzwaan GF. Preferential HLA usage in the influenza virus-specific CTL response. J Immunol 2004; 641–650.

61 Weiss SR, Navas-Martin S. Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. Microbiol Mol Biol Rev 2005; 69: 635–664.

62 Soo YO, Cheng Y, Wong R, Hui DS, Lee CK, Tsang KK et al. Retrospective comparison of convalescent plasma with continuing high-dose methylprednisolone treatment in SARS patients. Clin Microbiol Infect 2004; 10: 676–678.

63 Zhang L, Zhang F, Yu W, He T, Yu J, Yi CE et al. Antibody responses against SARS coronavirus in patients with disease outcome of infected individuals. J Med Virol 2006; 78: 1–8.

64 Traggiai E, Becker S, Subbarao K, Kolesnikova L, Uematsu Y, Gismondo MR et al. An efficient method to make human monoclonal antibodies from memory B cells: potent neutralization of SARS coronavirus. Nat Med 2004; 10: 871–875.

65 Sui J, Li W, Murakami A, Tamin A, Matthews LJ, Wong SK et al. Potent neutralization of severe acute respiratory syndrome (SARS) coronavirus by a human mAb to S1 protein that blocks receptor association. Proc Natl Acad Sci USA 2004; 101: 2536–2541.

66 Zhou J, Wang W, Zhong Q, Heu W, Yang Z, Xiao SY et al. Immunogenicity, safety, and protective efficacy of an inactivated SARS-associated coronavirus vaccine in rhesus monkeys. Vaccine 2005; 23: 3202–3209.

67 Spruth M, Kistner O, Savids-Dach H, Hitter E, Crowe B, Gerencer M et al. A double-inactivated whole virus candidate SARS coronavirus vaccine stimulates neutralising and protective antibody responses. Vaccine 2006; 24: 652–661.

68 Liu YY, Massare MJ, Barnard DL, Kort T, Nathan M, Wang L et al. Chimeric severe acute respiratory syndrome coronavirus (SARS-CoV) S glycoprotein and influenza matrix 1 efficiently form virus-like particles (VLPs) that protect mice against challenge with SARS-CoV. Vaccine 2011; 29: 6606–6613.

69 Lu B, Huang Y, Huang L, Li B, Zheng Z, Chen Z et al. Effect of mucosal and systemic immunization with virus-like particles of severe acute respiratory syndrome coronavirus in mice. Immunology 2010; 130: 254–261.

70 See RH, Zakhartchouk AN, Petric M, Lawrence DJ, Mok CP, Hogan RJ et al. Comparative evaluation of two severe acute respiratory syndrome (SARS) vaccine candidates in mice challenged with SARS coronavirus. J Gen Virol 2006; 87: 641–650.

71 Bisht H, Roberts A, Vogel L, Subbarao K, Moss B. Neutralizing antibody and protective immunity to SARS coronavirus infection of mice induced by a soluble recombinant polyepitope containing an N-terminal segment of the spike glycoprotein. Virology 2005; 334: 160–165.

72 Chen J, Lau YF, Lamirande EW, Paddock CD, Bartlett JH, Zaki SR et al. Cellular immune responses to severe acute respiratory syndrome coronavirus (SARS-CoV) infection in senescent BALB/c mice: CD4+ T cells are important in control of SARS-CoV infection. J Virol 2010; 84: 1289–1301.

73 Zhao J, Perlmans S. T cell responses are required for protection from clinical disease and for virus clearance in severe acute respiratory syndrome coronavirus-infected mice. J Virol 2010; 84: 9318–9325.

74 Zhao J, Van Rooijen N, Perlmans S. Evasion by stealth: inefficient immune activation underlies poor T cell response and severe disease in SARS-CoV-infected mice. PLoS Pathog 2009; 5: e1000636.

75 Tang F, Qian Y, Xin ZT, Wrammert J, Ma MJ, Lu H et al. Lack of peripheral memory B cell responses in recovered patients with severe acute respiratory syndrome: a six-year follow-up study. J Immunol 2011; 186: 7264–7268.

76 Khanolkar A, Hartwig SM, Haag BA, Meyerholz DK, Epping LL, Haring JS et al. Protective and pathologic roles of the immune response to mouse hepatitis virus type 1: implications for severe acute respiratory syndrome. J Virol 2009; 83: 9268–9272.