Persistent memory CD4+ and CD8+ T-cell responses in recovered severe acute respiratory syndrome (SARS) patients to SARS coronavirus M antigen

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The membrane (M) protein of severe acute respiratory syndrome coronavirus (SARS-CoV) is a major glycoprotein with multiple biological functions. In this study, we found that memory T cells against M protein were persistent in recovered SARS patients by detecting gamma interferon (IFN-γ) production using ELISA and ELISpot assays. Flow cytometric analysis showed that both CD4+ and CD8+ T cells were involved in cellular responses to SARS-CoV M antigen. Furthermore, memory CD8+ T cells displayed an effector memory cell phenotype expressing CD45RO-CCR7-CD62L+. In contrast, the majority of IFN-γ+CD4+ T cells were central memory cells with the expression of CD45RO+CCR7+CD62L-. The epitope screening from 30 synthetic overlapping peptides that cover the entire SARS-CoV M protein identified four human T-cell immunodominant peptides, p21-44, p65-91, p117-140 and p200-220. All four immunodominant peptides could elicit cellular immunity with a predominance of CD8+ T-cell response. This data may have important implication for developing SARS vaccines.

INTRODUCTION

Severe acute respiratory syndrome (SARS) is a newly emerged infectious disease that led to thousands of infected patients and hundreds of deaths. The aetiological agent of the syndrome, a novel coronavirus termed SARS coronavirus (SARS-CoV), has been identified (Drosten et al., 2003; Ksiazek et al., 2003). Although the epidemic was eventually controlled, the high morbidity and mortality associated with SARS make it imperative to develop effective vaccines to prevent this disease.

The immune system plays a vital role in defence against SARS-CoV infection, since none of the drugs employed to treat SARS is able to inhibit viral replication in vivo. Strong humoral responses have been found in most SARS patients following SARS-CoV infection, with high titres of neutralizing antibodies (Abs) present in their convalescent sera (Li et al., 2003). Sera from recovered SARS patients could be used to treat newly infected individuals (Soo et al., 2004), and passive transfer immune serum can prevent replication of SARS-CoV in a mouse model (Subbarao et al., 2004), confirming the protective nature of the anti-SARS-CoV serum Abs. UV-inactivated SARS-CoV vaccine was able to elicit a high titre of antibody responses in mice (Takasuka et al., 2004). Recent studies have demonstrated that vaccines based on the S protein of SARS-CoV seem to induce a considerable neutralizing antibody response and protective immunity (Yang et al., 2004; Bisht et al., 2004; Bukreyev et al., 2004). In addition to humoral response, T cells are essential for adaptive immunity against viral infection. Antiviral CD4+ T cells help the production of virus-specific Abs by B cells, while CD8+ cytotoxic T lymphocytes (CTLs) can kill virus-infected host cells.

The genome of SARS-CoV encodes four major structural components including the spike protein (S), the nucleocapsid protein (N), the envelop protein (E) and the membrane protein (M). To date, more attention has been focused on the antibody response and cellular immunity induced by the S and N proteins (Yang et al., 2004; Bisht et al., 2004; Bukreyev et al., 2004; Gao et al., 2003). Many studies suggest that the S protein of SARS-CoV is a promising antigen for developing SARS vaccines since it can elicit protective humoral immunity (Yang et al., 2004; Bisht et al., 2004) and that the N protein may serve as an ideal antigen for SARS diagnosis since it can induce an
appreciable antibody response in SARS patients (Liu et al., 2004). In addition, human leukocyte antigen (HLA)-A2-restricted CD8+ T-cell epitopes have been identified in the S protein (Wang et al., 2004a, b; Zhi et al., 2005). We have demonstrated recently that memory CD4+ and CD8+ T cells specific for SARS-CoV S, E and N antigens from recovered SARS patients were persistent for a long period of time (Yang et al., 2006; Peng et al. 2006a, b). However, limited information is available on the immune response to the M protein of SARS-CoV.

The M protein of SARS-CoV is a major transmembrane glycoprotein of 220 aa. The interaction between the M protein and the S protein, as well as the N protein, is essential for viral assembly and budding. Recently, antibodies to the M protein that were detectable in convalescent-phase SARS patients and B-cell epitopes of the M protein have been identified (He et al., 2005). Although it has been demonstrated that DNA vaccine encoding the M protein of SARS-CoV can elicit antigen-specific T-cell responses in animal models (Jin et al., 2005; Wang et al., 2005), the protective effect of T cells directed to the M protein in humans remains undefined. Thus, it is essential to determine whether recovered SARS patients generate cell-mediated immunity against SARS-CoV and how long this immunity will persist. In this study, we analysed CD4+ and CD8+ T-cell responses to a pool of 30 overlapping SARS-CoV M peptides in recovered SARS patients, and characterized the phenotype of memory T-cell subpopulations, as well as sought to identify the immunodominant T-cell peptides on the M protein of SARS-CoV. Our study demonstrated that SARS-CoV M-specific memory CD4+ and CD8+ T cells were persistent in the peripheral blood of recovered SARS patients more than 1 year after infection. These memory T cells revealed effector and central memory phenotypes. Additionally, four human T-cell immunodominant peptides from the M protein of SARS-CoV were identified by eliciting gamma interferon (IFN-γ) production.

**METHODS**

**Donors.** Six fully recovered SARS patients, comprising four males and two females aged 25 to 32 years, worked in the Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China. All were infected with SARS-CoV between March and April 2003, these studies were performed 12–23 months after recovery. Four healthy donors, comprising two males and two females, whose ages ranged from 27 to 33 years, without any contact history with SARS-CoV, acted as healthy controls.

**Synthetic peptides.** A total of 30 peptides, spanning the entire sequence of SARS-CoV M protein, were kindly provided by the Vaccine Research Center, National Institute of Allergy and Infectious Disease, National Institutes of Health, USA. The peptides range from 15 to 18 aa in length overlapped by 10–11 aa.

**Reagents.** Anti-CD4 PerCP (peridinin chlorophyll protein) and anti-CD8 PerCP were purchased from BD Biosciences Immunocytometry Systems. Mouse anti-human CD28, anti-human CD49d, anti-CD4 FITC, anti-CD8 PE-Cy7 (cyanin 7), anti-CD45RO FITC, anti-CCR7 PE (phycoerythrin), anti-CD62L PE, anti-IFN-γ PE, anti-IFN-γ APC (allophycocyanin), anti-interleukin (IL)-2 APC and isotype-matched control antibodies were obtained from BD Bioscience Pharmingen. RPMI 1640 medium, penicillin, streptomycin and 2-mercaptoethanol were purchased from GIBCO.

**Cell preparation.** Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood that was mixed with an equal volume of Hank’s balanced salt solution using Ficoll–Hypaque density gradient centrifugation. The cells were washed twice in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS) (HyClone), 100 U penicillin ml−1, 100 μg streptomycin ml−1 and 50 μM 2-mercaptoethanol. The cells were adjusted to a final concentration of 2 × 10^6 ml−1 in complete RPMI 1640 medium.

**Cell culture conditions and IFN-γ detection by ELISA.** Fresh PBMCs were isolated from recovered SARS patients and healthy donors, suspended in complete RPMI 1640 culture medium and stimulated with a pool of 30 overlapping SARS-CoV M peptides (1 μg ml−1) in the presence of 1 μg anti-CD28 ml−1 and 1 μg anti-CD49d ml−1 mAbs. The final concentration of each peptide was 1 μg ml−1. PBMCs were plated into a flat-bottomed 96-well plate at a total of 2 × 10^5 cells per well and incubated for 72 h at 37 °C with 5% CO2 in triplicate. The supernatants were harvested and assayed for IFN-γ production by ELISA according to the manufacturer’s protocol (BD Pharmingen). In each experiment, PBMCs were cultured with 1 μg anti-CD28 ml−1 plus 1 μg anti-CD49d ml−1 in the absence of SARS-CoV M peptides as a negative control. In addition, the cells were stimulated with 20 ng ml−1 phorbol-12-myristate-13-acetate (PMA) (Sigma-Aldrich) and 1 μg ionomycin ml−1 (Sigma-Aldrich) as a positive control.

**IFN-γ ELISpot assay.** Human IFN-γ ELISpot assays were performed using a commercially available set (BD Pharmingen) according to the manufacturer’s instructions. Briefly, 96-well nitrocellulose plates (Millipore) were coated overnight at 4 °C with 5 μg ml−1 anti-human IFN-γ capture antibody. The wells were then washed and blocked for 2 h at room temperature with 10% FCS-RPMI 1640 medium. A total of 2 × 10^6 PBMCs were added to each well and incubated for 20 h with a pool of SARS-CoV M peptides (1 μg ml−1), 1 μg anti-CD28 ml−1 and 1 μg anti-CD49d ml−1 in triplicate. Similarly, the cells supplemented with anti-CD28 (1 μg ml−1) and anti-CD49d (1 μg ml−1) in the absence of SARS-CoV M peptides were considered as negative controls, and the cells were cultured with polyclonal stimuli (20 ng PMA ml−1 plus 1 μg ionomycin ml−1) as positive controls. The plates were washed and subsequently incubated with 2 μg ml−1 biotinylated anti-human IFN-γ detection antibody for 2 h at room temperature. After washing, the wells were developed for 1 h with streptavidin-HRP, followed by addition of 3-amino-9-ethylcarbazole (AEC) substrate reagent. The spots were counted automatically using an ImmunoSpot image analyser (Cellular Technology). A spot represents an IFN-γ-producing cell. The average of spots in triplicate wells was calculated and considered as the number of specific spot-forming cells (SFC) per 2 × 10^5 cultured PBMCs.

**Cell surface, intracellular cytokine staining and flow cytometric analysis.** PBMCs were stimulated with or without a mixture of 30 overlapping SARS-CoV M peptides in the presence of 1 μg anti-CD28 ml−1 and 1 μg anti-CD49d ml−1 in 15 ml tubes. After the first 1 h incubation, 10 μg brefeldin A ml−1 (Sigma-Aldrich) was added to cultures to enable intracellular protein to accumulate in all stimulations. After incubation for a total of 5 h, the cells were washed, fixed, permeabilized using saponin (Sigma-Aldrich) and blocked with 25 μg human IgG ml−1 for 30 min at 4 °C. The cells were then stained with anti-CD4, anti-CD8, anti-IFN-γ, anti-IL-2, anti-CD45RO, anti-CCR7.
and anti-CD62L. After staining, all samples were washed twice in PBS buffer (containing 0.1 % saponin, 0.1 % BSA and 0.05 % NaN₃) and resuspended in 300 μl PBS for flow cytometry. More than 600,000 cells were acquired on a FACS Aria flow cytometer (Becton Dickinson) and FACS data were analysed using the CellQuest software (Becton Dickinson).

**Statistical analysis.** The levels of IFN-γ and numbers of IFN-γ-producing cells were compared using Student’s t-test between recovered SARS patients and healthy control donors in the same condition. P<0.05 was considered significant.

**RESULTS**

**Quantification of SARS-CoV M peptides-specific memory T cells**

To determine whether recovered SARS patients could maintain memory T-cell responses to the M protein of SARS-CoV more than 1 year after SARS-CoV infection, fresh PBMCs were isolated from fully recovered SARS patients and healthy donors and cultured with or without a pool of 30 SARS-CoV M peptides in the presence of anti-CD28 and anti-CD49d mAbs. After stimulation for 72 h, cell-free culture supernatants were harvested and assayed for the production of IFN-γ by ELISA. The results showed that PBMCs from healthy donors secreted very low levels of IFN-γ (0–78 pg ml⁻¹) when cultured with or without the SARS-CoV M peptides. Statistical analysis indicated that the production of IFN-γ between the two culture conditions was not significantly different (P>0.05) (Fig. 1a). In contrast, PBMCs from recovered SARS individuals produced significantly higher levels of IFN-γ (440±219 pg ml⁻¹) when stimulated with SARS-CoV M peptides (P<0.01) compared with the cells stimulated without SARS-CoV M peptides (50±31 pg ml⁻¹) (Fig. 1a). In addition, no marked difference in the production of IFN-γ between recovered SARS patients and healthy control individuals was observed when the PBMCs were stimulated with PMA plus ionomycin (P>0.05) (data not shown).

To assess the frequency of SARS-CoV M-specific T cells at the single-cell level, PBMCs were cultured in the presence or absence of a pool of 30 SARS-CoV M peptides. The frequency of IFN-γ-producing cells was detected by IFN-γ-specific ELISpot assay after stimulation for 20 h. As shown in Fig. 1(b), 2–5 IFN-γ-positive spots in 2×10⁵ PBMCs were detected in the cells without stimulation, either in healthy donors or in recovered SARS patients. The addition of SARS-CoV M peptides, however, induced high frequency of IFN-γ-secreting T-cell response in recovered SARS patients, with an average number of spot-forming cells (SFC) of 106 in 2×10⁵ cultured PBMCs (range from 36 to 180), which was significantly higher (P<0.01) than that in spot-forming cells in healthy donors (1–6 spots in 2×10⁵ PBMCs) when PBMCs were stimulated with SARS-CoV M peptides (Fig. 1b). Moreover, the PBMCs from recovered SARS patients revealed similar frequency of IFN-γ-forming cells (488 in 2×10⁵ PBMCs) compared with the PBMCs from healthy individuals (513 in 2×10⁵ PBMCs) when stimulated with PMA plus ionomycin (P>0.05) (data not shown). Taken together, SARS-CoV M-specific memory T cells persisted in peripheral blood of recovered SARS individuals and displayed the capacity to secrete IFN-γ upon subsequent stimulation with SARS-CoV M peptides in vitro.

![Fig. 1. SARS-CoV M peptides-specific IFN-γ production by PBMCs.](image-url)

PBMCs from healthy individuals (513 in 2×10⁵ PBMCs) when stimulated with PMA plus ionomycin (P>0.05) (data not shown). Taken together, SARS-CoV M-specific memory T cells persisted in peripheral blood of recovered SARS individuals and displayed the capacity to secrete IFN-γ upon subsequent stimulation with SARS-CoV M peptides in vitro.
Subpopulation of SARS-CoV M peptides-specific memory T cells

It is well known that cellular immune responses are mediated by CD4$^+$ and CD8$^+$ T cells. We next analysed IFN-$\gamma$-producing T-cell subpopulations that were involved in anti-SARS-CoV immunity by flow cytometry. PBMCs isolated from recovered SARS patients were stimulated with a pool of SARS-CoV M peptides (1 $\mu$g ml$^{-1}$) and co-stimulatory mAbs (1 $\mu$g anti-CD28 ml$^{-1}$ and 1 $\mu$g anti-CD49d ml$^{-1}$), in the presence of 10 $\mu$g brefeldin A ml$^{-1}$. After stimulation for 5 h, the cells were stained for surface expression of CD4 and CD8 and for intracellular IFN-$\gamma$ and IL-2, or their corresponding isotype controls. The results showed that addition of SARS-CoV M peptides to cultures resulted in the expression of IFN-$\gamma$ and IL-2 in both CD4$^+$ (Fig. 2a) and CD8$^+$ T-cell populations (Fig. 2b). In contrast, CD4$^+$ and CD8$^+$ T cells failed to secret IFN-$\gamma$ and IL-2 in the absence of SARS-CoV M peptides. In addition, the percentage of IFN-$\gamma$- and IL-2-producing CD4$^+$ and CD8$^+$ T cells was significantly higher in SARS-CoV M peptides-stimulated cells compared with that from un-stimulated cells ($P<0.05$) (Fig. 2c), whereas no difference was observed in the proportion of IL-2-producing CD8$^+$ T cells between those two groups ($P>0.05$). Based on the production of these two cytokines (IFN-$\gamma$ and IL-2), SARS-CoV M peptides-specific CD4$^+$ T cells could be divided into three distinct populations: IFN-$\gamma^+$ IL-2$^-$, IFN-$\gamma^+$ IL-2$^+$ and IFN-$\gamma^-$ IL-2$^+$ (Fig. 2d). Overall, these data demonstrated that both CD4$^+$ and CD8$^+$ memory T cells were generated and persisted more than 1 year after SARS-CoV infection, and that these cells displayed a heterogeneity of cytokine expression.

Fig. 2. Expression of intracellular IFN-$\gamma$ and IL-2 in PBMCs of recovered SARS patients after stimulation with SARS-CoV M peptides in vitro. Fresh PBMCs from recovered SARS patients were cultured for 5 h with a mixture of 30 SARS-CoV M peptides (1 $\mu$g ml$^{-1}$) and stained with anti-CD4, CD8, IFN-$\gamma$ and IL-2 mAbs. The expression of IFN-$\gamma$ and IL-2 by CD4$^+$ (a) and CD8$^+$ (b) T cells was analysed. The percentage (mean ± so) of IFN-$\gamma^-$ or IL-2-secreting CD4$^+$ and CD8$^+$ T cells from six recovered SARS individuals is shown (c) and the subsets of M peptides-specific CD4$^+$ T cells based on the expression of IFN-$\gamma$ and IL-2 (d) are also displayed. The numbers in each quadrant are indicated as percentages. Representative data from one out of six recovered SARS individuals with similar results are shown (d). Student’s t-test was used for statistical analysis. *, $P<0.05$; NS, not significant.
Characterization of memory CD4$^+$ and CD8$^+$ T cells specific for SARS-CoV M peptides

With regard to memory CD4$^+$ and CD8$^+$ T cells in humans, multiple phenotypes and a broad spectrum of functions have been reported in different viral infections. In this study, we also performed phenotypic characterization of antiviral T-cell responses specific for SARS-CoV M peptides on the basis of their ability to secrete IFN-γ and the expression of CD45RO, CCR7 and CD62L. Our results indicated that the total CD4$^+$ T cells expressed high levels of CCR7 (78%) and CD62L (75%) and that approximately 40% of CD4$^+$ T cells expressed CD45RO (Fig. 3a). In contrast, SARS-CoV M-specific IFN-γ-producing CD4$^+$ T cells had an increased expression of CD45RO (80%), but had decreased expression of CD62L (45%) compared with total CD4$^+$ T cells (Fig. 3a). Furthermore, most of antigen-specific CD4$^+$ IFN-γ$^+$ cells were CD45RO$^+$ CCR7$^+$ (66%) or CD45RO$^+$ CD62L$^-$ (42%) (Fig. 3a). A notable finding was that total CD8$^+$ T cells expressed low amounts of CCR7 and CD62L compared with total CD4$^+$ T cells (Fig. 3b). Furthermore, IFN-γ$^+$ CD8$^+$ T cells were predominantly CD45RO$^-$ (90%), and mainly defined as CD45RO$^-$ CCR7$^-$ (74%) or CD45RO$^-$ CD62L$^-$ (88%) (Fig. 3b). These results showed the heterogeneity of memory T cells and also demonstrated differences between memory CD4$^+$ and memory CD8$^+$ T-cell populations.

Mapping of immunodominant peptides on the M protein in recovered SARS patients

To identify human T-cell immunodominant peptides on the M protein, a set of 30 synthetic overlapping peptides that span the entire sequence of the M protein were used as antigens to induce T-cell responses detected by IFN-γ ELISpot assays. A total of $2 \times 10^5$ PBMCs from recovered SARS patients were incubated for 20 h with individual peptides (1 μg ml$^{-1}$) in the presence of co-stimulatory mAbs, 1 μg anti-CD28 ml$^{-1}$ and 1 μg anti-CD49d ml$^{-1}$.

Representative results from two recovered SARS patients are shown in Fig. 4, four regions with high T-cell immune reactivity were located at 13–44, 60–110, 117–164 and 176–220, respectively, in the entire sequence of the M protein (Fig. 4). In other words, the T-cell response was observed in cultures stimulated with peptides corresponding to aa...

**CD4+ and CD8+ T-cell immunodominant peptides of SARS-CoV M protein were identified by intracellular cytokine expression in recovered SARS patients**

As described above, p21-44, p65-91, p117-140 and p200-220 could effectively induce IFN-γ production following short-term stimulation *in vitro*. Next, we investigated CD4+ and CD8+ T-cell response to these peptides using flow cytometry assay. The results showed that peripheral blood CD4+ and CD8+ T cells in recovered SARS patients were able to produce IFN-γ when stimulated with all these peptides (Fig. 5).

**DISCUSSION**

In the current study, we found that antigen-specific memory T cells were capable of secreting high levels of IFN-γ by ELISA assay upon *in vitro* stimulation with a pool...
of SARS-CoV M peptides. These memory T cells persisted for more than 1 year in recovered SARS individuals. These data were further confirmed by IFN-γ ELISpot assays showing a high frequency of SARS-CoV M-specific IFN-γ-producing T cells. Flow cytometric analysis showed that both CD4+ and CD8+ T cells were involved in SARS-CoV M-specific cellular immunity with a predominance of CD8+ T-cell response.

It is well known that CD4+ T cells can be divided into two major subpopulations, Th1 and Th2, based on cytokine production and biological functions (Abbas et al., 1996). Th1 cells are heterogeneous IFN-γ+ and IFN-γ− cells. The IFN-γ− Th1 cells may differentiate into memory cells in vivo (Wu et al., 2002). Consistent with the observations in an animal model (Jin et al., 2005), we found that 30 overlapping SARS-CoV M peptides induced a strong Th1 type response, reflected by expressing high levels of IFN-γ and IL-2. Based on the production of IFN-γ and IL-2, SARS-CoV M-specific CD4+ T cells could be divided into three distinct populations: single IFN-γ-secreting cells, double IFN-γ/IL-2-secreting cells and single IL-2-secreting cells. IL-2 may play an important role in sustaining memory T cells and IFN-γ may mediate an effector function. Thus, IL-2+ T cells may be able to differentiate into long-term memory T cells, whereas the single IFN-γ+ T cells may be the effector and short-lived memory T cells. In agreement with other observations (Mallard et al., 2004; Zimmerli et al., 2005), SARS-CoV M-specific CD8+ T cells are not the major producer of IL-2. In this study, the majority of IFN-γ+ CD8+ T cells were CD45RO− CCR7+. This is supported by evidence that virus-specific IFN-γ/IL-2-secreting CD8+ T cells were CD45RA− CCR7+, whereas single IFN-γ-secreting CD8+ T cells were either CD45RA− CCR7− or CD45RA+ CCR7− (Zimmerli et al., 2005).

It has been generally accepted that CD45RO expression defines activated or memory T cells. Here we found that SARS-CoV M-specific memory CD4+ T cells were mostly CD45RO+, while the majority of memory CD8+ T cells displayed CD45RO− phenotype. These findings suggest that, at least in humans, the capacity of memory CD8+ T cells to express CD45RO in vivo may be different from that of CD4+ T cells. Wills et al. (1999) showed that virus-specific memory CD8+ T cells revert from CD45ROhigh to CD45RAhigh in vivo. Since CD45RAhigh cells contain both naïve cells and memory cells, we conclude that CD45RA is not a reliable marker of naive CD8+ T cells in human and that CD45RO is a marker of cell activation that does not identify all CD8+ memory T cells.

Based on the expression of cell-homing receptors, memory T cells can be divided into two distinct subsets: central memory T cells (T_C and the producer of IL-2 (Wherry et al., 2003). Thus, we speculate that double IFN-γ/IL-2-secreting CD4+ T cells are T_C expressing CD45RO+ CCR7+ cells. These cells with CD45RO+ CCR7+ phenotype are typical of T_CM and the producer of IL-2 (Wherry et al., 2003). CD45RO− CCR7− T cells were CCR7−/IL-2-secreting CD45RA+ T cells. In contrast, SARS-CoV M-specific CD8+ T cells predominantly displayed CD45RO− and CCR7− phenotype, which might represent terminally differentiated effector memory T cells (Champagne et al., 2001). This may reflect differences in the inherent stability of CD4+ and CD8+ memory T cells that are CCR7− in normal steady-state conditions (Seder & Ahmed, 2003). The Ssp-1 (an HLA-A∗0201-restricted CD8+ T-cell epitope on SARS-CoV S protein)-specific CTL cells exhibited a differentiated effector phenotype, CD45RA+ CCR7− CD62L− (Chen et al., 2005). Consistent
with those observations, we found that the vast majority of SARS-CoV M-specific memory CD8\(^+\) T cells were mostly confined to the CD45RO\(^-\)CCR7\(^-\)CD62L\(^-\) population. However, SARS-CoV M-specific memory CD4\(^+\) T cells expressed a mixed CCR7\(^+\)CD62L\(^-\) phenotype that differs from classical CCR7\(^+\)CD62L\(^+\) T\(_{EM}\) and CCR7\(^-\)CD62L\(^-\) T\(_{EM}\) cells. These studies suggest that the expression of CCR7 and CD62L in memory CD4\(^+\) T cells may overlap only partially.

In further study, we identified four human T-cell immunodominant peptides on the M protein of SARS-CoV, p21-44, p65-91, p117-140 and p200-220, which were able to induce cellular immunity mediated by CD4\(^+\) and CD8\(^+\) T cells. These responses were predominantly of memory CD8\(^+\) T cells, compared with memory CD4\(^+\) T cells. Together with the identification of the T-cell immunodominant peptides of the M protein of SARS-CoV, this may provide the basis for the development of immunity-based prophylactic, therapeutic, and diagnostic techniques for SARS.

In conclusion, SARS-CoV infection elicited T-cell responses to the M protein in recovered SARS patients, and this immunity persisted for a long period of time. Both CD4\(^+\) and CD8\(^+\) T cells were involved in the maintenance of immune memory to SARS-CoV M protein, which may have important implications in developing SARS vaccines.

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Perspective memory T-cell responses to SARS-CoV M antigen


