

T cell-mediated immune response to respiratory coronaviruses

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Abstract Emerging respiratory coronaviruses such as the severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) pose potential biological threats to humans. SARS and MERS are manifested as severe atypical pneumonia associated with high morbidity and mortality in humans. The majority of studies carried out in SARS-CoV-infected humans and animals attribute a dysregulated/exuberant innate response as a leading contributor to SARS-CoV-mediated pathology. A decade after the 2002–2003 SARS epidemic, we do not have any approved preventive or therapeutic agents available in case of re-emergence of SARS-CoV or other related viruses. A strong neutralizing antibody response generated against the spike (S) glycoprotein of SARS-CoV is completely protective in the susceptible host. However, neutralizing antibody titers and the memory B cell response are short lived in SARS-recovered patients and the antibody will target primary homologous strain. Interestingly, the acute phase of SARS in humans is associated with a severe reduction in the number of T cells in the blood. Surprisingly, only a limited number of studies have explored the role of the T cell-mediated adaptive immune response in respiratory coronavirus pathogenesis. In this review, we discuss the role of anti-virus CD4 and CD8 T cells during respiratory coronavirus infections with a special emphasis on emerging coronaviruses.

Keywords Respiratory coronavirus · T cells · Immune response

Introduction

Coronaviruses belong to the family *coronaviridae* and are enveloped, positive-sense, single-stranded RNA viruses. The coronavirus genome is approximately 31 kb, making these viruses the largest known RNA viruses yet identified [1]. Coronaviruses infect a variety of hosts including humans and several other vertebrates. Coronaviruses are associated with several respiratory and intestinal tract infections. Respiratory coronaviruses have long been recognized as significant pathogens in domestic and companion animals and as the cause of upper respiratory tract infections in humans [2]. Thus, several human coronaviruses (HCoV) are

the etiological agents for mild respiratory illness, including the common cold and croup (e.g., HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU) [3, 4]. Human coronaviruses such as SARS-CoV and MERS-CoV are also associated with severe respiratory illness [5–9]. Coronaviruses that induce respiratory tract disease in other vertebrate animals include mouse hepatitis virus-1 (MHV-1) a natural mouse pathogen, infectious bronchitis virus (IBV) in chickens and other avian species, bovine coronavirus (BCoV) in cows and other ruminants, porcine respiratory syndrome virus (PRCV) in pigs and canine respiratory coronavirus (CRCoV) in dogs to name a few [10, 11].

Coronaviruses that induce mild respiratory illness are generally more prevalent in younger populations of humans and domestic animals [10, 11], while those that are responsible for severe disease, such as SARS-CoV and MERS-CoV, cause lethal disease in aged or immunocompromised individuals [8, 12]. Notable exceptions to this are IBV, a

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severe form of upper respiratory tract infection in young chicks [13], and HCoV-NL63, responsible for croup in children [14]. During the 2002–2003 epidemic, SARS-CoV infection resulted in an overall 10 % mortality. While 100 % survival was observed in young (<24 years old) SARS-CoV-infected patients, the mortality rate was >50 % in elderly individuals aged 65 and above [11]. To date, newly emerging MERS-CoV has infected 495 people with 141 deaths [15]. Several reports from the 2002–2003 SARS outbreak indicated that the acute respiratory distress syndrome (ARDS) developed in the majority of patients with severe disease. ARDS, a nonspecific end-stage process in patients with pulmonary disease caused by a variety of etiological agents, is most severe in elderly individuals and resulted in ~52 % mortality among elderly SARS patients [16]. Pathological investigation of patients with lethal SARS revealed acute pulmonary edema, extensive inflammatory cell infiltration, multi-organ failure, thromboembolic complications and septicemia [17]. Severe lung and systemic inflammation is believed to result from cytokine dysregulation; in patients with SARS, increased levels of cytokines such as TNF- α , IP10, IL-6 and IL-8 likely contributed to the poor outcome [17]. Such an exuberant innate cytokine response was attributed to hyper-activation of macrophage/monocyte lineage cells. Additionally, increased levels of type I interferon (IFN) and a dysregulated interferon-stimulated gene (ISG) response were observed in patients with severe SARS [18, 19]. Overall, it is still not known whether SARS in humans was the result primarily of type I IFN-independent exaggerated pro-inflammatory reaction or whether both IFN-dependent and IFN-independent aberrant cytokine production contributed to severe pathology. Similar to SARS in humans, MERS-CoV-infected patients exhibit symptoms of a flu-like illness followed by an atypical pneumonia, including fever, dry cough and severe shortness of breath [8]. However, we still do not know much about the innate or the adaptive immune response in MERS-CoV-infected individuals, mainly because only a small number of sporadic MERS cases reported to date, and there is a paucity of clinical data absence of any autopsy information.

To investigate SARS-CoV pathogenesis, several animal models have been developed [20, 21]. Soon after the 2002–2003 SARS epidemic, mice, cats and ferrets were used as animal models to study SARS pathogenesis. Human isolates of SARS-CoV could replicate in these hosts following intranasal infection, but in contrast to SARS in humans, no overt clinical signs were observed in cats while 50 % of ferrets showed evidence of mild disease [22]. Similarly, mice infected with the human Urbani strain of SARS-CoV developed only mild disease, although disease severity was greater in aged mice [23]. Several non-human primates were also experimentally infected with SARS-CoV with variable disease severity dependent upon

the primate model used [7, 21, 24]. Despite this variation, aged non-human primates were associated with exacerbated innate immune response and acute lung injury, similar to that in humans although without associated lethality [7, 21]. Because of this lack of useful animal models that mimicked human disease, several laboratories developed mouse- or rat-adapted strains of SARS-CoV that caused extensive and lethal pulmonary disease. One mouse-adapted strain of SARS-CoV, MA15, has been extensively used in pathogenesis studies. SARS-CoV-MA15 induces severe disease in young BALB/C and in aged mice (>20 weeks) of all strains [25]. Infection of aged mice with SARS-CoV-MA15 induced a robust up-regulation of pro-inflammatory cytokines (TNF- α , IL-6, IL-8, IP-10, MCP-1) and chemokines (CXCL-1, CXCL-2, CCL-3 and CCL-5) [21, 26]. Furthermore, in comparison with SARS-CoV-MA15-infected young C57BL/6 mice, infection of aged mice (>12 months) is associated with severe reduction in the number of virus-specific CD8 T cells in the lungs [27]. Since T cells are required for controlling exuberant innate immune responses, the absence of a potent anti-virus T cell response in aged hosts could lead to exacerbated/dysregulated innate responses and pathology [28, 29]. Additionally, virus-specific CD4 and CD8 T cells play a critical role in clearing virus by eliminating virus-infected cells. Several studies in both humans and animals have identified and discussed the host innate response to SARS-CoV and other respiratory coronaviruses. Despite these extensive efforts, there is limited information available on the role of the antigen-specific T cell-mediated immune response to respiratory coronaviruses. In this review, we will focus on the T cell-mediated immune response to SARS-CoV.

Primary T-cell response to respiratory virus infections

The majority of studies addressing T cell responses to respiratory virus infections come from mice infected with a variety of natural and mouse-adapted pathogens. A few studies use natural mouse pathogens such as Sendai virus, a mouse para-influenza type I pathogen and mouse hepatitis virus-1 (MHV-1). More commonly, mouse-adapted strains of human pathogens such as the A/Puerto Rico/8/1934 H1N1 (PR8) or A/WSN/33 H1N1 (WSN) strains of influenza and SARS-CoV-MA15 have been used to study innate and adaptive immune responses [25, 30]. Initiation of the immune response against invading pathogens begins with direct infection of airway epithelium. Following initial infection, lung-resident respiratory dendritic cells (rDCs) acquire the invading pathogen or antigens from infected epithelial cells, become activated, process antigen and migrate to the draining (mediastinal and cervical) lymph

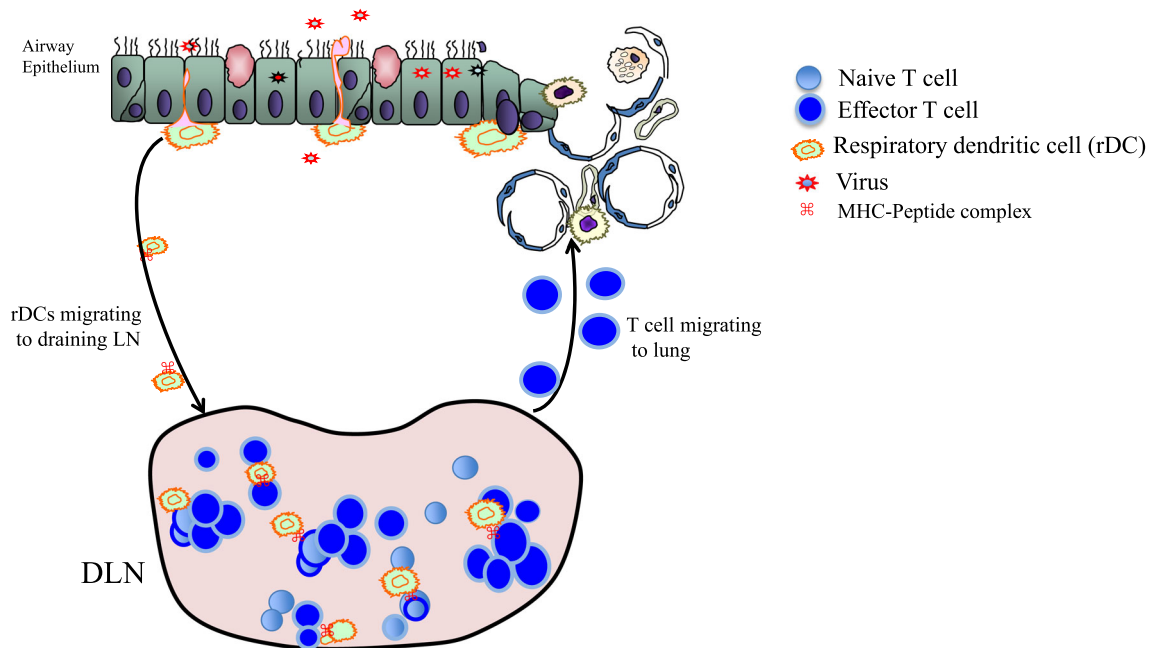


Fig. 1 Induction of T cell response to respiratory virus infection

nodes (DLN). Once in the DLNs, rDCs present the processed antigen in the form of MHC/peptide complex to naïve circulating T cells. Following engagement of the T cell receptor (TCR) with peptide–MHC complex and additional co-stimulatory signals, T cells become activated, proliferate vigorously and migrate to the site of infection [31–33] (Fig. 1). Once at the site of infection, activated virus-specific effector T cells produce antiviral cytokines (IFN- γ , TNF- α , IL-2), chemokines (CXCL-9, 10 and 11) and cytotoxic molecules (perforin and granzyme B) [34]. Effector cytokines such as IFN- γ directly inhibit viral replication and enhance antigen presentation [35]. The chemokines produced by activated T cells recruit more innate and adaptive cells to control pathogen burden. Cytotoxic molecules such as granzyme B directly kill infected epithelial cells and help eliminate the pathogen [36–39]. Despite a wealth of information on the T cell response to many respiratory pathogens, less is known about the respiratory coronavirus infections.

Virus-specific T cells and the primary immune response to SARS-CoV in humans

The acute phase of SARS in human patients was associated with marked leukopenia with severe lymphopenia (~80 % of patients), involving a dramatic loss of CD4 T cells (~90–100 % of patients) and CD8 T cells (~80–90 % patients) in comparison with healthy control individuals

[40–42]. Subsequent studies showed impaired CD4 and CD8 T cell activation in SARS-CoV-infected patients as determined by CD25, CD28 and CD69 expression on CD4 and CD8 T cell subsets [43, 44]. Severe SARS-CoV infection in humans was characterized by the delayed development of the adaptive immune response and prolonged virus clearance [45]. In addition, leukopenia and associated lymphopenia are also observed in MERS patients, albeit to a lesser degree than that observed in SARS patients. A detailed clinical study showed that 14 % of MERS patients were leukopenic, while 34 % of the patients had lymphopenia [46]. Decreased numbers of T cells strongly correlated with the severity of acute phase of SARS disease in humans [42, 47]. Although SARS-CoV is not known to productively infect T cells, altered antigen presenting cell (APC) function and impaired DC migration resulting in reduced priming of T cells likely contribute to fewer number of virus-specific T cells in the lungs [27, 48, 49]. Other possible explanations for T cell lymphopenia include an exuberant type I IFN response and high levels of glucocorticoids resulting from a normal stress response both of which might induce T cell apoptosis [50]. Currently, much less is known about the fate of T cells in MERS-CoV-infected patients.

Several HLA-A*02:01-restricted T cells recognizing SARS-CoV epitopes have been identified in the PBMC of SARS-recovered individuals. Most of these immunogenic epitopes were localized to the spike (S) and nucleocapsid

(N) protein of SARS-CoV, using ELISPOT and intracellular IFN- γ expression assay following peptide stimulation directly ex vivo [51, 52]. Additionally, several CD8 T cell epitopes were also identified and characterized in the M protein of SARS-CoV from PBMCs of the SARS survivors; however, HLA restriction of these is not known [53]. Subsequently, Lv et al. [54] demonstrated that immunization of HLA-A2.1/Kb transgenic mice with a recombinant DNA (rDNA) vaccine encoding the S protein induced peptide S958–966-specific IFN- γ release and target cell lysis by CD8 T cells. Several SARS-CoV specific T cell epitopes were identified in infected/recovered human PBMCs [55, 56]. To date, few studies have identified antigen-specific CD4 T cells in SARS-CoV-infected/recovered patients. In one of the studies, CD4 T cells specific for epitopes in the nucleocapsid (N) protein were identified in SARS survivors [57]. In the other study, Libraty et al. [58] detected HLA-DR restricted CD4 T cell epitopes in the S protein (S729–745, S358–374 and S427–444) in recovered patients. These results show that both S and N proteins of SARS-CoV contain immunogenic epitopes that can be recognized by CD4 and CD8 T cells. Since the S protein of SARS-CoV is capable of inducing neutralizing antibodies, CD4 and CD8 T responses and N protein can elicit T cell response in humans, both of these proteins are useful potential vaccine candidates able to generate a strong humoral and cell-mediated immune response against SARS-CoV. One caveat is that immunizations with some vaccines encoding the N protein induce an eosinophilic response [59], so these vaccines will need to be monitored carefully. No information about epitopes recognized in patients with MERS is currently available.

The T cell response in respiratory coronavirus infected animals

Identification of virus-specific CD4 and CD8 T cell epitopes in mice

In one of the first studies, Yang et al. [60] demonstrated the existence of SARS-CoV-specific T cells in mice immunized with recombinant DNA (rDNA) encoding the S protein. After stimulation with pools of overlapping S-peptides, antigen-specific CD4 and CD8 T cells were detected by IFN- γ expression. Subsequently, several studies have identified SARS-CoV-specific CD4 and CD8 T cells in mice immunized with rDNA or recombinant virus encoding S, N or M proteins. [61–64]. A detailed list of SARS-CoV-specific immunodominant CD4 and CD8 T cell epitopes identified in mice is provided in Table 1.

Identification of relevant T cell epitope in MERS-CoV has been hindered by the absence, until recently of a mouse model for infection. Dipeptidyl-peptidase (hDPP4) is

Table 1 List of SARS-CoV-specific CD4 and CD8 T cell epitopes found in C57BL/6 and BALB/C mice

Protein	Peptide sequence	MHC-restriction	References
<i>CD8 T cell epitopes</i>			
C57BL/6 mice			
Spike	S436–443	H2 ^b	[64]
	S525–532	H2 ^b	[64]
	S497–504	H2 ^b	[75]
	S627–642	H2 ^b	[75]
	S641–658	H2 ^b	[75]
Nucleocapsid	NP219–228	H2 ^b	[75]
M protein	M137–180	H2 ^b	[75]
BALB/C mice			
Spike	S366–374	H2 ^d	[64]
	S521–529	H2 ^d	[75]
	S1031–1047	H2 ^d	[110]
Nucleocapsid	NP80–99	H2 ^d	[111]
	NP84–101	H2 ^d	[111]
	NP92–101	H2 ^d	[111]
<i>CD4 T cell epitopes</i>			
C57BL/6 mice			
Nucleocapsid	NP11–25	H2 ^b	[75]
	NP51–65	H2 ^b	[75]
	NP61–75	H2 ^b	[75]
	NP111–125	H2 ^b	[75]
BALB/C mice			
Spike	S365–374	H2 ^d	[110]
	S435–443	H2 ^d	[110]
Nucleocapsid	NP80–99	H2 ^d	[111]
	NP353–370	H2 ^d	[111]
	NP241–258	IA ^d	[111]

identified as a functional MERS-CoV receptor in humans [65], but mouse DPP4 (mDPP4) is not a functional receptor [66]. As a result, mice are impervious to MERS-CoV infection. Recently, Zhao et al. [67] showed that BALB/c and C57BL/6 mice were sensitized for MERS-CoV infection by transduction with an adenovirus-5 (Ad5) expressing hDPP4. Following MERS-CoV infection, several H2^b-restricted CD8 T cell epitopes were detected in the S (S395, S434 and S1165) and M (M64 and M165) proteins in C57BL/6 mice, and H2^d-restricted CD8 T cell epitopes were localized to the (S291, S319, S448 and S647), N (N57, N101 and N214) and M (M110 and M159) proteins in BALB/C mice.

Infection with the murine coronavirus, MHV-1, induces a severe lung pathology in A/J and C3H/HeJ mice. [68, 69]. Following MHV-1 infection, virus-specific CD4 and CD8 T cells were identified both in susceptible (C3H/HeJ) and in resistant (C56BL/6) strains of mice. Using direct ex vivo

stimulation of splenocytes from infected mice with several individual overlapping peptides, IFN- γ production was detected by flow cytometry. In C57BL/6 mice, the immunodominant, IA^b-restricted CD4 T cell epitopes were localized to the S (S361–S375, S766–780) and M (M131–M145) proteins, while H2^b-restricted CD8 T cell epitopes were found in the S (S324–317, S532–539 and S587–594) and M (M184–191) proteins. Similarly, in C3H/HeJ mice, CD4 T cell epitopes (I-E^k restricted) were found in the S (S171–S185, S921–S935 and S881–S895), N (N376–390 and N346–N360) and M (M196–210) proteins, while the only dominant CD8 T cell epitope was in the N (N421–N428) protein [70].

Primary T cell response to respiratory coronaviruses in mice

Virus clearance during a primary response to virus infections such as influenza and para-influenza clearly depends on virus-specific CD4 and CD8 T cells and the rapidity of virus clearance correlates with the magnitude of CD4 and CD8 T cell response [71–74]. Although several SARS-CoV-specific CD4 and CD8 T cells have been detected in both infected/recovered patients and mice, very few studies have addressed the role of virus-specific T cells in SARS-CoV pathogenesis. In a study using BALB/C mice, Chen et al. [26] showed that intranasal inoculation of 12–14 month-old BALB/C mice with SARS-CoV (Urbani strain) induced interstitial pneumonitis and diffuse alveolar damage. In this study, depletion of CD4 T cells (but not CD8 T cells) delayed virus clearance and further enhanced immune-mediated interstitial pneumonitis. Depletion of CD4 T cell also resulted in reduced neutralizing antibody titers in the lungs of SARS-CoV (Urbani)-infected mice. Since the Urbani strain of SARS-CoV induces a nonlethal, self-limiting disease, the protective role of virus-specific CD4 and CD8 T cells could not be described in this study. On the other hand, infection of young BALB/c mice with the mouse-adapted strain of SARS-CoV (SARS-CoV-MA15) induces a severe disease, but generates a poor virus-specific CD4 and CD8 T cell response. Such a poor virus-specific CD4 and CD8 T cell response is attributed to an inefficient immune activation by SARS-CoV-MA15, particularly of respiratory DC (rDCs), as shown by reduced expression of MHC-II, CD86 and CD40 on cells harvested from the lungs. Activation of rDCs by the reversal of inhibitory mechanisms (such as depleting inhibitory alveolar macrophages or treating mice with poly I:C) resulted in greater numbers of anti-virus CD4 and CD8 T cells in the lungs, which ultimately correlated with better protection [49]. Subsequently, the direct evidence for the role of virus-specific CD4 and CD8 T cells in SARS-CoV clearance and host protection came from adoptive transfer

studies. In one study, Zhao et al. adoptively transferred SARS-CoV-specific effector CD4 and CD8 T cells (separately) into immunodeficient SCID and RAG^{-/-} mice or susceptible young BALB/C mice. Transfer of SARS-CoV-specific CD4 and CD8 T cells into these mice resulted in rapid virus clearance and amelioration of the clinical disease. Increasing the number of virus-specific CD8 T cells *in vivo* by immunization with S366-peptide-pulsed DCs also resulted in a robust T cell response, accelerated virus clearance and increased survival in SARS-CoV-MA15 challenged BALB/C mice [75]. Although the adoptive transfer of SARS-CoV-specific effector CD4 and CD8 T cells controlled SARS-CoV in the lungs, it is still not known whether natural, *in vivo* generated virus-specific CD4 and CD8 T cells would be equally protective.

With advancing age, both humans and animals become highly susceptible to SARS-CoV and other respiratory virus infections. Such an age-dependent increase in the susceptibility is associated with a significant reduction in the magnitude of virus-specific T cell response [12, 27, 76, 77]. Young (6 wk) C57BL/6 mice generate a SARS-CoV-specific CD8 T cell response that is approximately eight-fold greater than that observed in 12-month-old mice [25]. The reduction in the numbers of SARS-CoV-specific CD8 T cells in the lungs of aged mice is attributed in part to the impaired ability of rDCs to migrate to DLN and to prime sufficient numbers of antigen-specific CD8 T cells. Even though impaired migration of rDCs to DLN is an age-dependent phenomenon, it is much pronounced in aged mice infected with SARS-CoV as compared to those infected with other respiratory viruses such as influenza A virus or respiratory syncytial virus (RSV) [27]. Migration of rDCs to DLN requires CCR7 expression [78] and CCR7 expression on rDCs is inhibited by the prostaglandin, PGD2 [79], which in turn increases in the lungs as mice age and is even further increased, however, after SARS-CoV infection [27]. Local pharmacologic inhibition of the PGD2 receptor, DP-1, with a specific antagonist (BW A868C) resulted in enhanced migration of rDCs to DLN and a subsequent augmented SARS-CoV-specific CD8 T cell response in the lungs, associated with enhanced survival of aged mice [27]. It is important to note, however, that only a partial protection (as demonstrated by ~65 % survival) was observed in the DP-1 antagonist (BW A868C)-treated SARS-CoV-infected aged mice. These results suggest that other age-associated factors that impair one or more components of innate or adaptive arm of the immune system also likely contribute to the deficit observed.

Similar to SARS-CoV-specific T cells, MERS-CoV-specific CD8 T cells also play an important role in clearing MERS-CoV in both BALB/c and C57BL/6 mice. In a recent study, infection of Ad5-hDPP4-transduced, T cell (TCRa^{-/-})-deficient mice with MERS-CoV resulted in the

persistence of MERS-CoV in the lungs, while virus was cleared in control mice. Following MERS-CoV infection, the numbers of virus-specific CD8 T cells peaked on day 7 post-infection in the lungs of Ad5-hDPP4-transduced MERS-CoV-infected WT C57BL/6 and BALB/C mice. Additionally, effector CD8 T cells specific for immune-dominant epitopes (S1165 in C57BL/6 mice and S291 in BALB/C mice) efficiently killed peptide-pulsed target cells *in vivo* [67]. Although Ad5-hDPP4 transduction and subsequent MERS-CoV sensitization has its limitations, this study clearly demonstrates the importance of virus-specific CD8 T cells in clearing MERS-CoV.

As described earlier, MHV-1 infection of the respiratory tract induces pulmonary pathology in susceptible A/J and C3H/HeJ mice [70]. Unlike SARS-CoV-specific CD4 and CD8 T cells, *in vivo* depletion of MHV-1-specific CD4 and CD8 T cells in susceptible A/J and C3H/HeJ mice during primary infection significantly ameliorated disease severity and improved airway function, suggesting that MHV-1-specific T cells induce overt lung pathology in A/J and C3H/HeJ mice. Thus, unlike SARS-CoV-infected mice, the T cell response is apparently excessive in MHV-1-infected mice. In contrast, infection of C57BL/6 RAG1^{-/-} mice with MHV-1 leads to significant weight loss and persistence of MHV-1 in the lungs, liver, spleen and brain during the later stages of infection (day 10 post-infection). Intranasal infection with MHV-1 in susceptible C3H/HeJ mice generated robust and broad virus-specific CD4 T cell response, whereas in resistant C57BL/6 mice, antigen-specific CD8 T cell response dominated. The resistance displayed by C57BL/6 mice was probably not entirely due to greater virus-specific CD8 T cells, as equally robust MHV-1-specific CD8 T cell responses in C3.SW-H2(b)/SnJ mice, was associated with significant morbidity [68]. These results are consistent with those obtained from infecting mice with the neurotropic JHM strain of MHV. MHV-JHM infection induces encephalomyelitis with both acute and chronic demyelination in mice [80, 81]. Virus-specific CD8 T cells play a critical role in viral clearance and CD4 T cells provide necessary help for antiviral function of CD8 T cells [82]. Interestingly, MHV-JHM-specific CD8 T cells require distinct effector mechanisms to control virus replication in different cell types in the CNS. For instance, perforin-mediated cytolysis is crucial to control virus replication in microglia/macrophages, while virus clearance from oligodendrocytes requires IFN- γ dependent effector functions [83]. Adoptive transfer of antigen-specific CD4 and CD8 T cells into infected mice lacking T or B cells (RAG1^{-/-}) induces CNS demyelination, showing that the T cell response is required for both virus clearance and immunopathology. As in MHV-1-infected mice, T cells thus contribute to morbidity [84]. Studies from another coronavirus, MHV-3, which induces hepatic necrosis in

mice, showed that an effective T cell response ameliorates disease and the Th1/Th2 balance determines resistance and susceptibility in A/J and BALB/C mice, respectively [85].

These results suggest that the role of virus-specific primary CD4 and CD8 T cell responses to respiratory or other coronavirus infections are both virus and mouse strain dependent. Furthermore, it will be interesting to know whether MHV-1-mediated lung pathology in A/J and C3H/HeJ mice is due to an imbalance of Th1, Th2 and/or Th17 cell responses as shown in other respiratory virus infections such as RSV [85–87].

In an unrelated coronavirus infection, IBV infection is one of the leading causes of respiratory illness in young chicks. Infection of young chicks with the Gray strain of IBV induces S- and N protein-specific CD8 T cell responses. Adoptive transfer of virus-specific effector CD8 T cells (isolated from the spleen at 10-days post-infection) into a naïve chicks greatly reduced clinical illness and rapidly cleared virus from the lungs in comparison with those receiving naïve CD8 T cells [88]. These results show that coronavirus-specific CD8 T cells are also protective in this setting.

The memory T cell response to respiratory coronaviruses

The effector phase of an immune response is followed by a sharp contraction in the number of antigen-specific T cells, with 90–95 % of virus-specific T cells undergoing apoptosis [89]. The contraction phase is followed by a memory phase in which a stable pool of memory T cells is maintained for a prolonged period of time. Such memory T cells are programmed to counter subsequent infection with the same or related pathogen, mounting rapid responses on re-exposure to the pathogen in question, with minimal requirement for co-stimulatory signals [34]. It is well established now that memory T cells are unique in their anatomic distribution, function, maintenance and response to recall [34, 90–92]. Recent advances highlight the key role of virus-specific tissue-resident memory cells in effectively countering a local pathogen challenge. These tissue-resident memory T cells are well equipped to generate a rapid recall response as they are located at the site of infection and possess potent lytic activity and granzyme B expression [93]. The tissue-resident memory T cells secrete cytokines (IFN- γ) and chemokines (CXCL9-11) that activate innate cells and attract more memory T cells from the periphery [91, 92]. Following a respiratory tract infection, memory T cells reside both in the lung airways and in lung interstitial tissue. The lung airways contain a high proportion of antigen-specific T cells in comparison with those in the lung interstitial tissue. Conversely,

lung-airway-resident memory T cells lack constitutive cytolytic activity and do not proliferate in situ [94]. However, these cells are constantly replenished from the circulation, and recruitment of these cells from circulation depends on CXCR3 expression [95].

Virus-specific memory T cell responses in humans

Although difficult to address in humans, recent studies highlight the importance of virus-specific T memory cells in patients with respiratory disease. Thus, Sridhar et al. [96] showed that the presence of memory T cells correlated with protection during the recent epidemic caused by the H1N1 strain of influenza A virus. However, most of our understanding of virus-specific memory T cells in the lungs is derived from experimental studies using either influenza or Sendai virus in mice. In terms of patients with SARS, several studies have identified virus-specific memory CD4 and CD8 T cells in patients who recovered from the infection as long as four years after acute infection. In one such study, CD8 T cells specific for HLA-A*02:01-restricted epitopes in the spike protein (SSp-1, S978 and S1202) were identified in surviving patients over one year post-infection. These virus-specific CD8 T cells produced high levels of effector cytokines (IFN- γ and TNF- α) and cytotoxic molecules (perforin and granzyme B) after peptide stimulation in vitro [97]. Memory CD4 T cells specific for HLA-DR08- and HLA-DR15-restricted epitopes within the S protein of SARS-CoV were also identified in recovered individuals [58]. Using pools of overlapping peptides, N, M and E protein-specific CD4 and CD8 T cells were identified in PBMCs from SARS-recovered individuals at 2-years post-infection. Virus-specific CD4 T cells mainly exhibited a central memory phenotype (CD45RA⁻CCR7⁺CD62L⁻), whereas CD8 memory T cells were effector memory cells (CD45RA⁺CCR7⁻CD62L⁻) [53, 55, 98, 99]. In a phase I clinical trial, vaccination of healthy individuals with rDNA encoding spike (S) protein of SARS-CoV elicited both neutralizing antibodies and the spike-protein-specific T cell responses. The majority of SARS-CoV spike-protein-specific T cells were CD4 T cells (10/10 subjects), and a minority of subjects had detectable spike protein-specific CD8⁺ T cell responses (2/10 subjects) [100]. Collectively, these studies suggest a potential role for virus-specific memory T cells in broad and long-term protection against SARS-CoV infection. This is important as neutralizing Abs and the memory B cells response to SARS-CoV decline significantly after 1–2 years post-infection and are also strain specific.

Virus-specific memory T cell response in animals

Similar to the human studies, analysis of experimental animals has demonstrated the presence of SARS-CoV-specific CD4 and CD8 T cells following rDNA vaccination or recombinant virus immunizations. Intramuscular

immunization of rhesus macaques with recombinant adenovirus (rAd5) encoding the SARS-CoV-N protein, followed by a booster vaccination on day 28 induced N-specific-T cell responses [101]. Immunization of mice with rDNA vaccine encoding the S, N, M or E protein of SARS-CoV induced virus-specific memory CD4 and CD8 T cells [63, 102, 103]. Virus-specific memory CD4 and CD8 T cells were able to produce effector cytokines (e.g., IFN- γ) and cytolytic molecules upon in vitro peptide stimulation [102]. Although several groups demonstrated the presence of SARS-CoV-specific memory CD4 and CD8 T cells, very few studies have demonstrated their in vivo potential to clear virus. In one study, depletion of virus-specific memory CD4 and CD8 T cells in BALB/C mice immunized with rDNA (encoding S protein) did not have any effect on virus clearance on day 2 post-challenge [60]. However, caveats of this study were that the Urbani strain, which is largely non-pathogenic in mice, was used and that the authors did not examine the effect of CD4 and CD8 T cell depletion on virus clearance during later time points, when virus-specific effector memory CD4 and CD8 T cells would be expected to exert their antiviral functions. In another study, Ohno et al. [104] identified HLA-A*0201-restricted SARS-CoV-N-specific CTLs in infected HLA-A*0201 transgenic mice, using surface-linked liposomal peptides a CTL-based vaccine against SARS-CoV infection. These surface-linked liposomal peptides (derived from the N protein) effectively induced CTL responses, and upon challenge, these immunized mice rapidly cleared vaccinia virus (VV) expressing the SARS-CoV-N. In an unpublished study, we demonstrated the protective role for SARS-CoV-S-specific memory CD8 T cells following lethal SARS-CoV infection in 9- to 11-month-old C57BL/6 mice. In this study, intravenous DC-peptide (DC-S436/S525) immunization followed by intranasal boosting with recombinant vaccinia virus encoding S436/S525 generated large pool of S436/S525-specific memory CD8 T cells in the lungs of 9- to 11-month-old C57BL/6 mice. The virus-specific memory CD8 T cells in the lungs provided partial but significant protection against lethal SARS-CoV challenge. These data suggest that virus-specific memory CD8 T cells enhance the kinetics of virus clearance and protect the susceptible host from lethal SARS-CoV infection. However, the protection was not as effective as observed after the natural infection, suggesting a role for anti-virus CD4 T cells or anti-virus antibodies in providing optimal protection.

In contrast, adoptive transfer of MHV-1-specific memory CD4 and CD8 T cells (bulk memory splenocytes or purified memory CD4 and CD8 T cells isolated from spleen) enhanced morbidity and mortality in MHV-1-challenged C3H/HeJ mice [68]. The lung pathology in these mice was possibly not due to robust IFN- γ and other

pro-inflammatory cytokines production as even a sub-lethal dose of MHV-1 infection generates greater magnitude of virus-specific CD4 and CD8 T cell response. Another possibility is that in the absence of immune serum or a B cell response, virus-specific memory T cells cannot provide complete protection [105]. In mice infected with neurotropic MHV-JHM strain, antigen-specific memory CD8 T cells were generated after immunization with rVV expressing the immunodominant CD8 T cell epitope (pN9). Following CNS MHV-JHM challenge, pN9-specific CD8 T cells exhibited rapid recall in the lymphoid organs were rapidly recruited to the CNS in increased numbers and facilitated efficient MHV-JHM clearance from the CNS [106]. Memory virus-specific CD4 T cells are also protective in these infections [107].

In IBV infected chicks, adoptive transfer of memory CD8 T cells (isolated from the spleen at 3–6 weeks post IBV infection) but not CD4 protected young chicks from lethal IBV infection. In these studies, memory CD8 T cells showed cytolytic activity in *in vitro* assays and completely cleared the IBV from lungs and kidneys [108, 109].

Although there is a limited amount of data available on virus-specific memory T cell responses to emerging coronaviruses, the existing studies indicate that lung resident virus-specific memory CD8 T cells provide substantial protection following SARS-CoV challenge.

Summary and conclusion

In this review, we discussed our current understanding of the virus-specific T cell-mediated immune response to respiratory coronaviruses. Several lines of evidence from other respiratory virus infections such as influenza A and para-influenza have established that virus-specific CD4 and CD8 T cells generated during primary and memory response are able clear virus and protect the host from lethal infections. On the contrary, virus-specific T cell response to respiratory coronaviruses and their ability to clear virus depends on the type of pathogen in question and the host used in the study. In case of mice infected with SARS-CoV, virus-specific CD8 T cells in the absence of antibody or CD4 T cells responding to virus provide a partial but significant level of protection and effect virus clearance. On the other hand, MHV-1-specific T cells are detrimental and induce lung pathology in susceptible A/J and C3H/HeJ mice.

Follow-up studies from patients who recovered from SARS suggest that the SARS-CoV-specific antibody response is short lived. In these patients, SARS-CoV-specific IgM and IgA response lasted less than 6 months, while virus-specific IgG titer peaked four-month post-infection and markedly declined after 1 year. Despite the

lack of virus-specific memory B cell response, SARS-CoV-specific memory T cells persist in SARS-recovered patients for up to 6 years post-infection. Consistent with these human studies, results from animal studies also suggest that strong virus-specific T cell response are required to protect mice from lethal SARS-CoV-MA15 infection. The future vaccine interventions should also consider strategies to enhance T cell response to provide robust long-term memory. Since, tissue-resident memory T cells provide better protection, boosting a local and systemic memory T cell response would be a useful strategy than either of these interventions alone.

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