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Review Article

SARS-CoV-2-Specific T Cell Responses in Patients with COVID-19 and Unexposed Individuals

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Conflict of Interest
The authors declare no potential conflicts of interest.

Abbreviations
AA, amino acid; AIM, activation-induced marker; ARTE, Ag-reactive T cell enrichment;

ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection causes coronavirus disease 2019 (COVID-19), an ongoing pandemic disease. In the current review, we describe SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T-cell responses in acute and convalescent COVID-19 patients. We also discuss the relationships between COVID-19 severity and SARS-CoV-2-specific T-cell responses and summarize recent reports regarding SARS-CoV-2-reactive T cells in SARS-CoV-2-unexposed individuals. These T cells may be cross-reactive cells primed by previous infection with human common-cold coronaviruses. Finally, we outline SARS-CoV-2-specific T-cell responses in the context of vaccination. A better understanding of SARS-CoV-2-specific T-cell responses is needed to develop effective vaccines and therapeutics.

Keywords: SARS-CoV-2; COVID-19; T cell

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection causes coronavirus disease 2019 (COVID-19), which was declared a pandemic disease by the World Health Organization. As of January 3, 2021, there have been more than 84 million confirmed cases and more than 1.8 million deaths attributed to COVID-19 worldwide. SARS-CoV-2 infection results in diverse clinical manifestations, from asymptomatic or mild disease to severe disease that is associated with hyper-inflammatory responses (1-3). Although the case fatality rate of COVID-19 is lower than that of severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), it is much higher than that of seasonal influenza (4,5).

Patients with COVID-19 tend to have lymphopenia that preferentially affects T-cell populations (6,7), and T-cell lymphopenia is associated with severe COVID-19 (7,8). Early studies reported activation and exhaustion phenotypes for T cells in patients with COVID-19 and relationships between the T cell phenotype and disease severity (6,7,9-14). However, those studies examined pan-$CD4^+$ and/or $CD8^+$ T-cell populations without information on Ag specificity.

Later, SARS-CoV-2-reactive T-cell responses in acute and/or convalescent COVID-19 patients were reported. SARS-CoV-2-reactive T cells were examined by functional assays, including

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CCCoV, common cold coronavirus; CM, central memory; CMV, cytomegalovirus; COVID-19, coronavirus disease 2019; CSA, cytokine secretion assay; EBV, Epstein-Barr virus; EM, effector memory; HLA, human leukocyte antigen; ICS, intracellular cytokine staining; M, membrane; MERS, Middle East respiratory syndrome; N, nucleocapsid; ORF, open reading frame; S, spike; SARS, severe acute respiratory syndrome; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; scRNA-seq, single-cell RNA sequencing; TFH, follicular helper T.

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IFN-γ ELISpot assays, intracellular cytokine staining (ICS), and activation-induced marker (AIM) assays performed following *ex vivo* stimulation with SARS-CoV-2 proteins or peptides. More recently, SARS-CoV-2-specific $CD8^+$ T cells were detected, and their phenotypes were examined by an MHC-I multimer technique (15-21). In addition, SARS-CoV-2-reactive T cells were enriched using an Ag-reactive T cell enrichment (ARTE) technique and subsequently analyzed by single-cell RNA sequencing (scRNA-seq) (22).

In the present review, we summarize recent reports regarding SARS-CoV-2-specific T-cell responses in acute and convalescent COVID-19 patients and unexposed individuals. We also describe SARS-CoV-2-specific T-cell responses in the context of vaccination.

SARS-CoV-2-SPECIFIC T-CELL RESPONSES IN CONVALESCENTS

In COVID-19 convalescent individuals, SARS-CoV-2-specific T-cell responses are detected by IFN-γ ELISpot assays or ICS following *ex vivo* stimulation of PBMCs with recombinant proteins (23) or overlapping peptide pools (24). When AIM assays were performed with peptides predicted for MHC-I or MHC-II binding, SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T-cell responses were detected in 100% and up to 70% of COVID-19 convalescent individuals, respectively (25). These responses targeted not only spike (S) protein, but also membrane (M), nucleocapsid (N), and other open reading frames (ORFs). In these studies, SARS-CoV-2-specific T-cell responses significantly correlated with SARS-CoV-2 S-specific Ab titers (15,23,25,26). However, memory T-cell responses can be elicited even in the absence of SARS-CoV-2-specific Abs (16).

In COVID-19 convalescent individuals, SARS-CoV-2-specific $CD4^+$ T cells predominantly produce IFN-γ, IL-2, and TNF, whereas SARS-CoV-2-specific $CD8^+$ T cells predominantly produce IFN-γ and exert a cytotoxic function that can be evaluated by degranulation marker CD107a following *ex vivo* stimulation (16). $CD8^+$ T cells targeting different viral proteins exhibit different degrees of polyfunctionality. M- or N-specific $CD8^+$ T cells had higher polyfunctionality, a greater ability to produce multiple cytokines when stimulated, than S-specific $CD8^+$ T cells (15). $CD4^+$ T cells targeting different viral proteins exhibited different differentiation patterns in convalescents. S-specific $CD4^+$ T cells had a profile of follicular helper T (T_{FH}) cells, whereas M- or N-specific $CD4^+$ T cells had a profile indicative of Th1/Th17 cells (16). However, little or no Th2 cytokines were produced by S-, M-, or N-specific T cells from COVID-19 convalescents (25,27) although Th2 cell responses play a role in some pulmonary diseases.

The phenotypes of SARS-CoV-2-specific T cells was initially characterized by stimulation-based functional assays, such as ICS, and SARS-CoV-2-specific T cells exhibited mainly the effector memory (EM) phenotype (26). However, the phenotypes of T cells can change during *ex vivo* stimulation. More importantly, virus-specific, non-functioning T cells cannot be detected by stimulation-based functional assays. These limitations can be overcome by using MHC multimer techniques, which require information on the immunodominant epitopes and their MHC restriction. Recently, data from MHC multimer staining became available in terms of the phenotypes of SARS-CoV-2-specific T cells, particularly $CD8^+$ T cells. A list of SARS-CoV-2-specific MHC-I multimers with positive staining results is presented in **Table 1**.

Table 1. A list of SARS-CoV-2-specific MHC-I multimers with positive staining results

HLA allele	AA sequence	Protein	Position	Ref.
A*01:01	CTDDNALAY	ORF1ab	4163-4172	(17)
	FTSDYYQLY	ORF3a	207-215	(15,17)
A*02:01	YLQPRTFLL	S	269-277	(15,16,18-21)
	RLQSLQTYV	S	1000-1008	(20)
	FIAGLIAIV	S	1220-1228	(19)
	LLLDRLNQL	N	222-230	(21)
	YLFDESGEFKL	ORF1ab	906-916	(21)
	FLLNKEMYL	ORF1ab	3183-3191	(18)
	KLWAQCVQL	ORF1ab	3886-3894	(21)
	ALWEIQQVV	ORF1ab	4094-4102	(21)
	LLYDANYFL	ORF3a	139-147	(17,21)
A*03:01	KTFPPTEPK	N	362-370	(15)
A*11:01	KTFPPTEPK	N	362-370	(15)
B*07:02	SPRWYFYYL	N	105-113	(15-17)
B*40:01	MEVTPSGTWL	N	322-331	(15)
B*44:02/03	MEVTPSGTW	N	322-330	(17)
	SEFSSLPSY	ORF1ab	3946-3954	(17)

AA, amino acid; HLA, human leukocyte antigen.

MHC-I multimer staining revealed that SARS-CoV-2-specific CD8$^+$ T cells exhibit the EM (CCR7$^-$CD45RA$^-$; up to 50%) or central memory (CM) phenotypes (CCR7$^+$CD45RA$^-$; up to 20%) with early (CD27$^+$CD28$^+$; up to 40%) or intermediate (CD27$^+$CD28$^-$; up to 50%) differentiation in COVID-19 convalescent individuals (15). In another study, SARS-CoV-2-specific MHC-I multimer$^+$CD8$^+$ T cells exhibited an early differentiated memory phenotype (CCR7$^+$CD127$^+$CD45RA$^{-/+}$TCF1$^+$) in convalescents (16).

Our group also performed SARS-CoV-2-specific MHC-I staining in combination with the cytokine secretion assay (CSA) to examine the phenotypes and functions of SARS-CoV-2-specific CD8$^+$ T cells (19). SARS-CoV-2-specific MHC-I multimer$^+$CD8$^+$ T cells exhibited early differentiated EM phenotypes in the early convalescent phase. Interestingly, the frequency of CCR7$^+$CD45RA$^+$ cells among SARS-CoV-2-specific MHC-I multimer$^+$CD8$^+$ T cells increased in the late convalescent phase, suggesting the development of SARS-CoV-2-specific, stem-like memory CD8$^+$ T cells. In terms of their functions, SARS-CoV-2-specific MHC-I multimer$^+$CD8$^+$ T cells from convalescent patients exhibited a high proliferative capacity even though IFN-γ was produced by less than half of them. The relative frequency of IFN-γ-producing cells was significantly lower among SARS-CoV-2-specific CD8$^+$ T cells than among influenza A virus-specific CD8$^+$ T cells. However, SARS-CoV-2-specific MHC-I multimer$^+$CD8$^+$ T cells produced IFN-γ regardless of their PD-1 expression.

SARS-CoV-2-SPECIFIC T-CELL RESPONSES DURING ACUTE INFECTION

In patients with COVID-19, T-cell exhaustion or dysfunction has been reported since early in the emergence of SARS-CoV-2. Several studies reported an exhausted phenotype of CD8$^+$ T cells in patients with COVID-19, particularly in severe cases, with up-regulation of immune checkpoint inhibitory receptors, such as PD-1 (10-13). However, the expression of PD-1 in CD8$^+$ T cells can be up-regulated not only by functional exhaustion, but also recent activation by the T-cell receptor. Recently, our group demonstrated that IFN-γ is produced by SARS-CoV-2-specific MHC-I$^+$CD8$^+$ T cells regardless of PD-1 expression in acute and convalescent COVID-19 patients (19). This result indicates that SARS-CoV-2-specific PD-1$^+$CD8$^+$ T cells are functional, not exhausted.

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Delayed induction of SARS-CoV-2-specific T-cell responses during acute infection has also been reported (26). In this study, acute SARS-CoV-2 infection resulted in functional impairment of dendritic cells and delayed virus-specific T-cell responses comprising more of a $CD4^+$ T-cell response than a $CD8^+$ T-cell response while neutralizing Abs were rapidly developed.

In patients with acute moderate or severe COVID-19, the $CD4^+$ and $CD8^+$ T-cells responding upon *ex vivo* stimulation with SARS-CoV-2 overlapping peptides, as detected by IFN-γ or CD107a positivity, were $CD38^+$HLA-DR^+Ki-67^+PD-1^+ (16), indicating that they are fully activated and proliferating. MHC-I multimer staining confirmed this result. SARS-CoV-2-specific MHC-I $multimer^+CD8^+$ T cells expressed activation markers (CD38, HLA-DR, and Ki-67), inhibitory receptors (PD-1 and TIM-3), and cytotoxic molecules (perforin and granzyme B) during acute infection (16).

Our group also reported phenotypes of SARS-CoV-2-specific MHC-I $multimer^+CD8^+$ T cells in acute COVID-19 patients (19). SARS-CoV-2-specific $CD8^+$ T cells from patients in the acute phase exhibited an activated phenotype with high expression of CD38, HLA-DR, PD-1, perforin, and granzyme B. The proliferation marker Ki-67 was highly expressed in MHC-I $multimer^+$ cells in the acute phase, whereas CD127 was rarely expressed. In a kinetic analysis during the acute phase, the relative frequency of MHC-I $multimer^+$ cells among total $CD8^+$ T cells tended to follow the kinetics of viral titers in nasopharyngeal swabs. The relative frequency of Ki-67^+ proliferating cells and $CD38^+$HLA-DR^+ activated cells among MHC-I $multimer^+$ cells paralleled changes in the frequency of MHC-I $multimer^+$ cells. However, the relative frequency of $perforin^+$granzyme B^+ cells and PD-1^+ cells among MHC-I $multimer^+$ cells was sustained during the course of COVID-19.

A possibility of bystander activation of T cells, which has been shown in other viral infections (28, 29), was investigated in acute SARS-CoV-2 infection. Early studies reported hyper-activated T-cell phenotypes without information on Ag specificity (30,31). However, a study using MHC-I multimers in patients with COVID-19 demonstrated that the expression of HLA-DR and Ki-67 were not up-regulated in $CD8^+$ T cells specific to SARS-CoV-2-unrelated viruses, including cytomegalovirus (CMV) and Epstein-Barr virus (EBV), though the expression of CD38 was markedly increased (16), indicating that bystander $CD8^+$ T cells are activated to a limited degree during acute SARS-CoV-2 infection.

SEVERITY OF INFECTION AND SARS-CoV-2-SPECIFIC T-CELL RESPONSES

Regarding a relationship between SARS-CoV-2-specific T-cell responses and disease severity, there have been contradictory results. Among COVID-19 convalescent individuals, the magnitude of overall T-cell responses was significantly greater in individuals with severe disease compared to individuals with mild disease, as evaluated by IFN-γ ELISpot assays that measure both $CD4^+$ and $CD8^+$ T cell responses (15). However, the frequency of cytokine-producing cells among total $CD8^+$ T cells following stimulation with SARS-CoV-2 peptide pools is lower in severe cases compared with mild cases in the ICS results from the same study (15). More detailed analyses have been performed with several groups of individuals, including healthy individuals who donated blood before or during the COVID-19 pandemic, exposed family members of symptomatic patients, and convalescent individuals who experienced mild or severe COVID-19 (16). SARS-CoV-2-specific T-cell responses against S, M,

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and N were highest in convalescent individuals with severe disease, with a stepwise decrease in convalescent individuals with mild disease, exposed family members, blood donors during the pandemic, and blood donors before the pandemic. Similar results were observed when SARS-CoV-2-specific $CD4^+$ T-cell proliferation was evaluated by CTV dilution assays (16).

In our recent study, we examined the relative frequency of $CD127^+KLRG1^-$ cells, which are known as memory-precursor effector cells during viral infection and are able to differentiate into multiple memory cell lineages (32,33), among SARS-CoV-2-specific MHC-I $multimer^+CD8^+$ T cells from early COVID-19 convalescent individuals (19) and found that it was significantly lower in individuals who recently recovered from severe disease than in those who recovered from mild disease. These data suggest that the generation of long-term memory $CD8^+$ T cells after recovery from COVID-19 may be determined by peak disease severity in the acute phase.

A study has comprehensively evaluated all three arms of the adaptive immunity in acute and convalescent COVID-19 patients (27). In this study, SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T-cell responses were associated with mild disease, indicating the roles of $CD4^+$ and $CD8^+$ T cells in protective immunity against SARS-CoV-2 infection. Moreover, the coordination in SARS-CoV-2-specific adaptive immune responses, including Ab and $CD4^+$ and $CD8^+$ T-cell responses, was associated with mild disease. Interestingly, as a single parameter, the relative frequency of SARS-CoV-2-specific IFN-γ-producing cells among total $CD8^+$ T cells showed the strongest correlation with peak disease severity in acute COVID-19 patients.

SARS-CoV-2-REACTIVE T-CELL RESPONSES IN UNEXPOSED INDIVIDUALS

The presence of SARS-CoV-2-reactive T-cell responses in unexposed individuals has been reported by several studies (34). SARS-CoV-2-reactive T-cell responses in unexposed individuals were first detected by AIM assays using peptides predicted for MHC-I or MHC-II binding (25). In this study, SARS-CoV-2-reactive $CD4^+$ and $CD8^+$ T-cell responses were detected in up to 50% and 20% of unexposed individuals, respectively, suggesting that these responses may result from previous infection by cross-reactive common-cold coronaviruses (CCCoVs), including HCoV-OC43, HCoV-HKU1, HCoV-229E, and HCoV-NL63. In another study, a SARS-CoV-2 S-reactive $CD4^+$ T-cell response was detected in 35% of unexposed individuals (35). This response primarily targeted the C-terminal part of S, which has a similarity with the S protein of CCCoVs. The SARS-CoV-2-reactive $CD4^+$ T-cell response has been mapped at epitope levels across the SARS-CoV-2 proteome (36). In this study, several epitopes of the memory $CD4^+$ T-cell response in unexposed individuals were cross-reactive to SARS-CoV-2 and CCCoVs with comparable affinity.

Le Bert et al. (24) examined individuals who recovered from SARS 17 years ago and detected long-lasting T-cell responses cross-reactive to SARS-CoV-2. In this study, SARS-CoV-2-reactive T-cell responses were detected against N, NSP7, and NSP13 proteins in unexposed individuals. Intriguingly, NSP7-specific T-cell response among unexposed individuals showed reactivity to regions conserved among animal β-coronaviruses rather than human CCCoVs (24), suggesting that exposure to unknown coronaviruses of animal origin other than the four CCCoVs may induce memory T-cell responses cross-reactive to SARS-CoV-2.

Given the presence of a certain degree of homology between SARS-CoV-2 and CCCoVs, we need to consider a similarity and difference in the amino acid sequence of epitope peptides when we focus on T-cell response at a single epitope level. **Table 2** shows a list of SARS-CoV-2-derived CD4+ T-cell epitopes that have been tested in unexposed individuals or examined for cross-reactivity with human CCCoVs (24,36,37).

It is unclear whether pre-existing SARS-CoV-2-reactive T cells contribute to protective immunity against SARS-CoV-2 during acute infection or interfere with the development of a competent SARS-CoV-2-specific T-cell response by an 'original antigenic sin' phenomena (38). Intriguingly, a recent study reported that COVID-19 patients with a recent history of CCCoV infection have significantly milder disease than those without a recent history of CCCoV infection (39). This result suggests that pre-existing SARS-CoV-2-reactive memory T cells primed by recent CCCoV infection may prevent the development of severe disease when hosts encounter SARS-CoV-2 infection. Further studies are required to clarify a role of SARS-CoV-2-reactive memory T cells primed by previous CCCoV infection in SARS-CoV-2 infection.

VACCINE-INDUCED T-CELL RESPONSES

Since December 2020, COVID-19 prophylactic vaccines have been approved and started to be administered to high-risk populations. Although various vaccine platforms were used, including mRNA, viral vectors, and recombinant proteins, vaccinations were shown to successfully elicit not only neutralizing Abs, but also SARS-CoV-2-specific CD4+ and CD8+ T-cell responses (40-43).

Table 2. A list of SARS-CoV-2-derived CD4+ T-cell epitopes that have been tested in unexposed individuals or examined for cross-reactivity with human CCCoVs

Protein	AA sequence	Position	HLA allele	Epitope-reactive T cell response in unexposed individuals	Confirmed cross-reactivity with CCCoVs	Detection method	Ref.
N	MKDLSPRWYFYYLGT	101-115	ND	+	ND	*Ex vivo* IFN-γ ELISpot and ICS assay	(24)
N	PRWYFYYLGTGPEAG	106-120	ND	+	ND		
ORF1a	SKLWAQCVQLHNDIL	26-40	ND	+	ND		
ORF1a	HNDILLAKDTTEAFE	36-50	ND	+	–		
S	ITRFQTLLALHRSYL	235-249	DRB1*01	+	ND	Pre-expansion → IFN-γ ELISpot	(37)
M	LSYYKLGASQRVAGD	176-190	DRB1*04, DRB1*07	–	ND		
N	ASWFTALTQHGKEDL	50-64	DRB1*04, DRB1*11	+	ND		
N	IGYYRRATRRIRGGD	84-98	DRB1*04, DRB1*11	–	ND		
N	RWYFYYLGTGPEAGL	107-121	DRB1*04	+	ND		
N	KDGIIWVATEGALNT	127-141	DRB1*01, DRB1*04, DRB1*11	+	ND		
N	AIVLQLPQGTTLPKG	156-170	DRB1*01, DRB1*03	–	ND		
N	YKHWPQIAQFAPSAS	298-312	DRB1*01, DRB1*04, DRB1*11	–	ND		
N	ASAFFGMSRIGMEVT	311-325	DRB1*01, DRB1*04, DRB1*07, DRB1*11	+	ND		
N	GTWLTYTGAIKLDDK	328-342	DRB1*01, DRB1*07, DRB1*15	+	ND		
N	AIVLQLPQGTTLPKG	156-170	DRB1*01, DRB1*03	–	ND		
E	FYVYSRVKNLNSSRV	56-70	DRB1*04, DRB1*11	+	ND		
ORF1	LDDFVEIIKSQDLSV	6751-6765	DRB1*11	+	ND		
ORF3	FMRIFTIGTVTLKQG	4-18	DRB1*01, DRB1*03, DRB1*07	+	ND		
ORF6	IWNLDYIINLIIKNL	26-40	DRB1*04, DRB1*07, DRB1*15	+	ND		
ORF7	QEEVQELYSPIFLIV	90-104	DRB1*01, DRB1*07	+	ND		
ORF8	SKWYIRVGARKSAPL	43-57	DRB1*01, DRB1*11	+	ND		
ORF10	INVFAFPFTIYSLLL	4-18	DRB1*01, DRB1*04	–	ND		
N	LLLLDRLNQLESKMS	221-235	DRB1*04, DRB1*15	+	ND		

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Table 2. (Continued) A list of SARS-CoV-2-derived CD4+ T-cell epitopes that have been tested in unexposed individuals or examined for cross-reactivity with human CCCoVs

Protein	AA sequence	Position	HLA allele	Epitope-reactive T cell response in unexposed individuals	Confirmed cross-reactivity with CCCoVs	Detection method	Ref.
N	LLLLDRLNQLESKMS	221-235	DRB1*11:01, DQA1*01:01, DQB1*05:01	+	ND	Pre-expansion → IFN-γ/IL-5 FluoroSPOT	(36)
S	SLLIVNNATNVVIKV	116-130	DRB1*07:01, *13:01, DQA1*01:03/DQB1*06:03	+	–		
S	NNATNVVIKVCEFQF	121-135	ND	+	ND		
S	CEFQFCNDPFLGVYY	131-145	DQA1*02:01/DQB1*02:02, *01:01/*05:01	+	ND		
S	CTFEYVSQPFLMDLE	166-180	DQA1*01:02/DQB1*06:04	+	–		
S	IGINITRFQTLLALH	231-245	DRB1*07:01, *11:02, *13:01, *13:03, DQA1*01:03/DQB1*06:03	+	ND		
S	TRFQTLLALHRSYLT	236-250	DRB1*01:01, *07:01, *08:03, DRB1*11:02, *13:01, *13:02, *13:03, DRB1*15:01, DQA1*01:01/DQB1*05:01, *01:02/*06:02, *01:03/*06:03	+	–		
S	LLALHRSYLTPGDSS	241-255	DRB1*01:01, *07:01, *11:02, *15:01	+	ND		
S	FTVEKGIYQTSNFRV	306-320	ND	+	ND		
S	SNFRVQPTESIVRFP	316-330	DRB1*03:01	+	ND		
S	QPTESIVRFPNITNL	321-335	DRB1*08:03, *15:01	+	–		
S	IVRFPNITNLCPFGE	326-340	ND	+	–		
S	CPFGEVFNATRFASV	336-350	DRB1*01:01, *08:03, *11:01, *11:02, *13:03, DQA1*05:01/DQB1*03:01, *01:02/*06:02	+	–		
S	VFNATRFASVYAWNR	341-355	DRB1*01:01, *08:03, *11:01, *13:03, *15:01, DQA1*05:01/DQB1*03:01, *01:02/*06:02	+	–		
S	SIIAYTMSLGAENSV	691-705	DRB1*01:03, *07:01, DQA1*02:01/DQB1*02:02, *01:01/*05:01	+	ND		
S	AYSNNSIAIPTNFTI	706-720	DQA1*01:03/DQB1*06:03	+	–		
S	NLLLQYGSFCTQLNR	751-765	DRB1*01:01, *15:01, DQA1*01:01/DQB1*05:01	+	ND		
S	TQLNRALTGIAVEQD	761-775	DRB1*01:01, *09:01, DQA1*03:01/DQB1*03:03, *03:01/*04:02, *01:02/*06:02, *01:02/*06:04	+	–		
S	VFAQVKQIYKTPPIK	781-795	ND	+	–		
S	NFSQILPDPSKPSKR	801-815	ND	+	ND		
S	KPSKRSFIEDLLFNK	811-825	DRB1*03:01, DQA1*05:01/DQB1*02:01, *02:01/*02:02, *01:01/*05:01	+	ND		
S	SFIEDLLFNKVTLAD	816-830	DRB1*01:01, *01:03, *04:04, *11:02, *13:03, *14:01, *15:01, *16:02, DQA1*01:01/DQB1*05:01, *01:02, *05:02	+	+		
S	AQYTSALLAGTITSG	871-885	ND	+	ND		
S	WTFGAGAALQIPFAM	886-900	DRB1*01:01, DQA1*05:01/DQB1*03:01	+	ND		
S	AQALNTLVKQLSSNF	956-970	DRB1*11:01, *13:03, DQA1*01:02/DQB1*06:02	+	ND		
S	GAISSVLNDILSRLD	971-985	DQA1*01:01/DQB1*05:01	+	–		
S	VQIDRLITGRLQSLQ	991-1005	DRB1*01:01, *03:01, *11:01, *13:03	+	–		
S	APHGVVFLHVTYVPA	1056-1070	DRB1*04:04, *10:01, DQA1*01:01/DQB1*05:01	+	ND		
S	ELDKYFKNHTSPDVD	1151-1165	ND	+	–		
S	GINASVVNIQKEIDR	1171-1185	DQA1*05:01/DQB1*03:01	+	–		
S	LNEVAKNLNESLIDL	1186-1200	DRB1*13:02	+	–		
S	YEQYIKWPWYIWLGF	1206-1220	ND	+	+		
N	DAALALLLLDRLNQL	216-230	DRB1*11:01, DQA1*01:01/DQB1*05:01	+	–		
N	PSGTWLTYTGAIKLD	326-340	DRB1*01:03, *07:01	+	+		
E	LAILTALRLCAYCCN	31-45	DRB1*01:01, *11:01	+	ND		
ORF1a	PLNSIIKTIQPRVEK	276-290	DRB1*01:01, *07:01	+	–		
ORF1a	EEIAIILASFSASTS	471-485	DRB1*04:10, *07:01, DQA1*02:01/DQB1*02:02, *03:01/*03:02	+	–		
ORF1a	SPLYAFASEAARVVR	531-545	DRB1*07:01, DQA1*02:01/DQB1*02:02	+	–		
ORF1a	QTFFKLVNKFLALCA	676-690	DRB1*01:03, *07:01, DQA1*01:01/DQB1*05:01	+	–		
ORF1a	GETFVTHSKGLYRKC	706-720	DRB1*07:01	+	ND		
ORF1a	KVTFFPDLNGDVVAI	1956-1970	DQA1*02:01/DQB1*02:02, *01:01/*05:01	+	ND		
ORF1a	SHNIALIWNVKDFMS	2706-2720	DRB1*04:10, DQA1*02:01/DQB1*02:02, *03:01/*03:02, *03:01/*04:02	+	ND		

(continued to the next page)

Table 2. (Continued) A list of SARS-CoV-2-derived CD4+ T-cell epitopes that have been tested in unexposed individuals or examined for cross-reactivity with human CCCoVs

Protein	AA sequence	Position	HLA allele	Epitope-reactive T cell response in unexposed individuals	Confirmed cross-reactivity with CCCoVs	Detection method	Ref.
ORF1a	KHFYWFFSNYLKRRV	3151-3165	DRB1*04:10, *0701, *09:01, DQA1*02:01/DQB1*02:02, *03:01/*04:02	+	−		
ORF1a	NHNFLVQAGNVQLRV	3326-3340	DRB1*01:01, *07:01, *08:03, *15:01, DQA1*05:01/ DQB1*03:01, *01:02/*06:02	+	−		
ORF1a	QNCVLKLKVDTANPK	3346-3360	DRB1*04:10	+	ND		
ORF1a	NRYFRLTLGVYDYLV	3801-3815	DRB1*01:01, *01:03, *07:01, *08:03, *15:01, DQA1*02:01/DQB1*02:02, *01:01/*05:01, *01:02/*06:02	+	−		
ORF1a	VLKKLKKSLNVAKSE	3976-3990	DRB1*11:01, *13:03, DQA1*01:02/DQB1*06:02	+	+		
ORF1b	KLLKSIAATRGATVV	4966-4980	DRB1*04:04, *10:01	+	+		
ORF1b	EFYAYLRKHFSMMIL	5136-5150	DRB1*07:01, *09:01, *13:01, DQA1*03:01/DQB1*04:02	+	+		
ORF1b	GLVASIKNFKSVLYY	5166-5180	DRB1*08:03, *12:02, DQA1*01:03/DQB1*06:01	+	ND		
ORF1b	LMIERFVSLAIDAYP	5246-5260	DRB1*01:03, *07:01, *08:03, *12:02, DQA1*02:01/ DQB1*02:02, *05:01/*03:01, *01:01/*05:01, *01:03/*06:01	+	+		
ORF1b	TSHKLVLSVNPYVCN	5361-5375	DRB1*07:01, *13:01, DQA1*01:03/DQB1*06:03	+	+		
ORF1b	ISPYNSQNAVASKIL	5836-5850	DRB1*01:01, DQA1*01:02/DQB1*06:02	+	ND		
ORF1b	NVNRFNVAITRAKVG	5881-5895	DRB1*08:03, *15:01, DQA1*05:01/DQB1*03:01, *01:02/*06:02	+	+		
ORF1b	REEAIRHVRAWIGFD	6001-6015	DRB1*01:03, *07:01	+	ND		
ORF1b	TQLCQYLNTLTLAVP	6846-6860	DRB1*01:01, *15:01, DQA1*01:01/DQB1*05:01, *01:02/*06:02	+	ND		
ORF3a	SDFVRATATIPIQAS	26-40	DRB1*07:01, DQA1*02:01/DQB1*02:02	+	−		
ORF3a	ALLAVFQSASKIITL	51-65	DRB1*07:01, *13:01, DQA1*01:03/DQB1*06:03	+	−		
ORF6	MFHLVDFQVTIAEIL	1-15	DRB1*13:03	+	−		
ORF6	TFKVSIWNLDYIINL	21-35	DRB1*01:03, *07:01, *15:01, DQA1*02:01/DQB1*02:02, *01:01/*05:01	+	−		
ORF6	YIINLIIKNLSKSLT	31-45	DRB1*11:02, *13:03	+	−		
ORF7	VKHVYQLRARSVSPK	71-85	DRB1*01:03, *07:01	+	−		
ORF8	FYSKWYIRVGARKSA	41-55	DRB1*01:01, *07:01	+	−		

AA, amino acid; HLA, human leukocyte antigen; E, envelope; +, positive response; −, negative response; ND, not determined.

A recent study using a SARS-CoV-2 infection model of rhesus macaques examined the contribution of Ab and T-cell immunity to protection against SARS-CoV-2 infection (44). They demonstrated the importance of Abs by adoptive transfer of purified IgG from convalescent animals to naïve animals, which protects recipients against SARS-CoV-2 challenge. They also showed the importance of CD8+ T cells in a depletion study. Depletion of CD8+ T cells in convalescent animals partially abolished the protective immunity against SARS-CoV-2 re-challenge. They suggested that T-cell responses are important, particularly when Ab responses are suboptimal. Although this study was performed using animals recovered from previous SARS-CoV-2 infection, the conclusion can be applied to the setting of vaccination. Further studies are required to determine the individual roles of Abs and CD4+ and CD8+ T cells in the protective immunity against SARS-CoV-2 infection following vaccination. This question is also important for determining immune correlates of protection against SARS-CoV-2 infection.

PERSPECTIVES

Ending the current COVID-19 pandemic relies on herd immunity that blocks the spread of SARS-CoV-2 within the population. Herd immunity can be achieved by eliciting protective immunity via vaccination or natural infection in a considerable proportion of the population and the maintenance of this protective immunity. In SARS-CoV-2 infection, the roles of virus-

specific $CD4^+$ and $CD8^+$ T cells in protective immunity are not as well clarified as the roles of neutralizing Abs. In addition, the longevity of SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cells needs to be investigated further. Whether pre-existing SARS-CoV-2-reactive T cells elicited by CCCoVs are beneficial or harmful to hosts remains to be elucidated. Immunological research of these questions will help us end the current COVID-19 pandemic and prepare for the next pandemic by developing effective prophylactics and therapeutics and establishing proper epidemiological strategies.

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