SARS-CoV-2 Specific T Cell Epitopes

Subjects: Immunology

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The COVID-19 pandemic has caused extensive loss of lives and economic hardship. In response, infectious disease experts and vaccine developers promptly responded by bringing forth candidate vaccines, some of which have been listed in the World Health Organization's Emergency Use Listing. Differences in the human leukocyte antigen (HLA) in variation of the T cell epitopes of SARS-CoV-2 genetic mutations left room for improvement of the vaccines.

infectious diseases SARS-CoV-2 COVID-19 vaccine

1. Introduction

The COVID-19 pandemic has impacted our lives not only physically and mentally, but also economically. To date, approximately 580 million confirmed cases of COVID-19 and 6.4 million deaths have been recorded globally. The impact is no less devastating in Malaysia: with over 5000 confirmed cases daily, the total number of confirmed COVID-19 cases is approaching 5 million with almost 36,000 deaths [1].

The SARS-CoV-2 virus is the etiological agent responsible for several pneumonia-like cases that began in Wuhan, China [2]. Being the hub of transportation and industry for central China, the outbreak that started in early November, or December 2019, rapidly spread to become a pandemic [3]. Similar to other viruses that are transmitted through direct, indirect, or close contact with respiratory secretions or droplets from infected people [4], this *Betacoronavirus* spreading was greatly facilitated by international air travel [4]. The enveloped SARS-CoV-2 virus bears a large (approximately 30+ kb) single-stranded-positive sense RNA genome consisting of up to 14 open reading frames (ORFs) that are translated into the spike (S) protein, matrix (M) protein, envelope (E) protein, nucleocapsid (N) and about 16 non-structural proteins (nsps) [5]. Similar to other RNA viruses, SARS-CoV-2 also accumulates genomic mutations as it replicates owing to natural selections [6]. A number of mutations contribute to the augmented ability of the virus to replicate as well as to evade the host immune responses [6].

With the growing number of cases and the emergence of new SARS-CoV-2 mutants, infectious disease experts, epidemiologists, and public health officers have worked relentlessly to control the spread of the infection and at the same time to deduce the consequences of SARS-CoV-2 mutations. Just within a year since the COVID-19 pandemic started, vaccines have been manufactured and used by millions around the world. The exact mechanism of how SARS-CoV-2 caused severe COVID-19 disease, however, is still not known. The Major Histocompatibility complex (MHC) system or human leukocyte antigen (HLA) complex in humans is located on the short arm of chromosome 6 (6p21.3) [7]. Normally inherited as an en bloc from each parent in a no recombination event, linked

HLA genes (HLA-A, -B, -C, -DR, -DQ, -DP) are combined as a HLA haplotype and transmitted on a single parental chromosome [8]. Abiding by its imperative functions in self-recognition, eliciting the immune response to an antigenic stimulus and to the regulation of cellular and humoral immunity, HLA class I antigens (HLA-A, -B, and -C) are expressed on the surface of all nucleated cells and platelets (except those of the central nervous system) [9] while the HLA class II antigens (HLA-DR, -DP, and -DQ) are expressed on antigen-presenting cells (APC) [9]. These highly polymorphic HLA loci are involved in antigen presentation to CD8+ T cells (HLA class I), natural killer cells, and CD4+ T cells (HLA class II) [10].

The fate of the SARS-CoV-2 virus and the outcomes of the infection are highly dependent on the efficiency of one's immune system, particularly the T-cell immunity. Considering that the HLA haplotype occurs differently in different populations, the efficiency in SARS-CoV-2 viral clearance and disease progression in return are speculated to be varied. Studies associated with SARS-CoV-2 and HLA have focused on the involvement of cytotoxic CD8+ T and helper CD4+ T lymphocytes as their responses are vital for initial viral clearance, the development of immunologic memory, and eventually for orchestrating the adaptive immune responses [11].

2. SARS-CoV-2 Specific T Cell Epitopes

The search for potential vaccine targets has led to numerous studies to decipher the T cell epitopes that can evoke the MHC-I and MHC-II responses. **Table 1** present the distribution of SARS-CoV-2 T cell epitopes as predicted from the combinations of the cohort (unexposed and convalescent individuals), bioinformatics, and mathematical modeling studies. Presentation of multiple SARS-CoV-2 epitopes is deemed critical in the induction of vaccine-based and natural infection immunity [12][13][14][15]. Detection of post-infectious T cell immunity is feasible through the employment of SARS-CoV-2-specific peptides even in seronegative convalescent individuals [12][16]. In the absence of antibody responses, specific T cell responses were observed in seronegative convalescent donors but not in unexposed donors, hence emphasizing the activation of T cell immunity upon infection. The SARS-CoV-2 CD4+ T cell is essential in evoking persistent and robust immune responses compared to the HLA class I T cell epitopes [12]. CD4+ T cell recognizes multiple dominant HLA-DR T cell epitopes [12]. The SARS-CoV-2 M protein was recognized by specific CD4+ T cells in COVID-19 cases [14]. The inadequacy of quality class II epitopes from the M protein is contributed to by its small size [17]. Although class II epitopes are predominantly available across the SARS-CoV-2 genomes, it appears that highly expressed proteins are preferred by memory CD4+ T cells [18].

Table 1. Distribution of CD4+ and CD8+ epitopes based on SARS-CoV-2 proteins.

No.	Protein(s) and Their (Respective Numbers of Epitopes)	Subset	Ref.
1	N (4) non-RBD-S (90), RBD-S (23), E (2), ORF3a (7), ORF7a (3), ORF6 (7), ORF8 (4), nsp1 (1), nsp2 (7), nsp3 (11), nsp4 (10), nsp5 (4), nsp6 (9), nsp8 (2), nsp10 (2), nsp12 (9), nsp13 (4), nsp14 (4), nsp15 (1), nsp16 (2)	CD4	[18]
2	S (29), N (15), M (27), ORF7a (1)	CD4	[19]
3	S (11), N (21), ORF3a (4)	CD8	[19]

No.	Protein(s) and Their (Respective Numbers of Epitopes)	Subset	Ref.
4	ORF9+N (50), ORF5+M (11), ORF2+S (3), ORF3 (4), ORF4+E (1), ORF6 (1), ORF8 (6)	CD4	[12]
5	ORF1 (4), ORF2+S (2), ORF9+N (5), ORF5+M (1)	CD8	[12]
6	S (1), ORF1ab (4)	CD8	[20]
7	M (2), S (5), ORF1ab (24), ORF3 (1), ORF6 (1), ORF7 (2), ORF8 (1)	CD4	[20]
8	nsp1 (2), nsp2 (14), PLpro (34), nsp4 (22), 3CL (6), nsp6 (18), nsp7 (4), nsp8 (4), nsp9 (2), nsp10 (2), RdRpol (19), Hel (14), nsp14 (19), nsp15 (4), nsp16 (9), S (20), ORF3a (10), E (8), M (8), ORF6 (6), ORF7a (4), ORF8 (3), N (7), ORF10 (1)	CD4	[21]
9	nsp1 (13), nsp2 (40), PLpro (128), nsp4 (40), 3CL (15), nsp6 (17), nsp7 (6), nsp8 (17), nsp9 (13), nsp10 (7), RdRpol (68), Hel (38), nsp14 (33), nsp15 (25), nsp16 (16), S (86), ORF3a (20), E (2), M (15), ORF6 (2), ORF7a (8), ORF8 (3), N (13), ORF10 (3)	CD8	[21]
10	N (8), nsp7 (1)	CD4	[14]
11	N (3)	CD8	[14]
12	ORF1ab (40), M (3), S (6), N (2), ORF3a (3), ORF7a (1)	CD8	[22]
13	ORF1ab (40), M (3), S (6), N (2), ORF3a (3), ORF7a (1)	CD4	[22]
14	ORF1ab (1478), S (248), ORF3a (69), E (18), M (72), ORF6 (8), ORF7a (26) ORF8 (22), N (60), ORF10 (12)	CD8	[23]
15	ORF1ab (1002), S (154), ORF3a (74), E (11), M (57), ORF6 (28), ORF7a (16) ORF8 (18), N (32), ORF10 (7)	CD4	[23]
16	M (5), N (2), S (5)	CD4	[24]
17	N (2)	CD8	[24]

T cell memory responses were observed more in patients recovering from severe COVID-19 infections than mild cases, with a greater magnitude of SARS-CoV-2-specific CD8+ T cells observed in the latter [19]. Detection of spike-specific antibodies in some donors with low (receptor-binding domain) RBD-specific antibodies indicates that antibodies were capable of targeting the non-RBD regions of the spike [19]. Establishments of T cell responses in correlation with milder cases will provide insight into protective immunity [19]. On that account, immense CD8+ T cell responses (to spike, M and N proteins) were observed in donors recovering from mild forms of the disease compared to severe cases [13][19].

CD4: cluster of differentiation 4, CD8: cluster of differentiation 8, N: nucleocapsid, S: spike, RBD-S: receptor-binding domain-spike, E: envelope, ORF: open reading frame, nsp: non-structural protein, M: matrix, PLpro: papain-like protease, RdRpol: RNA-dependent RNA polymerase, Hel: helicase.

These findings coincided with different subsets of SARS-CoV-2-specific T cells. Cytotoxic CD4+ T cells might not be a major contributor to SARS-CoV-2 clearance, since, unlike in influenza virus infections, CD107a+ CD4+ T cells (of cytotoxic potential) are scarcely detected [19]. Incorporating non-spike proteins such as N, M, and ORFs proteins in future vaccine design is perhaps beneficial as central memory and effector memory CD8+ T cells were identified in response to those proteins [19]. Ferretti et al., acknowledged in their study that next-generation vaccines incorporated with shared SARS-CoV-2 epitopes residing outside the spike protein will not only be

independent of mutational variation but will also be better at eliciting SARS-CoV-2-specific CD8+ T cell immunity [15].

Heterologous immunity in SARS-CoV-2 infection is characterized by the pre-existing T cell responses against SARS-CoV-2 peptides [12]. The immunity is cross-reactive with common cold coronaviruses in 81% of unexposed individuals [12]. Mateus et al., 2020 also demonstrated the capability of SARS-CoV-2-specific memory CD4+ T cells to cross-react with corresponding ~67% homologous sequences from any of the many different commonly circulating common cold human coronaviruses (HCoV)-OC43, -229E, -NL63, and -HKU1 [18]. However, this event seems to happen uniquely in one direction and not vice versa [18]. Despite being highly speculative and vague, the implications of pre-existing HCOVs memory CD4+ T cells on the magnitude of SARS-CoV-2 infection are ascertained [18]. Although the magnitude of T cell responses is not associated with disease severity, severely ill patients possibly lack pre-existing SARS-CoV-2 T cells. This is demonstrated by lower recognition rates of SARS-CoV-2 T cell epitopes in individuals with more severe COVID-19 symptoms compared to non-hospitalized patients with high antibody titers [12].

While bioinformatics- and mathematical modeling-type studies have limitations of their own [20][21][22][23], cohort and case-control studies also come with some drawbacks [12][13][14][18][19][24]. The cohort and case-control studies discussed in their review are largely affected by the number of donors. Meticulous evaluation in comparing mild and severe cases is inconceivable without taking diverse T cell receptors, peptide-MHC affinities, and antigen sensitivities for different epitopes into consideration [12][13][14][18][19][24]. The significance of these factors is worthy of being addressed in future studies. Different techniques applied to determine IFN-γ-producing SARS-CoV-2-specific T cell responses yield contrasting results. This drawback is a result of detection method discrepancies as demonstrated by peptide-stimulated activation-induced marker (AIM) assays and ELISpots and ICS assays in a recent immunogenicity study of recombinant adenovirus type-5-vectored COVID-19 vaccine human phase I trial [13][25]. Although both methods are valid, the functional relevance is different.

The geographical regions where the studied donors are recruited also influenced cross-reactive responses as different coronaviruses (in both humans and animals) are circulating in different populations [26][27][28][29]. Pre-existing T cells exhibiting cross-reactivity do not necessarily imply previous coronavirus infections, but they could potentially be primed by other microbes too [30]. Thus, further detailed investigations based on this factor are necessary. Furthermore, the cohort studies focused on T cell responses in PBMCs instead of memory T cells at the site of infection most likely contributes to effective protection as observed in influenza virus infection [19].

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