Short communication

BCG vaccine may generate cross-reactive T cells against SARS-CoV-2: *In silico* analyses and a hypothesis

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ABSTRACT

The world is facing the rising emergency of SARS-CoV-2. The outbreak of COVID-19 has caused a global public health and economic crisis. Recent epidemiological studies have shown that a possible association of BCG vaccination program with decreased COVID-19-related risks, suggesting that BCG may provide protection against COVID-19. Non-specific protection against viral infections is considered as a main mechanism of BCG and clinical trials to determine whether BCG vaccine can protect healthcare workers from the COVID-19 are currently underway. We hypothesized that BCG may carry similar T cell epitopes with SARS-CoV-2 and evaluated the hypothesis by utilizing publicly available database and computer algorithms predicting human leukocyte antigen (HLA) class I-binding peptides. We found that BCG contains similar 9-amino acid sequences with SARS-CoV-2. These closely-related peptides had moderate to high binding affinity for multiple common HLA class I molecules, suggesting that cross-reactive T cells against SARS-CoV-2 could be generated by BCG vaccination.

1. Introduction

The world is facing the rising emergency of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) since December 2019 and the outbreak of coronavirus disease 2019 (COVID-19) has caused a global public health and economic crisis.

Many countries are being forced to introduce strict limitations in order to reduce risk of contagion and COVID-19 is considered pandemic since 11th March 2020 [1–3]. Given the severity of the disease, vaccines and therapeutics for SARS-CoV-2 are urgently needed [1]. Accumulating evidence suggests that the human CD4 + and CD8+ T cells can response to SARS-CoV-2 [4–7], however, the knowledge about immune reactions against SARS-CoV-2 in human body is still partial [1].

Bacillus Calmette-Guérin (BCG) is a live-attenuated vaccine strain of *Mycobacterium bovis* that protects against tuberculosis. A preclinical study has shown that BCG vaccination induces genome-wide epigenetic reprogramming of human monocytes that correlates with protection against experimental viral infection [8]. Although conflicting results exist [9–11], recent epidemiological studies have shown that a possible association of BCG vaccination program with decreased COVID-19-related risks, suggesting that BCG vaccination may provide protection against COVID-19 [2,3,10,12,13].

Clinical trials to determine whether BCG vaccine can protect healthcare workers from the COVID-19 are currently underway (NCT04327206, NCT04328441, NCT04348370) [14]. These clinical studies have been initiated based on the idea that BCG vaccine may have non-specific immune protective effects against viral infections including COVID-19, highlighting the importance of innate immunity [2,3]. Studies have shown that BCG vaccination induces histone modifications and epigenetic reprogramming of innate immune cells, resulting in a more active innate immune response upon re-stimulation and a decreased susceptibility to respiratory tract infections, which has been termed trained immunity [8,15,16].

We thought that understanding the mechanism of protective effect of BCG vaccine against SARS-CoV-2 may guide the development of COVID-19 vaccine. To defend the body against viral infections, the cooperation between innate (non-specific) and adaptive

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(specific) immune system plays a crucial role [1,17]. Adaptive immunity, which is characterized by three unique features: specificity, diversity, and memory, is essential for regulating viral infections [17]. Therefore, we hypothesized that BCG may carry similar T cell epitopes with SARS-CoV-2. If similar T cell epitopes with SARS-CoV-2 are included in the amino-acid sequences of *Mycobacterium bovis*, BCG vaccination may activate not only non-specific innate immune system but also adaptive immune system and induce memory T cells specific to SARS-CoV-2, which leading to a lower COVID-19 growth rate in countries with known BCG policy.

Cross-reactivity is a term that was used to indicate unexpected reactivity to targets that differed from those used to initially define the T cell clone [17,18]. Studies revealed that T cells can recognize dissimilar epitopes [17]. Importantly, T cell cross-reactivity have been reported for cytotoxic T lymphocyte (CTL) responses to viruses including coronaviruses [5,6,17]. It has been shown that CTL elicited during a primary viral infection also can respond when the same host is re-infected with unrelated viruses in preclinical murine model [18]. By mapping the different viral epitopes to which a particular T cell clone responds, it was demonstrated that T cells can recognize similar but not identical peptides, suggesting that T cells specific to SARS-CoV-2 can be generated by BCG vaccination if *Mycobacterium bovis* contains similar T cell epitopes with SARS-CoV-2 [17,19].

2. Materials and methods

Similar amino acid sequences between SARS-CoV-2 and *Mycobacterium bovis* were searched by using protein BLAST (Basic Local Alignment Search Tool). Entire amino acid sequences of SARS-CoV-2 (MN908947.3) were annotated to the BLAST nr database of *Mycobacterium bovis* BCG str. Tokyo 172 (taxid:561275).

The human leukocyte antigen (HLA) class I molecules typically bind peptides 8–11 amino acids (mainly 9 amino acids) in length. Thus, similar 9-amino acids sequences between SARS-CoV-2 and *Mycobacterium bovis* (BCG str. Tokyo 172 [taxid:561275]) were analyzed by two computer algorithms; IEDB analysis resource (T cell epitope prediction tool, http://tools.iedb.org/main/tcell/) and NetMHCpan 4.1 (http://www.cbs.dtu.dk/services/NetMHCpan/) to evaluate whether these peptides can bind to common HLA class I (HLA-A, -B, or -C alleles) and can be recognized by CTL. We selected the 15 frequent HLA class I alleles in Japan or the United States of America from a allele frequency cutoff > 6%. The specific alleles tested for peptide binding affinity were: HLA-A (A*01:01, A*02:01, A*03:01, A*11:01, A*24:02, A*33:03), HLA-B (B*07:02, B*08:01, B*44:03, B*52:01) and HLA-C (C*01:02, C*03:04, C*05:01, C*06:02, C*07:01). Frequency of HLA alleles among humans were searched by a publicly available database (Allele Frequency Net Database, http://www.allelefrequencies.net/hla.asp). As of July 11th, 2020, total confirmed deaths per million population caused by COVID-19 in Japan and the United States of America were 7.7 and 412, respectively. Data of COVID-19 cases and death per country were obtained from World Health Organization (WHO) coronavirus disease (COVID-2019) situation reports (https://www.who.int/emergencies/diseases/novel-coronavirus-2019-situation-reports) on July 11th, 2020. Data of total population of countries were obtained from WHO website (https://www.who.int/countries/en/).

3. Results

We evaluated the hypothesis by utilizing publicly available database and two computer algorithms predicting HLA class I-binding peptides. We first searched similar amino acid sequences between SARS-CoV-2 and *Mycobacterium bovis* by using protein BLAST (Basic Local Alignment Search Tool). Entire amino acid sequences of SARS-CoV-2 (MN908947.3) were annotated to the BLAST nr database of *Mycobacterium bovis* BCG str. Tokyo 172 (taxid:561275).

Intriguingly, we found that *Mycobacterium bovis* has total seven similar 9-amino acids sequences, which include only one amino acid substitution, with SARS-CoV-2. Six out of 7 sets of different but closely related peptides between SARS-CoV-2 and *Mycobacterium bovis* are shown in Table 1. Unexpectedly, we also found that *Mycobacterium bovis* contains many 7-amino acid sequences completely identical to SARS-CoV-2. More than 40 identical heptamer amino acid sequences with SARS-CoV-2 were found in *Mycobacterium bovis* BCG str. Tokyo 172 (data not shown).

To evaluate whether the seven similar 9-amino acids sequences can bind to common HLA class I (HLA-A, -B, or -C alleles) and can be recognized by CTL, the amino acid sequences of both SARS-CoV-2 and *Mycobacterium bovis* were analyzed by two computer algorithms; IEDB analysis resource (T cell epitope prediction tool, http://tools.iedb.org/main/tcell/) and NetMHCpan 4.1 (http://www.cbs.dtu.dk/services/NetMHCpan/).

One out of 7 sets of these similar peptides (SARS-CoV-2, ELAKMVSLD; *Mycobacterium bovis*, ELAKLVSLD) was not predicted to bind to any HLA class I molecules tested. However, analyses using IEDB analysis resource demonstrated that six of 7 sets of different but closely related peptides between SARS-CoV-2 and *Mycobacterium bovis* had moderate to high binding affinity for multiple common HLA class I molecules (Table 1). Analyses using NetMHCpan 4.1 demonstrated that four sets of these similar peptides had weak to high binding affinity for common HLA class I molecules (Table 2). These results suggest that the similar peptides between SARS-CoV-2 and *Mycobacterium bovis* can be presented by common HLA class I and have the potential to induce cross-reactive T cells.

4. Discussion

Recent studies have reported that BCG vaccination has the potential to provide protective effects against viral infections [8,16]. In addition, epidemiological studies suggested a possible association of BCG vaccination program with decreased COVID-19-related risks [2,8,10,12–14]. Clinical studies have been initiated based on the idea that BCG vaccine has non-specific immune protective effects against COVID-19 [10,14].

However, the essential role of vaccination is to generate immunological memory, which is an exclusive property of "adaptive" or acquired" immune system, to viral infections [17]. Antigen-specific T cell receptor expressing T cells proliferate and differentiate in response to a primary infection and remain in the host after resolution of the infection. When host immune system encounter reinfection, memory T cell clones equipped with antiviral effector functions can rapidly expand and confer resistance to reinfection. Effector CD8+ T cells (called cytotoxic T lymphocytes, or CTLs) play a crucial role in destroying virus-infected cells. CTLs recognize viral-derived peptide-HLA complexes and kill specifically viral-infected cells. Importantly, recent studies suggested that cross-reactive T cell recognition between "common cold" coronaviruses and SARS-CoV-2 can be exist [5,6]. Cross-reactive CTL epitopes have been shown to exist between human immunodeficiency virus and *Mycobacterium tuberculosis* [17,19]. Therefore, we hypothesized that BCG may carry similar T cell epitopes with SARS-CoV-2, which leading to the potential to generate cross-reactive T cells response to SARS-CoV-2.

We investigated the similarity of amino acid sequences between SARS-CoV-2 and *Mycobacterium bovis* and found that

Table 1
HLA binding affinity of similar 9-amino acids sequences between SARS-CoV-2 and *Mycobacterium bovis* predicted by a IEDB analysis resource T cell epitope prediction tool.

| Amino acid sequence | SARS-CoV-2 | BCG | Gene name in SARS-CoV-2 | Protein name in BCG | HLA allele | Predicted HLAbinding affinity | | HLA allele | Predicted HLA binding affinity | |
						SARS-CoV-2	BCG		SARS-CoV-2	BCG
Sequence 1	VLGSLAATV	VLGGLAATV	*ORF1ab* (nsp9)	UDP-N-acetylmuramoylalanyl-D-glutamate-2,6-diaminopimelate ligase	*HLA-A*02:01*	1.1	1.4	*HLA-B*52:01*	2.2	1.9
Sequence 2	LQGPPGTGK	LLGPPGTGK	*ORF1ab* (nsp13)	Type VII secretion AAA-ATPase EccA	*HLA-A*11:01*	4.2	3.6	*HLA-A*33:03*	5.4	3.3
Sequence 3	QPTSEAVEA	RPTSEAVEA	*ORF1ab* (nsp2)	PPOX class F420-dependent enzyme	*HLA-B*07:02*	8.9	2.2	*Not predicted*		
Sequence 4	LAPLLSAGI	LAPLLCAGI	*ORF1ab* (nsp3)	Alcohol dehydrogenase	*HLA-B*52:01*	1.1	4.8	*HLA-C*01:02*	1.3	5.1
Sequence 5	LAPLLSAGI	LAPLLSAGA	*ORF1ab* (nsp3)	Metal-transporting ATPase	*HLA-C*03:04*	1.4	3.9	*Not predicted*		
Sequence 6	LVPFWITIA	LGPFWITIA	*ORF1ab* (nsp4)	Sugar ABC transporter permease	*HLA-C*03:04*	5.5	8	*Not predicted*		

Different amino acid residues between SARS-CoV-2 and *Mycobacterium bovis* are underlined. Common *HLA-A* (*A*01:01, A*02:01, A*03:01, A*11:01, A*24:02, A*33:03*), *HLA-B* (*B*07:02, B*08:01, B*44:03, B*52:01*) and *HLA-C* (*C*01:02, C*03:04, C*05:01, C*06:02, C*07:01*) were tested for peptide binding affinity. The amino acid sequences of both SARS-CoV-2 and *Mycobacterium bovis* were analyzed by a computer algorithm (IEDB analysis resource, T cell epitope prediction tool, http://tools.iedb.org/main/tcell/). Only the alleles which showed moderate to high binding affinity (Percentile Rank < 10) for both closely similar peptides are shown in this Table 1. *Not predicted* indicates that these is no HLA allele which can bind to peptide sequences among tested HLA alleles. Frequency of HLA alleles among humans were searched by a publicly available database (Allele Frequency Net Database, http://www.allelefrequencies.net/hla.asp).

Table 2
HLA binding affinity of similar 9-amino acids sequences between SARS-CoV-2 and *Mycobacterium bovis* predicted by NetMHCpan 4.1.

| Amino acid sequence | SARS-CoV-2 | BCG | Gene name in SARS-CoV-2 | Protein name in BCG | HLA allele | Predicted HLA binding affinity | | HLA allele | Predicted HLA binding affinity | |
						SARS-CoV-2	BCG		SARS-CoV-2	BCG
Sequence 1	VLGSLAATV	VLGGLAATV	*ORF1ab* (nsp9)	UDP-N-acetylmuramoylalanyl-D-glutamate-2,6-diaminopimelate ligase	*HLA-A*02:01*	0.36 (High)	0.32 (High)	*Not predicted*		
Sequence 2	LQGPPGTGK	LLGPPGTGK	*ORF1ab* (nsp13)	Type VII secretion AAA-ATPase EccA	*HLA-A*03:01*	0.42 (High)	0.03 (High)	*HLA-A*11:01*	0.47 (High)	0.50 (High)
Sequence 3	QPTSEAVEA	RPTSEAVEA	*ORF1ab* (nsp2)	PPOX class F420-dependent enzyme	*HLA-B*07:02*	1.89 (Weak)	0.65 (Weak)	*Not predicted*		
Sequence 4	LAPLLSAGI	LAPLLCAGI	*ORF1ab* (nsp3)	Alcohol dehydrogenase	*HLA-C*01:02*	0.36 (High)	1.6 (Weak)	*Not predicted*		
Sequence 5	LAPLLSAGI	LAPLLSAGA	*ORF1ab* (nsp3)	Metal-transporting ATPase	*Not predicted*			*Not predicted*		
Sequence 6	LVPFWITIA	LGPFWITIA	*ORF1ab* (nsp4)	Sugar ABC transporter permease	*Not predicted*			*Not predicted*		

Different amino acid residues between SARS-CoV-2 and *Mycobacterium bovis* are underlined. Common *HLA-A* (*A*01:01, A*02:01, A*03:01, A*11:01, A*24:02, A*33:03*), *HLA-B* (*B*07:02, B*08:01, B*44:03, B*52:01*) and *HLA-C* (*C*01:02, C*03:04, C*05:01, C*06:02, C*07:01*) were tested for peptide binding affinity. The amino acid sequences of both SARS-CoV-2 and *Mycobacterium bovis* were analyzed by a computer algorithm (NetMHCpan 4.1, http://www.cbs.dtu.dk/services/NetMHCpan/). High indicates strong binding peptides. Weak indicates weak binding peptides. *Not predicted* indicates that these is no HLA allele which can bind to peptide sequences among tested HLA alleles. Frequency of HLA alleles among humans were searched by a publicly available database (Allele Frequency Net Database, http://www.allelefrequencies.net/hla.asp).

BCG contains closely similar 9-amino acid sequences with SARS-CoV-2. HLA binding affinity analyses *in silico* demonstrated that these closely similar 9-mer peptides can be T cell epitopes. It has been known that HLA-class I molecules can bind peptides 8 amino acids in length and induce antigen-specific T cells [20,21]. In addition to seven similar 9 amino acids sequences, we found more than 40 identical heptamer amino acids sequences with SARS-CoV-2 in *Mycobacterium bovis* BCG str. Tokyo 172. Although we did not assess the binding capacity of the 8-amino acids sequences of BCG including the identical heptamer amino acids sequences with SARS-CoV-2, our study implies that BCG may contains amino acids sequences which have the potential to induce cross-reactive T cells against SARS-CoV-2 other than the similar 9-mer peptides.

In a recent study, NetMHCpan 4 has been used to predict potential T cell epitopes derived from SARS-CoV-2 [21,22]. Thus, we have used two computer algorithms, NetMHCpan 4.1 and IEDB analysis resource, to predict HLA class I-binding peptides. We have analyzed the closely related 9-mer amino acids sequences between

SARS-CoV-2 and *Mycobacterium bovis* by using these two computer algorithms and the analyses showed different results (Tables 1 and 2). Among these similar peptides, both computer algorithms demonstrated that the sequence 1, 2, 3, and 4 have good binding affinity for common HLA class I molecules, suggesting that these four closely related 9-mer peptides are candidates for further investigation.

Different countries use different strains of the *Mycobacterium bovis* [23]. We analyzed amino acid sequences of only BCG str. Tokyo 172 (taxid:561275), thus other strains of *Mycobacterium bovis* may show the different results from current study.

Viruses enter human cells by two ways. A virus infects cells directly through the interactions between virus particles and their receptors at the cell surface. After a virus infects a cell, the virus use the protein-synthesis machinery of the host cell to synthesize its own proteins [17,24]. Some of the synthesized viral proteins are degraded into 8–11-mer peptide fragments, which bind to HLA class I molecules if they have sufficient binding affinity. Then, the

HLA class I-peptide complex is presented on the cell surface of an infected cell. The viral-peptide specific activated CD8+ T cells can recognize the HLA class I-peptide complex and induce apoptosis of the infected cell. Antigen-presenting cells, such as dendritic cells engulf viral particles or remnants of infected cells and process them and present viral-derived peptides on MHC class I molecules via the cross-presentation pathway [17,24]. The dendritic cells stimulate viral antigen-specific naïve CD8+ T cells, and these activated CD8+ T cells differentiate into effector T cells, so-called CTLs that can recognize the HLA class I-peptide complex on infected cells. Recent studies have shown that SARS-CoV-2-specific CD8+ T cells can be detected in peripheral blood mononuclear cells of COVID-19 patients [5,6], which suggesting that some SARS-CoV-2-derived peptides can be naturally processed in human cells, presented on HLA-class I molecules and induce SARS-CoV-2-specific CTLs. In current study, we evaluate whether the similar 9-amino acids sequences can bind to common HLA class I molecules by using publicly available computer algorithms predicting HLA class I-binding peptides. However, we did not evaluate natural processing of the closely similar 9-mer peptides or binding to specific HLA class I molecules on human cells, which need be validated by future research.

All of the similar peptide sequences between SARS-CoV-2 and *Mycobacterium bovis* identified in current study are derived from open reading frame1ab (ORF1ab) gene of SARS-CoV-2. The ORF1ab gene expresses a polyprotein, which is comprised of 16 nonstructural proteins [25]. Thus, neutralizing antibody response to SARS-CoV-2 can not be generated. Recent studies have shown that COVID-19 patients mounted CD4+ and CD8+ T cells specific for peptides derived from ORF1ab of SARS-CoV-2 [5,6]. Although the presence of CD8+ T cells specific for the similar 9-mer peptide sequences have not been detected in COVID-19 patients, these accumulating evidences suggest immunogenicity of the peptides derived from ORF1ab gene and may support our hypothesis.

CD8+ T cells control viral infection through its direct cytotoxic activity and pro-inflammatory cytokine productions [24]. CD4+ T cells play essential roles in controlling viral infection through multiple mechanisms including enhancement of CD8+ T cell responses and promotion of memory responses. Thus, optimal protection against viral infection requires CD4+ T cell activation [17,26]. BCG vaccine has been shown to induce human memory CD4+ T cells [27], suggesting BCG vaccine may have the potential to induce cross-reactive memory T cells efficiently.

Data shown in this study are entirely originated form *in silico* analyses. Thus, our hypothesis needs to be further investigated. Although we are unable to prove our hypothesis at this time, our hypothesis proposed here could be validated by in vitro experiments. First, we need to induce HLA-class I-restricted CTL clones that specifically recognize *Mycobacterium bovis*-derived peptides by using candidate peptides (Tables 1 and 2) and healthy donor-derived peripheral blood mononuclear cells. Second, SARS-CoV-2 infected target cells need to be prepared from the same donor-derived and SARS-CoV-2 entry receptor ACE2 (angiotensin converting enzyme 2)-expressing cells. Third, cytotoxicity and cytokine-release assays need to be done to evaluate the specific SARS-CoV-2-infected cell recognition and cytotoxic function of *Mycobacterium bovis*-specific CTL clones by using these effector and target cells.

In conclusion, although the mechanisms of protective effect of BCG vaccine against SARS-CoV-2 need further investigation, our results and accumulating evidence suggest that BCG vaccine has the potential to generate cross-reactive T cells against SARS-CoV-2. The present study may provide new insights into the immune response to SARS-CoV-2 and guide the development of COVID-19 vaccine.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical statement

The present study was approved by the Kumamoto University Institutional Review Board (IRB number, 2020; Approval Date, April 17, 2020). This study was prepared in accordance with the Helsinki Declaration.

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