Identification of similar epitopes between SARS-CoV-2 and Bacillus Calmette–Guérin: potential for cross-reactive adaptive immunity

Running head: Epitope similarity between SARS-CoV-2 and BCG

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Abstract

Objectives. Bacillus Calmette–Guérin (BCG) vaccination has been implicated in protection against SARS-CoV-2 and as a non-specific immunization method against the virus. We therefore decided to investigate T cell and B cell epitopes within the BCG Pasteur strain proteome for similarity to immunogenic peptides of SARS-CoV-2. **Methods.** We used a bioinformatic approach and analyzed the BCG-Pasteur proteome to identify similar peptides to established and novel SARS-CoV-2 T cell and B cell epitopes. **Results.** We found 112 BCG MHC-I restricted T cell epitopes similar to MHC-I restricted T cell SARS-CoV-2 epitopes and 690 BCG B cell epitopes similar to SARS-CoV-2 B cell epitopes. The SARS-CoV-2 T cell epitopes represented 16 SARS-CoV-2 proteins, the SARS-CoV-2 B cell epitopes represented 5 SARS-CoV-2 proteins, including the receptor binding domain of the spike glycoprotein. **Conclusion.** Altogether our results provide a mechanistic basis for the potential cross-reactive adaptive immunity that may exists between the two microorganisms.

Keywords

COVID-19, SARS-CoV-2, 2019-nCoV, Bacillus Calmette–Guérin, BCG

Introduction

The current pandemic caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) has led to exponentially rising morbidity and mortality worldwide. Apart from aggressive quarantine and hygiene control measures, the most effective way to inhibit SARS-CoV-2 spread is a population wide vaccination campaign. Since the date of the introduction and overall effectiveness of SARS-CoV-2 vaccines are not known, alternative approaches for active immunization against SARS-CoV-2 are under consideration. The role of BCG vaccination in the prevention of SARS-CoV-2 infection and in the epidemiology of COVID-19 has been frequently implicated [1]. BCG is an attenuated *Mycobacterium bovis* strain, a species of the *Mycobacterium tuberculosis* complex and is used worldwide for vaccination against *Mycobacterium tuberculosis*. Interestingly, BCG appears to have multiple effects. Thus, it was observed that BCG immunization could also induce a so-called heterologous immune response against viruses such as human papilloma virus, influenza A, live yellow fever vaccine, and hepatitis B (HBsAg) as well as organisms such as *Candida albicans* (*C. albicans*), and *Staphylococcus aureus* (*S.aureus*) [2, 3, 4].

The adaptive component of heterologous immunity is thought to originate from the epitope similarity between distant microorganisms coupled with the polyspecificity of T cell and B cell responses. In this study we decided to explore if BCG immunization induced heterologous adaptive immunity could theoretically play a role in the protection against SARS-CoV-2. We compared the T cell and B cell epitopes of the BCG strain Pasteur 1173P2 (BCG-Pasteur) with T cell and B cell epitopes of SARS-CoV-2 and identified numerous highly similar epitopes between the two microorganisms.

Results

General description of peptide similarity between the BCG-Pasteur and SARS-CoV-2 proteomes. To explore the similarity of the BCG-Pasteur proteome to other proteomes, we compared the 9-mer and 15-mer peptides of the BCG-Pasteur proteome to 40 viral proteomes and the *Candida albicans* and *Staphylococcus aureus* proteomes. We chose the 9-mer and 15-mer peptide lengths as characteristic lengths for MHC-I restricted epitopes and MHC-II restricted epitopes/ linear B cell epitopes. Our data showed that the number of both the 9-mer and 15-mer peptides with high identity (>=67%) to BCG-Pasteur peptides were strongly correlated with the proteome sizes of the microorganisms (Figure 1 a-b). In addition to SARS-CoV-2, the comparison included RSV, HPV, yellow fever virus, influenza-A, HSV-1, *Candida albicans* and *Staphylococcus aureus*. These pathogens served as positive controls, as the prevalence/severity of the infections caused have been shown to be reduced by BCG vaccination and/or BCG induced cross-immunity was observed previously [2,5]. These pathogens, including SARS-CoV-2, correlate very well and follow the trend between genome length and the prevalence of similar peptides across a wide range of proteome sizes. These data indicate that the BCG-mediated protection does not require a particularly high degree of sequence similarity coverage between the proteomes of BCG and other microorganisms. Comparison of the similarity between BCG-Pasteur proteome and the original SARS-CoV-2 or random proteomes showed that the original SARS-CoV-2 proteome contained a significantly higher number of similar 9-mer peptides than all of the 50 random proteomes. For the 15-mer peptides, the SARS-CoV-2 proteome demonstrated higher numbers of similar peptides than most of the random proteomes (Figure 2a). Next we explored how the peptide length and degree of identity between the peptide pairs influence the number of similar peptides between the BCG-Pasteur and SARS-CoV-2 proteomes (Figure 2b). Very large numbers of 9-mer and 15-mer peptides with limited (<3 aa) identity were found but when using >=67% identity as a threshold level, the numbers of 9-mer peptides with 6 or higher identical amino acids were 40352 and the numbers of 15-mer peptides with 10 or more identical amino acids were 24 (Figure 2b insets).

Identification of BCG-Pasteur epitopes that are highly similar to the putative SARS-CoV-2 T cell epitopes. Firstly, we mapped the experimentally verified SARS-CoV-2 T cell MHC-I restricted epitopes to the BCG-Pasteur proteome using an identity threshold of >=67%. Further NetMHC 4.0 analysis identified 217 “strong binder” BCG-Pasteur peptides that could be presented by at least one representative allele of an HLA-A or HLA-B supertype. A third analysis step compared the BCG-Pasteur peptide associated HLA alleles with the associated HLA allele(s) of the similar SARS-CoV-2 peptide. This selection resulted in 112 BCG-Pasteur peptides that are 1; highly similar to the MHC-I restricted SARS-CoV-2 epitope and 2; are presented by an HLA allele related to the same HLA supertype (Figure 3a) (Supplementary Table 1). These 112 BCG-Pasteur peptides had a similar counterpart in 16 different SARS-CoV-2 proteins (Figure 3b) resulting the multiple representation of SARS-CoV-2 epitopes by BCG-Pasteur epitopes. SARS-CoV-2 epitopes such as LLSAGIFGA, FLLPSLATV, ALLADKFPV and LLLDRLNQL had 9, 8, 7 and 7 similar BCG-Pasteur peptide pairs respectively. An extreme example was the SARS-CoV-2 nsp05 protein epitope VLAWLYAAV which was the single epitope of the nsp05 protein but had 23 BCG-Pasteur epitope counterparts with 66% identity and the 78% identical ALAWLVAAV epitope. The 24 BCG-Pasteur epitope pairs of VLAWLYAAV represented 23 different BCG-Pasteur proteins. The best represented SARS-CoV-2 proteins were the nsp05, a chymotrypsin-like protease (M pro) and the spike (surface) glycoproteins. A similar analysis was performed for SARS-CoV-2 MHC-II restricted T cell epitopes but we found no highly similar epitopes between the two microorganisms, primarily due to the longer length of most peptides presented via MHC-II to T cells.

Identification of BCG-Pasteur epitopes that are highly similar to the putative SARS-CoV-2 B cell epitopes. We applied a similar selection scheme as before to identify highly similar BCG-Pasteur sequences with the experimentally verified and putative SARS-CoV-2 B cell epitopes. We mapped the SARS-CoV-2 B cell epitopes to the BCG-Pasteur proteome, and collected the highly similar sequences (>=62.5% identity). The similarity between SARS-CoV-2 and BCG-Pasteur sequences ranged between 62.5% and 100%. The identified BCG-Pasteur sequences were further analyzed for potential antigenicity using the BepiPred 2.0 B cell epitope prediction

method [6]. BepiPred analysis revealed that 690 BCG-Pasteur sequences were located within a BCG-Pasteur protein region that is likely to be recognized by antibodies (Supplementary Table 2). These 690 BCG-Pasteur peptides were similar to 17 putative SARS-CoV-2 B cell epitopes located in the nsp12, orf4 (envelope protein), orf5 (membrane glycoprotein), orf9 (nucleocapsid) and the spike glycoprotein (Figure 4a), indicating a high level of representation of these SARS-CoV-2 B cell epitopes. As an example of a prevalent epitope, the potential SARS-CoV-2 nucleocapsid epitope LLPAAD had 290 similar and likely antigenic BCG-Pasteur counterparts, including an identical counterpart. The high similarity and the existence of several similar shared BCG-Pasteur epitopes with SARS-CoV-2 B cell epitopes could therefore lead to the induction of cross-specific antibodies. Interestingly, the SARS-CoV-2 spike epitope FGEVFNAT, which is located in the receptor binding domain and is likely to elicit neutralizing antibodies, had five similar BCG-Pasteur counterparts (Figure 4b-c).

Conclusion

We systematically mapped numerous SARS-CoV-2 epitopes to the BCG-Pasteur proteome to find similar epitopes that might induce adaptive cross immunity and explain the protective qualities of BCG vaccination against SARS-CoV-2. Our analysis of similar peptides of BCG-Pasteur and other proteomes revealed that the occurrence of epitope similarity is strongly correlated to the proteome sizes of the microorganisms. As expected, the SARS-CoV-2 proteome behaved similarly, indicating that the coexistence of cross immunity with BCG-Pasteur is likely not due to exceptional similarity between these evolutionary distant microorganisms. The fact that the SARS-CoV-2 proteome contained higher number of similar epitopes than all (9-mer) or most of (15-mer) the random proteomes further supports that the similarity does not arise by chance between the two proteomes. Rather, short conservative protein sequences exist even between these distant microorganisms. The number of highly similar peptides between the two proteomes strongly depended on the length of the peptides. Thus, the numbers of similar 9-mer peptide pairs were 3 orders of magnitude higher than that of the 15-mers. The immunological consequence of these findings would be that the adaptive cross-immunity between these microorganisms has a higher chance to be induced between shorter epitopes and are therefore

inherently directed towards a MHC-I restricted T cell response. Alternatively, these data could reflect MHC-II restricted T cell responses to shorter epitopes and B cell recognition of short linear epitopes or smaller conformational epitopes. Our immunological analyses of the similar peptides supported the former possibility since we identified 112 similar MHC-I restricted T cell epitope pairs but did not identify similar MHC-II restricted epitopes. However, these findings do not completely rule out MHC-II restricted cross-immunity between BCG and SARS-CoV-2, since epitopes with low level of full-length sequence similarity can indeed induce cross-immunity [7]. We found that SARS-CoV-2 B cell epitopes having BCG-Pasteur counterparts were present mostly in structural proteins including the spike glycoprotein, a dominant target for protective antibodies. Among the 8 spike glycoprotein epitopes which had similar BCG-Pasteur peptide counterparts, one was located in the receptor binding domain, the target of neutralizing antibodies [8].

Overall, we have shown that there are shared T cell and B cell epitopes between SARS-CoV-2 and BCG-Pasteur which suggests that BCG induced immunity could influence the adaptive immune response against SARS-CoV-2. Although this *in silico* study has not demonstrated functional cross-reactive immunity, our work does support the further investigation of heterologous immunity between BCG and SARS-CoV-2. The similar BCG-Pasteur peptides identified in this study could be used as a reference set to assist the evaluation of BCG induced SARS-CoV-2 directed T cell and B cell responses.

Methods

Proteome comparison of BCG-Pasteur and SARS-CoV-2. SARS-CoV-2 protein sequences were compared to the BCG-Pasteur proteome (*Mycobacterium tuberculosis* variant bovis BCG p-1173P2 (GCF_000009445.1)). The following SARS-CoV-2 protein sequences were used: nsp1 (YP_009742608), nsp2 (YP_009742609), nsp3 (YP_009742610), nsp4 (YP_009742611), nsp5 (YP_009742612), nsp6 (YP_009742613), nsp7 (YP_009742614), nsp8 (YP_009742615), nsp9 (YP_009742616), nsp10 (YP_009742617), nsp11 (YP_009725312), nsp12 (YP_009725307), nsp13 (YP_009725308), nsp14 (YP_009725309), nsp15 (YP_009725310), nsp16 (YP_009725311), spike glycoprotein (YP_009724390), ORF3a (YP_009724391), ORF4 (YP_009724392), ORF5

(YP_009724393), ORF6 (YP_009724394), ORF7a (YP_009724395), ORF7b (YP_009725318), ORF8b (YP_009724396), ORF9 (YP_009724397), ORF10 (YP_009725255). For sequence comparison the previously described sequence identity measure was used [7]. BCG-Pasteur and SARS-CoV-2 peptide pairs that shared a >=67% identity in their peptide sequences were collected. The same identity measure was used to compare the BCG-Pasteur proteome to 39 viral proteomes and the *Candida albicans* and *Staphylococcus aureus* proteomes. The same identity measure was used to compare the BCG-Pasteur proteome to 50 randomly generated proteomes consisting of same number of peptides with the same amino acid compositions but scrambled order the SARS-CoV-2 protein sequences used before. To identify whether the SARS-CoV-2 proteome contained more similar peptides than the random proteomes two methods were applied: 1, Inter-Quartile Range (IQR, IQR = Q3 - Q1) based outlier detection (Q1: first quartile of the dataset , Q3: third quartile of the dataset), which marks data points as outlier, if a data point is above Q3 + 1.5 * IQR; 2, Normal distribution of the data was verified using the Shapiro-Wilk normality test, then Grubbs' test was applied to test if the maximum value is an outlier at the 0.05 significance level.

Mapping the experimentally verified SARS-CoV-2 T cell and B cell epitopes to the BCG-Pasteur proteome. The experimentally verified SARS-CoV-2 T cell and B cell epitopes (linear, human, positive T cell assays, MHC-I restricted and MHC-II restricted) were downloaded from the Immune Epitope Database (IEDB). Due to the low number of MHC-II restricted SARS-CoV-2 T cell and B cell epitopes, experimentally verified SARS-CoV T cell and B cell epitopes with identical sequence to SARS-CoV-2 were also included in the analysis as putative SARS-CoV-2 epitopes. SARS-CoV-2 epitopes were compared to the BCG-Pasteur proteome using the above mentioned identity measure. BCG-Pasteur peptides with identity (same amino acid at the same position) of >=67% were used for further T cell epitope selection, because recently it was showed that if there is a >=67% identity between a SARS-CoV-2 epitope and another coronavirus epitope than there is a 57% chance that adaptive cross immunity can be observed [7]. Similar threshold was observed in SARS-CoV-2 MHC-1 restricted T cell cross immune responses [9]. The threshold of the identification of cross binding linear B cell epitopes has not been described, therefore an

identity threshold similar to the T cell analysis (>=62.5%) was used in this study. BCG-Pasteur peptides similar to MHC-I-restricted epitopes were further analyzed with NetMHC 4.0 software [10] using predefined alleles representative of the HLA-A and HLA-B supertypes. BCG-Pasteur peptides similar to MHC-II restricted SARS-CoV-2 peptides were analyzed with NetMHCII 2.3 [11]. The identified "strong binder" epitopes were collected (threshold for strong binders: =<0.5 % Rank for NetMHC 4.0 and =<2 % Rank for NetMHCII 2.3). BCG-Pasteur proteins with identity of >=62.5% to a SARS-CoV-2 B cell epitopes were further analyzed with the BepiPred 2.0 software [6]. Peptides that were located entirely in an epitope region by BepiPred 2.0 were collected. 3D molecular surface model of SARS-CoV-2 spike protein homotrimer was created with the Maestro GUI of the Schrodinger program suit (Schrodinger Inc., New York, NY, USA) using the 6X29 pdb structure from the Protein Data Bank crystallographic database (www.rcsb.org).

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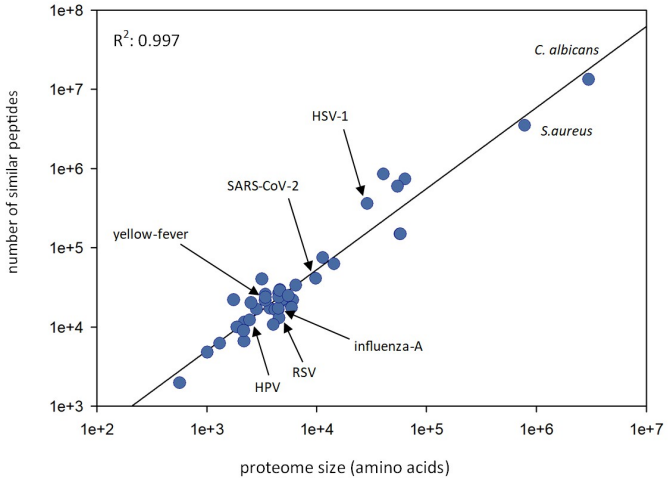
Figures

(a)



(b)

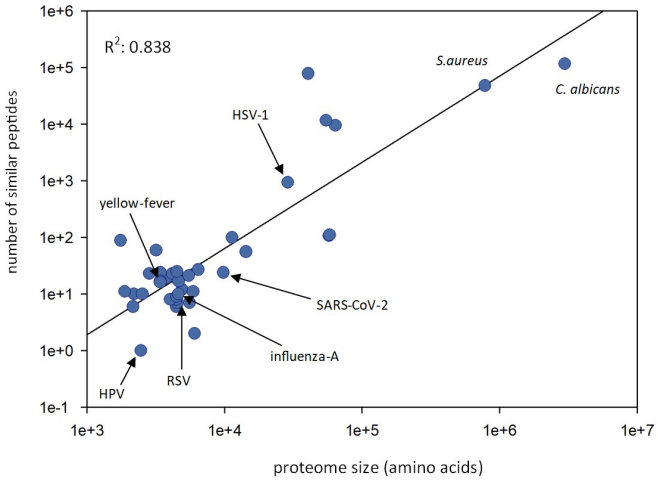


Figure 1. Exploration of peptide similarity between the BCG-Pasteur proteome and other proteomes. All possible 9-mer and 15-mer BCG-Pasteur peptides were compared to the same-length peptides of a variety of 40 viral proteomes and the *C. albicans* and *S. aureus* proteomes, ranging in size from < 1e+3 to > 1e+6. (a) 9-mer peptides with >=67% identity (b) 15-mer peptides with >=67% identity. Pearson correlation coefficients between the number of similar peptides and the proteome lengths are also shown. RSV: respiratory syncytial virus, HPV: human papilloma virus, HSV: herpes simplex virus.

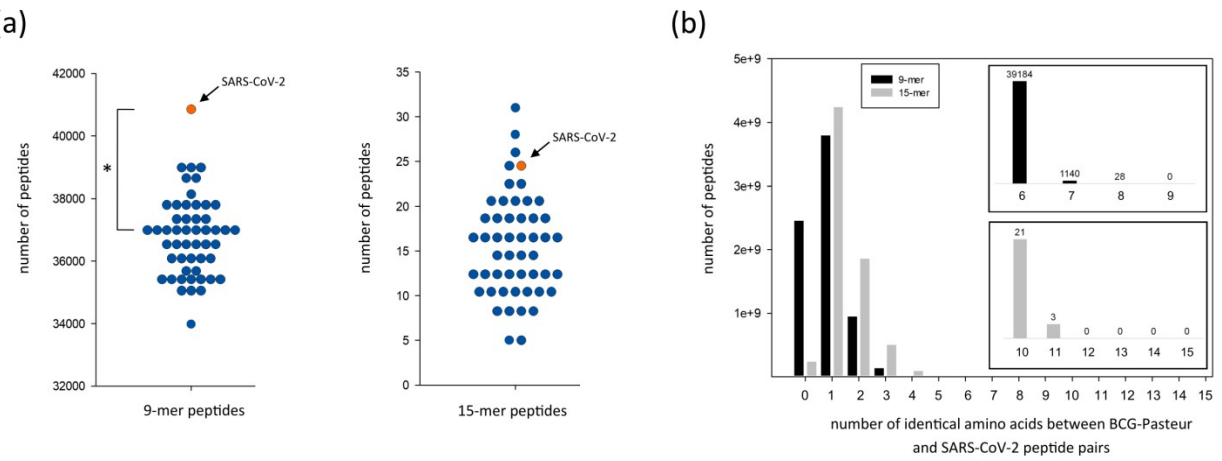


Figure 2. Exploration of peptide similarity between the BCG-Pasteur proteome and the SARS-CoV-2 proteome. (a) Comparison of the BCG-Pasteur proteome to the SARS-CoV-2 proteome and randomized proteomes. 9-mer and 15-mer peptides of the BCG-Pasteur proteome were mapped to the SARS-CoV-2 proteome and 50 random proteomes containing the same number and length proteins as the original SARS-CoV-2 proteome but with scrambled amino acid composition. The number of 9-mer and 15-mer BCG-Pasteur peptides >=67% identical to a SARS-CoV-2 peptide are shown. Inter-Quartile Range analysis of outliers identified values higher than 40222 as an outlier for 9-mer sequences and 30.25 for 15-mer sequences. According to this analysis, the SARS-CoV-2 9-mer value (40848) was an outlier, but the SARS-CoV-2 15-mer value (24) was not. A Grubbs' test for outliers was applied to calculate the significance of the 40848 value as an outlier. *: $P < 0.05$

(b) Exploration of the identity between BCG-Pasteur peptides and SARS-CoV-2 peptides. 9-mer and 15-mer BCG-Pasteur peptides were mapped to the SARS-CoV-2 proteome. The number of BCG-Pasteur peptides with different level of identity to a SARS-CoV-2 peptide is shown. Inset graphs show the occurrence of the 9-mer and 15-mer peptides with >=67% identity.

(a)

	HLA	SARS-CoV-2 protein	SARS-CoV-2 epitope	BCG-Pasteur epitope	%Rank	identity %
1	A02	NSP03	LLSAGIFGA	LLLAGIFGV	0,01	77,8
2	A02	NSP03	LLSAGIFGA	LLSAMIAGA	0,4	77,8
3	A02	NSP03	YIFFASFYY	YIFGFSFYT	0,03	66,6
1	A02	NSP04	FLLNKEMYL	RLLNVEDYL	0,4	66,6
1	A02	NSP05	VLAWLYAAV	ALAWLVAAV	0,1	77,8
2	A02	NSP05	VLAWLYAAV	VLADLVAEV	0,01	66,6
3	A02	NSP05	VLAWLYAAV	VLAELVARV	0,06	66,6
1	A02	NSP06	FLLPSLATV	RLLPNLATV	0,08	77,8
2	A02	NSP06	FLLPSLATV	LLLPELHTV	0,03	66,6
3	A02	NSP06	FLLPSLATV	LLLPRLTTV	0,07	66,6
1	A03	NSP08	ALRANSAVK	ALREFSSVK	0,08	66,6
2	A03	NSP08	ALRANSAVK	AIAAFSAVK	0,15	66,6
3	A03	NSP08	ALRANSAVK	ALRANGIPK	0,2	66,6
1	A02	NSP12	LLMPILTLT	LLAPILTPV	0,01	66,6
2	A02	NSP12	FVDGVPFVV	FVDDISFVV	0,03	66,6
3	A02	NSP12	RILGAGCFV	RILGWGWLV	0,1	66,6
1	A03	NSP13	VVYRGTTTY	VVYRVTGTK	0,02	66,6
2	B07	NSP13	IPRRNVATL	QPRRALATL	0,02	66,6
3	B07	NSP13	IPRRNVATL	IPRRMIATT	0,17	66,6
1	A02	NSP14	ALLADKFPV	YLLADAFTV	0,01	66,6
2	A02	NSP14	ALLADKFPV	ALLALQHPV	0,03	66,6
3	A02	NSP14	ALLADKFPV	ALLAASVPV	0,04	66,6
1	B58	NSP15	TTLPVNVAF	TTLPVTGVF	0,4	66,6
2	B58	NSP15	TTLPVNVAF	VTLSVAVAF	0,4	66,6
1	A03	NSP16	KMQRMLLEK	KVQRMLLAK	0,17	77,8
2	A02	NSP16	YLNTLTLAV	LLLTLNLAV	0,08	66,6
3	A02	NSP16	YLNTLTLAV	YLPTLLLAA	0,25	66,6
1	A02	SPIKE	FIAGLIAIV	FIAWLIWLV	0,01	66,6
2	A02	SPIKE	FIAGLIAIV	FIAHHIAPV	0,01	66,6
3	A02	SPIKE	FIAGLIAIV	FIAPLVAMV	0,06	66,6
1	B44	ORF4	SEETGTLIV	IEITGTLTV	0,2	66,6
2	B44	ORF4	SEETGTLIV	SEWRATLIV	0,5	66,6
3	B44	ORF4	SEETGTLIV	SEWRATLIV	0,5	66,6
1	A24	ORF5	RYRIGNYKL	RYPIGLYNL	0,06	66,6
1	A02	ORF6	HLVDFQVTI	HLVDFPVTL	0,4	77,8
2	A02	ORF6	HLVDFQVTI	SLVDSQVTV	0,2	66,6
1	A02	ORF7A	KLFIRQEEV	KLFCRMAEV	0,03	66,6
2	A02	ORF7A	KLFIRQEEV	RLFARQMEV	0,09	66,6
3	A02	ORF7A	KLFIRQEEV	KLLQRPEEV	0,5	66,6
1	A02	ORF9	LLLDRLNQL	YLLDRLAEL	0,01	66,6
2	A02	ORF9	LLLDRLNQL	GLLDRLVYL	0,03	66,6
3	A02	ORF9	LLLDRLNQL	LLLDFLGGL	0,1	66,6

(b)

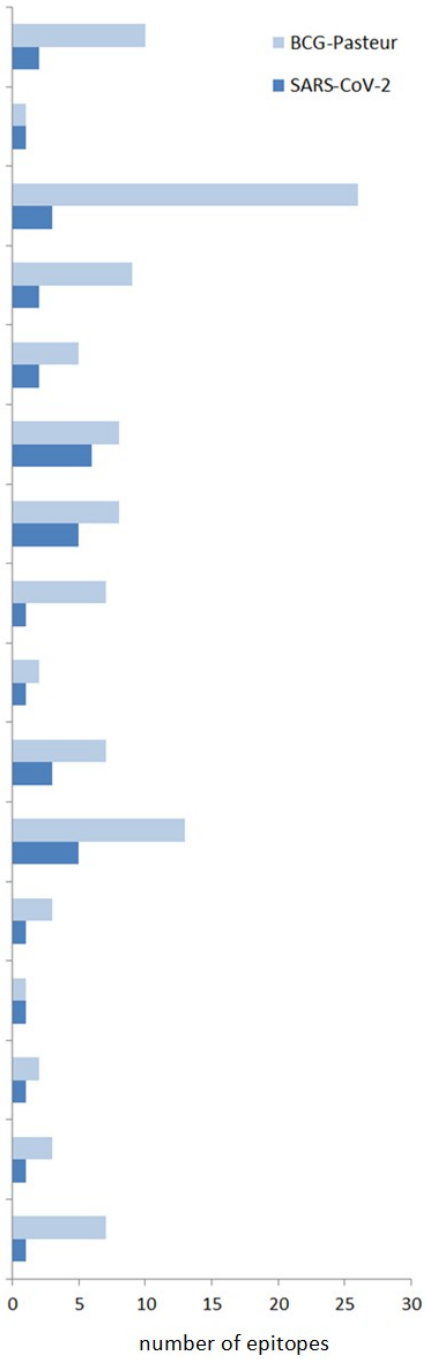


Figure 3. Similarity between MHC-I restricted BCG-Pasteur and SARS-CoV-2 T cell epitopes. (a) Top ranked BCG-Pasteur epitopes with the highest similarity/ NetMHC %Rank to an epitope of a

SARS-CoV-2 protein. The NetMHC %Rank, which corresponds to the predicted HLA binding affinity are shown (lower NetMHC %Rank value indicates stronger HLA binding). Note that the SARS-CoV-2 epitope SEETGTLIV has two identical epitope counterparts from two BCG-Pasteur proteins. (b) Distribution of SARS-CoV-2 epitopes among the viral proteins. The number of SARS-CoV-2 epitopes and the number of their similar BCG-Pasteur epitope counterparts are shown. SG: spike glycoprotein

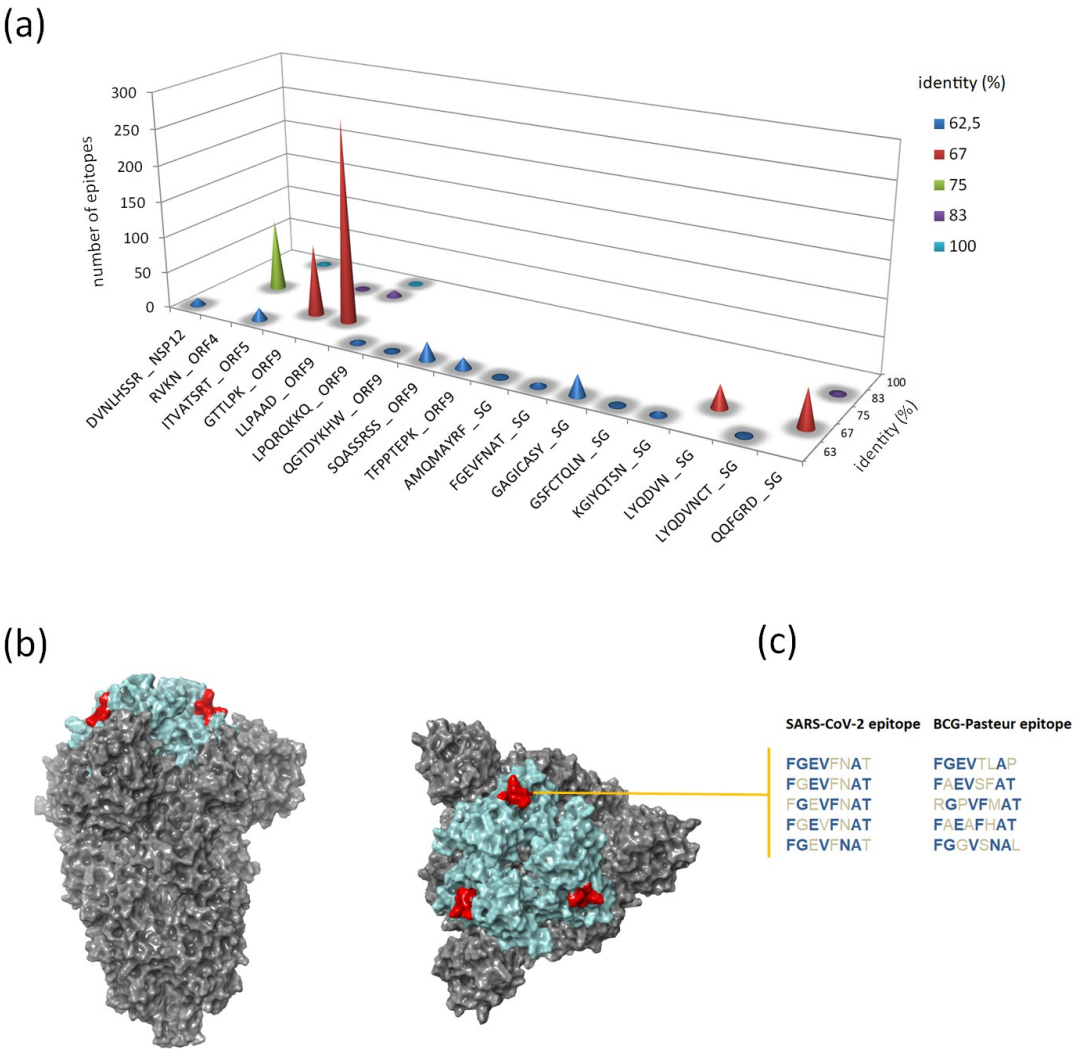


Figure 4. Similarity between BCG-Pasteur and SARS-CoV-2 B cell epitopes. a, Individual SARS-CoV-2 B cell epitopes (putative and experimentally verified) were compared with all the possible same length peptides of the BCG-Pasteur proteome. BCG-Pasteur proteins containing the highly

similar peptides (>=62.5% identity) were analyzed with BepiPred 2.0 to identify putative B cell epitopes. b, Localization of the SARS-CoV-2 B cell epitope FGEVFNAT (red) in the receptor binding domain (turquise) of the homotrimer SARS-CoV-2 spike glycoprotein (side view and top view). c, FGEVFNAT and the similar BCG-Pasteur B cell epitopes identified. SG: spike glycoprotein

Supplementary material

Supplementary Table 1. Similar MHC-I restricted BCG-Pasteur T cell epitopes and SARS-CoV-2 T cell epitopes

Supplementary Table 2. Similar BCG-Pasteur epitopes and SARS-CoV-2 B cell epitopes