In silico studies suggest T-cell cross-reactivity between SARS-CoV-2 and less dangerous coronaviruses

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Short Report

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Abstract

So far, it is impossible to explain the diverse individual and population responses to SARS-CoV-2 infection. Many factors may be involved, including genetics, diet, vaccinations, the innate immune response, viral load, and other phenomena. Further, immune responses raised against pathogens other than SARS-CoV-2 (cross-reactivity) may also be involved. In this work, we analyzed the potential for T-cell cross-reactivity between less contagious coronaviruses (HCoV-OC43, HCoV-HKU1, HCoV-229E, and HCoV-NL63) and SARS-CoV-2. *In silico* research suggests that SARS-CoV-2 and less dangerous coronaviruses share identical peptides, which can be presented on MHC class I molecules. Those T-cells epitopes belong to several coronavirus proteins localized inside the viral envelope, including helicase, RNA polymerase, proofreading exoribonuclease, and 2'-O-methyltransferase. Our data suggest that a milder course of COVID-19, in some populations, may be related to the cross-reactivity of T cells.

Introduction

Over the course of the COVID-19 pandemic thus far, it has become clear that there are diverse individual and population responses to the SARS-CoV-2 virus that influence its pathogenicity. Many people who come into contact with COVID-19 patients, such as family members, never exhibit any symptoms, or only mild symptoms, of the disease, despite having no previous exposure to SARS-CoV-2. It is possible that this protection against SARS-CoV-2 may be based on selected and expanded T-cell populations that originated not after SARS-CoV-2 infection, but rather after infection with pathogens that contain antigens similar to those found in SARS-CoV-2. Indeed, recombinant vaccines are sometimes created using pathogens other than the one being targeted, and immunity against a pathogen does not have to result from exposure to that particular pathogen. Moreover, genes encoding viral antigens can be mutated to create vaccines with increased efficacy ^{1,2}. Recently published data identified cross-reactions between SARS-CoV-2 and HCoV-OC43, HCoV-229E, HCoV-NL63, and HCoV-HKV1 antigens ^{1,3}. Although studies show that the T lymphocyte response is very complex in comparison to the humoral response, bioinformatics models can be used to analyze T lymphocyte epitopes. With a large number of positive results, these models can be used to provide useful directions for future research.

Results

Available protein sequences for several common human coronaviruses and the novel SARS-CoV-2 were downloaded from the Swiss-Prot manually curated and annotated database.

Using netCTL 1.2 software ⁴, we were able to predict over 500 cytotoxic T-lymphocyte (CTL) epitopes in the proteomes of each of the viruses for different MHC supertypes (A1, A2, A3, A24, A26, B7, B8, B27, B39, B44, B58, and B62). A relatively large number of these were common between SARS-CoV-2 and other human coronaviruses (see Tables 2-5 and complete data in Supplementary Table S2 online). All CTL epitopes common between SARS-CoV-2 and the other human CoVs were identified in replicase polyprotein 1ab. SARS-CoV-2 replicase polyprotein 1ab is post-translationally cleaved at multiple sites by

either 3CL-PRO or PL-PRO protease to form several accessory proteins (see Fig. 1). The CTL epitopes were only identified in the RNA polymerase, helicase, proofreading exoribonuclease, and 2'-O-methyltransferase regions of the protein. These four proteins were highly conserved among the analyzed human coronavirus species. Table 1 shows the percentage of identical positions in pairwise and multiple sequence alignments among the human coronaviruses.

Materials And Methods

Protein sequences

Sequences of human coronaviruses HCoV-OC43, HCoV-HKU1, HCoV-229E, HCoV-63NL, and SARS-CoV-2 proteins were downloaded from the Uniprot (Swiss-Prot) database. Complete list of sequences can be found in supplementary Table S1.

MHC epitope prediction

Predictions of cytotoxic T-lymphocyte (CTL) epitopes were obtained using netCTL 1.2 software ⁴, which is an implementation of an integrative prediction algorithm and includes predictions of proteasomal cleavage, TAP transport efficiency, and MHC class I affinity. The predictions are currently limited to MHC supertypes A1, A2, A3, A24, A26, B7, B8, B27, B39, B44, B58, and B62.

Sequence alignment

Pairwise and multiple sequence alignments were calculated with Clustal Omega ⁵ using default parameters – Gonnet transition matrix, gap opening penalty 6 bits, and extension 1 bit.

Discussion

Four common human coronaviruses, HCoV-OC43, HCoV-229E, HCoV-NL63, and HCoV-HKV1, circulate among the global population, but are comparatively less dangerous than SARS-CoV-2 ⁶. We hypothesized that a cross-response of cytotoxic cells ⁷, selected during an immune response to these common viruses, may play an auxiliary role during elimination of SARS-CoV-2. Unfortunately, T-lymphocyte responses are much more difficult to study *in vitro* than B-lymphocyte responses. To this end, and because of the importance of T-cells during viral infections, we focused on the T-cell response ⁷. We tested the above hypothesis using *in silico* models. We assumed that the MHC I peptides were properly selected during our *in silico* studies. Next, we performed a search for identical, or nearly identical, peptides presented by MHC I molecules during infection with the four common human coronaviruses and SARS-CoV-2. Our *in silico* analysis suggests that T lymphocytes selected during infection with the mild coronaviruses exhibit cross-reactivity with SARS-CoV-2, as they share antigenic peptides with SARS-CoV-2 peptides. Indeed, several SARS-CoV-2 proteins share peptides/epitopes with HCoV-HKU1 HCoV-OC43, HCoV-229E, and HCoV-NL63, including epitopes from helicase, RNA polymerase, proofreading exoribonuclease, and 2'-O-methyltransferase. Importantly, these molecules are not virion surface proteins and are not optimal

targets for antibodies. We are aware of the many limitations in the use of *in silico* testing to predict which peptides are recognized/presented after infection^{8,9}. However, the number of common peptides identified in this study was relatively large, and the common epitopes were found in all four mild coronaviruses. Therefore, it cannot be ruled out that T-cell cross-reactivity may offer some protection against SARS-CoV-2 for people who have been infected with other, less dangerous coronaviruses; a similar conclusion was recently published¹⁰. Therefore, we suggest that the T-lymphocytes selected and expanded during infection with SARS-CoV-2, as well as cross-reactive T-lymphocytes propagated during mild coronavirus infections, can support the immune response to SARS-CoV-2.

Abbreviations

CTL—Cytotoxic T-lymphocyte

MHC—Major Histocompatibility Complex

NSP—Non-structural protein

PL-PRO—Papain-like proteinase

3CL-PRO—3C-like proteinase

HCoV—Human coronavirus

Declarations

Author contributions

MP carried out presented analyses and interpreted the results. PR conceived and supervised the study. MP and PR wrote the main manuscript text and MP prepared figure and tables. Both authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

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Tables

Table 1 Percent of identical positions in pairwise and multiple sequence alignments of SARS-CoV-2 and other human coronavirus protein sequences.

SARS-CoV-2 protein	HCoV-OC43	HCoV-HKU1	HCoV-229E	HCoV-NL63	Multiple
RNA polymerase	65.9	66.8	58.5	58.5	45.3
Helicase	67.4	65.1	59.1	60.1	47.8
Proofreading exoribonuclease	57.2	57.6	51.6	52.4	37.7
2'-O-methyltransferase	65.9	63.2	56.5	57.8	43.5

Table 2 Predicted CTL epitopes shared by SARS-CoV-2 and HCoV-229E.

HCoV-229E	MHC supertype	CTL epitope	SARS-CoV-2 protein
	A1	TTAYANSVF	RNA polymerase
		GSEYDYVIF	Helicase
	A2	SLAIDAYPL	RNA polymerase
		AIMTRCLAV	Proofreading exoribonuclease
	A24	TTAYANSVF	RNA polymerase
		AYANSVFNI	
	A26	TTAYANSVF	RNA polymerase
	B27	NRFNVAITR	Helicase
	B39	HEFCSQHTM	RNA polymerase
		SLAIDAYPL	
		GVAPGTAVL	2'-O-methyltransferase
	B44	HEFCSQHTM	RNA polymerase
	B58	TTAYANSVF	RNA polymerase
	B62	TTAYANSVF	RNA polymerase
		SLAIDAYPL	
		GVAPGTAVL	2'-O-methyltransferase
	B7	LPYPDPSRI	RNA polymerase
		IPARARVEC	Helicase
		GVAPGTAVL	2'-O-methyltransferase

Table 3 Predicted CTL epitopes shared by SARS-CoV-2 and HCoV-HKU1.

HCoV-HKU1	MHC supertype	CTL epitope	SARS-CoV-2 protein
	A1	FVDGVPFVV FVSLAIDAY	RNA polymerase
	A2	FVDGVPFVV RLANECAQV SVFNICQAV RILGAGCFV SLAIDAYPL	RNA polymerase
	A24	LYYQNNVFM YYQNNVFMS EFCSQHTML PYPDPSRIL	RNA polymerase
		VYIGDPAQL	Helicase
		KYTQLCQYL SWNADLYKL	2'-O-methyltransferase
	A26	FVSLAIDAY	RNA polymerase
	A3	NLKYAISAK CSQHTMLVK	RNA polymerase
		ALVYDNKLK	Helicase
	B27	NRARTVAGV ARTVAGVSI SRILGAGCF	RNA polymerase
		NRFNVAITR	Helicase
	B39	FVDGVPFVV HEFCSQHTM SLAIDAYPL	RNA polymerase
		SLYVNBKHA	Proofreading exoribonuclease
	B44	HEFCSQHTM IERFVSLAI	RNA polymerase
	B58	SLYVNBKHA	Proofreading exoribonuclease

B62	FQTVKPGNF	RNA polymerase
	VLYYQNNVF	
	FVSLAIDAY	
	SLAIDAYPL	
	LYLGGMSYY	Helicase
	SLYVNBKHF	Proofreading exoribonuclease
B7	SAKNRARTV	RNA polymerase
	LPYPDPSRI	
B8	VCRFDTRVL	Proofreading exoribonuclease
	SAKNRARTV	RNA polymerase
	VLYYQNNVF	
	IERFVSLAI	
	RLKLFAAET	Helicase
	SLYVNBKHF	Proofreading exoribonuclease

Table 4 Predicted CTL epitopes shared by SARS-CoV-2 and HCoV-NL63.

HCoV-NL63	MHC supertype	CTL epitope	SARS-CoV-2 protein
	A1	SSQGSEYDY	Helicase
	A2	SLAIDAYPL	RNA polymerase
	A24	LYYQNNVFM YYQNNVFMS PYPDPSRIL	RNA polymerase
		KYTQLCQYL	2'-O-methyltransferase
	A26	YLRKHFSMM	RNA polymerase
	B27	LRKHFSMMI RKHFSSMMIL	RNA polymerase
		NRFNVAITR	Helicase
	B39	RKHFSMMIL HEFCSQHTM SLAIDAYPL	RNA polymerase
		SLYVNKHAF	Proofreading exoribonuclease
	B44	HEFCSQHTM	RNA polymerase
	B58	SLYVNKHAF	Proofreading exoribonuclease
	B62	YLRKHFSMM SLAIDAYPL	RNA polymerase
		SSQGSEYDY	Helicase
		SLYVNKHAF	Proofreading exoribonuclease
	B7	YLRKHFSMM LPYPDPSRI	RNA polymerase
		IPARARVEC	Helicase
	B8	YLRKHFSMM	RNA polymerase
		SLYVNKHAF	Proofreading exoribonuclease

Table 5 Predicted CTL epitopes shared by SARS-CoV-2 and HCoV-OC43.

HCoV-OC43	MHC supertype	CTL epitope	SARS-CoV-2 protein
	A1	FVDGVPFVV	RNA polymerase
		FVSLAIDAY	
		IVDTVSAIV	Helicase
	A2	FVDGVPFVV	RNA polymerase
		RLANCAQV	
		SVFNICQAV	
		RILGAGCFV	
		SLAIDAYPL	
		IVDTVSAIV	Helicase
		AIMTRCLAV	Proofreading exoribonuclease
	A24	LYYQNNVFM	RNA polymerase
		YYQNNVFMS	
		EFCSQHTML	
		VYIGDPAQL	Helicase
		KYTQLCQYL	2'-O-methyltransferase
	A26	FVSLAIDAY	RNA polymerase
		EIVDTVSAIV	Helicase
	A3	NLKYAISAK	RNA polymerase
		CSQHTMLVK	
		ALGGSVAIK	2'-O-methyltransferase
	B27	NRARTVAGV	RNA polymerase
		ARTVAGVSI	
		SRILGAGCF	
		NRFNVAITR	Helicase
	B39	FVDGVPFVV	RNA polymerase
		HEFCSQHTM	
		SLAIDAYPL	
		EIVDTVSAIV	Helicase

	SLYVNBKHF	Proofreading exoribonuclease
B44	HEFCSQHTM	RNA polymerase
	IERFVSLAI	
B58	SLYVNBKHF	Proofreading exoribonuclease
B62	FQTVKPGNF	RNA polymerase
	VLYYQNNVF	
	FVSLAIDAY	
	LYLGGMSYY	Helicase
	SLYVNBKHF	Proofreading exoribonuclease
B7	SAKNRARTV	RNA polymerase
	VCRFDTRVL	Proofreading exoribonuclease
	VPLKSATCI	
B8	SAKNRARTV	RNA polymerase
	VLYYQNNVF	
	IERFVSLAI	
	RLKLFAAET	Helicase
	SLYVNBKHF	Proofreading exoribonuclease

Figures

FIGURE 1

Figure 1

SARS-CoV-2 replicase polyprotein 1ab is post-translationally cleaved into several accessory proteins by PL-PRO and 3CL-PRO

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTables.pdf](#)