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, 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 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 SAR-CoV-2. We hypothesized that a cross-response of cytotoxic cells, 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. 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. 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 published. 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

| Abbreviation | Description                      |
|--------------|----------------------------------|
| 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.

### References

1. Clark, T. G. & Cassidy-Hanley, D. Recombinant subunit vaccines: potentials and constraints. *Dev. Biol. (Basel)*. **121**, 153–163 (2005).
2. Doria-Rose, N. A. & Joyce, M. G. Strategies to guide the antibody affinity maturation process. *Current Opinion in Virology* **11**, 137–147 (2015).
3. Parren, P. W. & Burton, D. R. The antiviral activity of antibodies in vitro and in vivo. *Adv. Immunol.* **77**, 195–262 (2001).
4. Larsen, M. V. et al. Large-scale validation of methods for cytotoxic T-lymphocyte epitope prediction. *BMC Bioinformatics* **8**, (2007).

5. Söding, J. Protein homology detection by HMM–HMM comparison. *Bioinformatics* **21**, 951–960 (2004).

6. Chen, B. et al. Overview of lethal human coronaviruses. *Signal Transduct. Target. Ther.* **5**, 89 (2020).

7. Welters, M. J. P. et al. Multiple CD4 and CD8 T-cell activation parameters predict vaccine efficacy in vivo mediated by individual DC-activating agonists. *Vaccine* **25**, 1379–1389 (2007).

8. Melief, C. J. M. & Van Der Burg, S. H. Immunotherapy of established (pre)malignant disease by synthetic long peptide vaccines. *Nature Reviews Cancer* **8**, 351–360 (2008).

9. Purcell, A. W., McCluskey, J. & Rossjohn, J. More than one reason to rethink the use of peptides in vaccine design. *Nature Reviews Drug Discovery* **6**, 404–414 (2007).

10. Grifoni, A. et al. Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. *Cell* **181**, 1489-1501.e15 (2020).

**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     | RNA polymerase    |
|           | FVSLAIDAY     |                   |
| A2        | FVDGVPFVV     | RNA polymerase    |
|           | RLANECAQV     |                   |
|           | SVFNICQAV     |                   |
|           | RILGAGCFV     |                   |
|           | SLAIDAYPL     |                   |
| A24       | LYYQNNVFM     | RNA polymerase    |
|           | YYQNNVFMS     |                   |
|           | EFCSQHTML     |                   |
|           | PYPDPSRIL     |                   |
|           | VYIGDPAQL     | Helicase          |
|           | KYTEQLCQYL    | 2′-O-methyltransferase |
|           | SWNADLYKL     |                   |
| A26       | FVSLAIDAY     | RNA polymerase    |
| A3        | NLYKAISAK     | RNA polymerase    |
|           | CSQHTMLVK     |                   |
|           | ALVYDNKLK     | Helicase          |
| B27       | NRARTVAGV     | RNA polymerase    |
|           | ARTVAGVSI     |                   |
|           | SRLGAGCF      |                   |
|           | NRFNVAITR     | Helicase          |
| B39       | FVDGVPFVV     | RNA polymerase    |
|           | HEFCSQHTM     |                   |
|           | SLAIDAYPL     |                   |
|           | SLYVNKHAF     | Proofreading exoribonuclease |
| B44       | HEFCSQHTM     | RNA polymerase    |
|           | IERFVSLAI     |                   |
| B58       | SLYVNKHAF     | Proofreading exoribonuclease |
### Table 4 Predicted CTL epitopes shared by SARS-CoV-2 and HCoV-NL63.

| B62   | FQTVKPGNF | RNA polymerase |
|-------|-----------|----------------|
|       | VLYYQNNVF |                |
|       | FVSLAIDAY |                |
|       | SLAIDAYPL |                |
|       | LYLGGMSYY | Helicase       |
|       | SLYVNHAF  | Proofreading exoribonuclease |
| B7    | SAKNRARTV | RNA polymerase |
|       | LPYPDPSRI |                |
|       | VCRFDTRVL | Proofreading exoribonuclease |
| B8    | SAKNRARTV | RNA polymerase |
|       | VLYQNNVF  |                |
|       | IERFVLAI  |                |
|       | RLKLFAAET | Helicase       |
|       | SLYVNHAF  | Proofreading exoribonuclease |
## Table 5 Predicted CTL epitopes shared by SARS-CoV-2 and HCoV-OC43.

| HCoV-NL63 | MHC supertype | CTL epitope | SARS-CoV-2 protein |
|-----------|--------------|------------|-------------------|
| A1        | SSQGSEYDY    | Helicase   |
| A2        | SLAIDAYPL    | RNA polymerase |
| A24       | LYYQNNVFMS   | RNA polymerase |
|           | YYQNNVFM     | RNA polymerase |
|           | PYPDPSRIL    | 2′-O-methyltransferase |
| A26       | YLRKHFSMM    | RNA polymerase |
| B27       | LRKHFSSMIL   | RNA polymerase |
|           | NRFNVAITR    | Helicase |
| B39       | RKFHSMMIL    | RNA polymerase |
|           | HEFCSQHTM    | Proofreading exonucleolyase |
|           | SLAIDAYPL    | RNA polymerase |
|           | SLYVNKHAF    | Proofreading exonucleolyase |
| B44       | HEFCSQHTM    | RNA polymerase |
| B58       | SLYVNKHAF    | Proofreading exonucleolyase |
| B62       | YLRKHFSMM    | RNA polymerase |
|           | SLAIDAYPL    | Helicase |
|           | SSQGSEYDY    | RNA polymerase |
|           | SLYVNKHAF    | Proofreading exonucleolyase |
| B7        | YLRKHFSMM    | RNA polymerase |
|           | LPYPDPSRI    | Helicase |
| B8        | YLRKHFSMM    | RNA polymerase |
|           | SLYVNKHAF    | Proofreading exonucleolyase |
| HCoV-OC43 | **MHC supertype** | **CTL epitope** | **SARS-CoV-2 protein** |
|-----------|-----------------|-----------------|----------------------|
| A1        | FVDGVPFVV       | RNA polymerase  |
|           | FVSLAIDAY       |                 |
|           | IVDTVSAVLV      | Helicase        |
| A2        | FVDGVPFVV       | RNA polymerase  |
|           | RLANECAQV       |                 |
|           | SVFNICQAV       |                 |
|           | RILGAGCFV       |                 |
|           | SLAIDAYPL       |                 |
|           | IVDTVSAVLV      | Helicase        |
|           | AIMTRCLAV       | Proofreading exoribonuclease |
| A24       | LYYQNNVFM       | RNA polymerase  |
|           | YYQNNVFMS       |                 |
|           | EFCSQHTML       |                 |
|           | VYIGDPAQL       | Helicase        |
|           | KYTQLCQYL       | 2'-O-methyltransferase |
| A26       | FVSLAIDAY       | RNA polymerase  |
|           | EIVDTVSAVL      | Helicase        |
| A3        | NLKYAISAK       | RNA polymerase  |
|           | CSQHTMLVK       |                 |
|           | ALGGSVAIK       | 2'-O-methyltransferase |
| B27       | NRARTVAGV       | RNA polymerase  |
|           | ARTVAGVISI      |                 |
|           | SRILGAGCF       |                 |
|           | NRFNVAITR       | Helicase        |
| B39       | FVDGVPFVV       | RNA polymerase  |
|           | HEFCSQHTM       |                 |
|           | SLAIDAYPL       |                 |
|           | EIVDTVSAVL      | Helicase        |
|     |     |                          |                      |                      |
|-----|-----|--------------------------|----------------------|----------------------|
|     |     | SLYVNKHAF                | Proofreading exoribonuclease |
| B44 |     | HEFCSQHTM                | RNA polymerase       |
|     |     | IERFVSLAI                |                      |
| B58 |     | SLYVNKHAF                | Proofreading exoribonuclease |
| B62 |     | FQTVKPGNF                | RNA polymerase       |
|     |     | VLYYQNNVF                |                      |
|     |     | FVSLAIDAY                |                      |
|     |     | LYLGGMSYY                | Helicase             |
|     |     | SLYVNKHAF                | Proofreading exoribonuclease |
| B7  |     | SAKNRARTV                | RNA polymerase       |
|     |     | VCRFDTRVL                | Proofreading exoribonuclease |
|     |     | VPLKSATCI                |                      |
| B8  |     | SAKNRARTV                | RNA polymerase       |
|     |     | VLYYQNNVF                |                      |
|     |     | IERFVSLAI                |                      |
|     |     | RLKLFAAET                | Helicase             |
|     |     | SLYVNKHAF                | Proofreading exoribonuclease |

**Figures**
Figure 1

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

Supplementary Files

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- SupplementaryTables.pdf