

The possible role of molecular mimicry in SARS-CoV-2-mediated autoimmunity: an immunobiochemical basis

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ABSTRACT

Coronavirus Disease 2019 (COVID-19), caused by the novel Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), persists as a threat to global health and continues to be a rapidly evolving condition. Although COVID19 is negatively correlated with the existing comorbidities in terms of the clinical outcome, the ability of SARS-CoV-2 to mediate the novel, or to exacerbate the existing autoimmune conditions, has generated considerable interest, due to its potential implications both with regard to patients suffering from autoimmune conditions, as well as to the long-term consequences of the disease. However, although molecular mimicry has been postulated as a potential causative factor in post-COVID19 autoimmunity and multi-organ damage, a substantial body of research needs to emerge in order to achieve a more definitive conclusion. We investigated the possibility of SARS-CoV-2 peptide sequences behaving as molecular mimics with a potential to trigger an autoimmune response. Thus, on the basis of analysis *in silico*, we were able to develop a plausible case for the molecular mimicry as a potential aetiological mechanism of SARS-CoV-2-mediated autoimmunity, both in a multi-organ damage context or outside of the viral phase of infection. Interestingly, this is the first time that the peptide sequence of MACROD1 has been implicated in the COVID-19 autoimmunity. Additionally, we also confirm that PARP9 and PARP14 may be involved in the process.

Introduction

Coronavirus Disease (COVID-19) Pandemic, resulting from by the novel Severe Acute Respi-

ratory Syndrome Coronavirus-2 (SARS-CoV-2), persists as a threat to global health and continues to be a rapidly developing situation [1]. Due to the potential implications both with regard to

patients suffering from autoimmune conditions, as well as to the long-term consequences of COVID-19, COVID-19 has generated considerable attention, although it has been negatively correlated with the existing comorbidities in terms of the clinical outcome, the ability of SARS-CoV-2 to mediate the novel, or to exacerbate the existing autoimmune conditions [2–4]. In spite of the respiratory involvement, as the most prominent symptom, the prospect of the virus triggering the novel autoimmune conditions, or exacerbating the existing ones, is becoming increasingly substantiated by a growing body of research [5–8]. It has been established that, depending on the disease severity, COVID-19 is characterised by cytokine dysregulation, which results in a tissue-damaging cytokine storm in the critically ill patients [5, 9–12]. Single cell RNA sequencing revealed that proinflammatory cytokines are predominantly secreted by monocyte-derived inflammatory macrophages [13]. This causes the onset of acute respiratory distress syndrome (ARDS), with interleukin (IL)-6, IL-1 β and tumour necrosis factor (TNF)- α closely correlated with severe ARDS cases, accompanied by multi-organ damage [1, 6, 14–16]. Considering that IL-6 and IL-1 β strongly mediate the recruitment of neutrophils and T cells, it is not at all surprising that these cytokines have been particularly strongly correlated with a severe immunopathology [17–19]. In fact, most COVID-19 patients are either asymptomatic or exhibit mild-to-modest symptomatology. However, the subpopulations predisposed to autoimmunity, or those with pre-existing autoimmune conditions, may experience detrimental effects; either in an acute clinical context of COVID-19, or through a potential triggering of novel autoimmune conditions via a number of virus-mediated mechanisms [4, 20, 21].

Research concerning the underlying mechanisms is currently developed, although clinical reports suggest a link between SARS-CoV-2 and autoimmunity, both as a potential causative factor and an exacerbator. For instance, a recent study reported the presence of antiphospholipid antibodies (aPLs) in 47% (31/66) of critically ill COVID-19 patients, as compared to the non-critically ill ones [22]. Moreover, SARS-CoV-2-induced autoimmunity is also indicated by reports of anti-nuclear antibodies (ANAs), lupus anticoagulant, anti-interferon (IFN) and anti-melanoma

differentiation-associated protein 5 (MDA5) antibodies in severely ill patients. A study conducted by Vojdani et al. 2020 demonstrated a potential cross-reactivity of anti-SARS-CoV-2 spike antibodies and the following human tissue proteins, i.e. transglutaminase 3, transglutaminase 2, myelin basic protein, mitochondria, nuclear antigen, α -myosin, thyroid peroxidase, collagen, claudin 5+6, and S100B. This, in turn, further contributes to the hypothesis that SARS-CoV-2 may be an aetiological factor of autoimmunity [23]. Furthermore, it has been estimated that approximately 4% of uninfected patients over the age of 70 possess anti-IFN autoantibodies which contribute to ~20% of COVID-19 fatalities. This fact has been attributed to mutations in genes involved in the regulation of type I and type III IFN immunity [24, 25]. However, whether SARS-CoV-2 is able to elicit the production of *de novo* anti-IFN autoantibodies, remains to be substantiated. Nevertheless, it would be tempting to suggest that mutations in genes associated with IFN immunity may contribute to the ability of SARS-CoV-2 to trigger the production of autoreactive antibodies which target the IFN pathways and IFN itself.

Autoimmune conditions, such as Guillain-Barré syndrome (GBS), Miller Fisher syndrome, paediatric immune thrombocytopenia (ITP), immune thrombocytopenic purpura (ITPP), systemic lupus erythematosus (SLE) and Kawasaki disease (KD), have been reported in COVID-19 patients regardless of the acute phase of infection [26–33]. Additionally, multisystem inflammatory disorders in children, phenotypically consistent with the clinical presentation of the Kawasaki disease, have been substantially correlated with acute COVID-19 in a recent report by Rubens et al. [34].

The possible correlation between ITP and COVID-19 is particularly remarkable. In view of two thirds of children diagnosed with ITP have suffered a viral infection, such as cytomegalovirus, hepatitis C, herpes, varicella zoster, rubella, Epstein-Barr virus, approximately one month prior to the diagnosis, it is impossible to exclude that SARS-CoV-2 could potentially be a novel aetiological factor of this autoimmune condition [29, 35–39]. The aforementioned paediatric case report of COVID-19-associated ITP could be indicative of the fact that SARS-CoV-2 may act as a viral trigger for autoimmunity in younger indi-

viduals, or it may exacerbate the existing ITP. The idea of SARS-CoV-2 which would exacerbate the stable ITP (and possibly other autoimmune conditions) may be supported by the case report of a previously stable ITP with infrequent flare-ups transitioning towards acute ITP after immunization with the Pfizer-BioNTech mRNA COVID-19 vaccine [40].

A possible mechanism by which SARS-CoV-2 may trigger or exacerbate autoimmunity is the phenomenon of molecular mimicry, which presumably occurs when T or B cells, induced by pathogen-derived peptides become cross-activated by self-peptides (of the host). In theory, therefore, it is possible that sequence similarities between foreign and self-peptides are sufficient to result in the aforementioned cross-activation [41, 42]. Structural homology is very important in the theory of molecular mimicry as confirmed by findings of single antibodies or TCR (T cell receptor) being activated by merely a few crucial residues [42, 43]. In fact, molecular mimicry may occur at the following levels:

- 1) complete identification of the viral and host protein,
- 2) homology between the host and viral protein/s,
- 3) common or sufficiently similar native or modified (glycosylated) amino acid sequences/epitopes between the virus and the host,
- 4) structural similarities between viral or environmental agents. Furthermore, viral infections may be followed by epitope spreading, which is a process of an immune response being elicited against viral epitopes which are not pathogenicity factors and display no cross-reactivity with such epitopes [44–47].

Nevertheless, although molecular mimicry has been postulated as a potential causative factor in post-COVID-19 autoimmunity and multi-organ damage, more research is necessary with regard to this hypothesis. By means of *in silico* analysis, we investigated whether SARS-CoV-2 peptide sequences could behave similarly to molecular mimics, with a potential to trigger an autoimmune response. The immunogenic potential of the retrieved homologous sequences was validated in terms of their potential to elicit T and B cell responses in terms of their sequential and structural information. In

particular, the study comprised the analysis of binding affinity between human leukocyte antigen (HLA)-encoded proteins and the molecular mimics, along with the immunogenicity of continuous and discontinuous predicted B cell epitopes which share ≥ 3 amino acid sequences (viral proteins).

Methods

Protein sequences

SARS-CoV-2 reference protein sequences were retrieved from the National Center for Biotechnology Information (NCBI) database. All of the 28 SARS-CoV-2 proteins were queried when running the BLASTp tool (Table 1).

Table 1. List of NCBI database protein accession numbers used in the BLASTp query. Accession numbers presented in bold are SARS-CoV-2 proteins for which the BLASTp program returned significant E values.

Protein Accession Number	Protein name
YP_009724389.1	ORF1ab polyprotein
YP_009725295.1	ORF1a polyprotein
YP_009724390.1	surface glycoprotein
YP_009724391.1	ORF3a protein
YP_009724392.1	envelope protein
YP_009724393.1	membrane glycoprotein
YP_009724394.1	ORF6 protein
YP_009724395.1	ORF7a protein
YP_009725318.1	ORF7b
YP_009724396.1	ORF8 protein
YP_009724397.2	nucleocapsid phosphoprotein
YP_009725255.1	ORF10 protein
YP_009742617.1	nsp10
YP_009742616.1	nsp9
YP_009742615.1	nsp8
YP_009742614.1	nsp7
YP_009742613.1	nsp6
YP_009742612.1	3C-like proteinase
YP_009742611.1	nsp4
YP_009742610.1	NSP3
YP_009742609.1	nsp2
YP_009742608.1	leader protein
YP_009725312.1	nsp11
YP_009725311.1	2'-O-ribose methyltransferase
YP_009725310.1	endoRNase
YP_009725309.1	3'-to-5' exonuclease
YP_009725308.1	helicase
YP_009725307.1	RNA-dependent RNA polymerase

Homology search

The BLASTp online tool was utilized to compare SARS-CoV-2 protein accessions (**Table 1**) to the human proteome, and the query was limited to *Homo sapiens* (taxid: 9606) in the UniProtKB/Swiss-Prot database. Both default and modified parameters (expected threshold modified to 1) were used in the analysis. BLASTp results were visualized using Kablamm. The retrieved human proteins with homologous sequences were queried using the Open Targets online repository for human proteins implicated in the disease in order to establish whether any of the retrieved human proteins have been correlated with either autoimmunity, or general inflammatory phenotypes.

Prediction of B cell epitopes

In order to explore the possibility whether the conserved regions may act as B cell epitopes, the prediction was conducted on the basis of the Immune epitope database (IEDB). From a variety of algorithms available on IEDB, Emini surface accessibility, Kolaskar and Tongaonkar and Bepipred were used. Homologous SARS-CoV-2 amino acid sequences retrieved from the BLASTp results were queried in their full length. Validation of whether the retrieved sequences could act as B cell epitopes on a structural level, was performed using the ElliPro server, which uses a protein data bank (PDB) format as input. PDB crystal structures for each of the 28 SARS-CoV-2 proteins were retrieved from PDB and prepared for docking using PyMOL [48]. ElliPro retrieves both linear and discontinuous antibody epitopes based on the provided crystal structure, using algorithms rooted in the protein shape approximation along with the clustering of neighbouring residues, and the residue protrusion index. Default search parameters were maintained during the utilization of each of the aforementioned tools.

Prediction of T cell epitopes

The selected viral protein sequences were subjected to HLA-II binding analysis on 3/16/2021 using the IEDB analysis resource SMM-Align (ver. 1.1) tool [49]. Outputs generated by the SMM-Align method are given in units of inhibitory concentration 50 nM, therefore, lower IC₅₀ values indicated high binding affinity. According to the guidelines provided on IEDB, inhibitory concen-

tration (IC) 50 values < 50nM are considered to be binders of high affinity, < 500 nM of intermediate affinity, whereas < 5000 nM of low affinity.

Potential binders (IC₅₀ < 100) generated by the MHC-II binding prediction tool were further analysed and visualized using LigPlot⁺, a tool which generates 2D ligand-protein interaction diagrams (50). Docking simulations between the potential binders and MHC-II alleles were conducted through the GalaxyPepDock online docking server, using crystal structures of HLA-DRB1 (PDB ID: 6BIN), HLA-DQ2.3 (PDB ID: 4D8P) and HLA-DR (PDB ID: 4H26) (51). Prior to docking simulations, the resident peptide, the accompanying solvent (water), and any other ligands provided within the PDB files, were removed.

Results

Homology between SARS-CoV-2 proteins and human proteins implicated in autoimmune and non-autoimmune conditions

Interestingly, out of the 28 SARS-CoV-2 proteins which were queried against the human proteome, homology was found between the viral open reading frame (ORF) 1a, ORF1ab, ORF7b and the multi-domain non-structural protein 3 (NSP3), as well as the human proteins mono-ADP-ribosyltransferase (PARP14), mono-ADP-ribosyltransferase (PARP9), ADP-ribose glycohydrolase (MACROD1) and the low-density lipoprotein receptor-related protein 2 (LRP2) (refer to **Table 1**).

In terms of the default BLASTp search parameters, ORF1a and ORF1ab and NSP3 were found to have sequences homologous with PARP14, PARP9 and MACROD1 (**Figures 1–9**), whereas homology between ORF7b and LRP2 was observed only after running a second query where the expected threshold was modified to 1 (**Figure 10**). The homologous regions between ORF7b and LRP2 returned no significant results once analysed for their ability to act as T and B cell epitopes.

PARP9 and PARP14 play pivotal roles in the eukaryotic physiology, and have been strongly implicated in COVID-19. In spite of the fact that PARP protein family is generally relatively obscure with regard to their functionality under normal physiological conditions, both

Table 2. BLASTp results obtained following the query of 38 SARS-CoV-2 proteins against the human proteome

Viral protein	Human protein	Max Score	Total Score	Query Cover	E value	Per. ident	Acc. Len	Accession
ORF1a	PARP14	56.2	56.2	2%	5.00E-07	32.26	1801	Q460N5.3
	PARP9	50.1	50.1	2%	3.00E-05	30.99	854	Q8IXQ6.2
	MACROD1	42.7	42.7	3%	0.003	28	325	Q9BQ69.2
ORF1ab	PARP14	56.2	56.2	1%	8.00E-07	32.26	1801	Q460N5.3
	PARP9	50.4	50.4	1%	4.00E-05	30.99	854	Q8IXQ6.2
	MACROD1	42.7	42.7	1%	0.005	28	325	Q9BQ69.2
ORF7b	LRP2	26.2	26.2	51%	0.55	60.87	4655	P98164.3
NSP3	PARP14	57.4	57.4	5%	9.00E-08	32.26	1801	Q460N5.3
	PARP9	51.2	51.2	6%	7.00E-06	30.99	854	Q8IXQ6.2
	MACROD1	41.6	41.6	7%	0.003	27.81	325	Q9BQ69.2



Figure 1. Diseases and phenotypes associated with PARP9

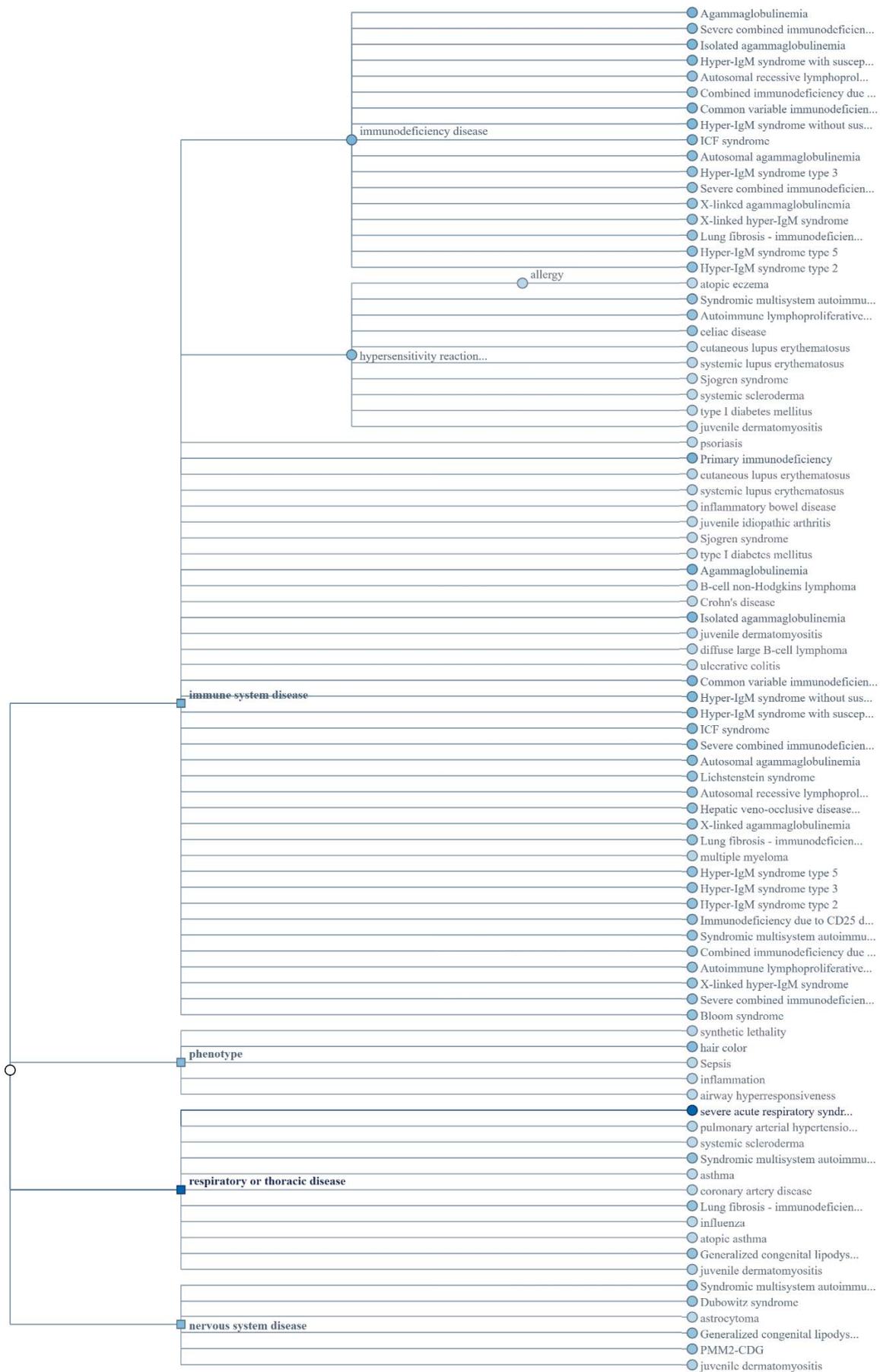


Figure 2. Diseases and phenotypes associated with PARP14

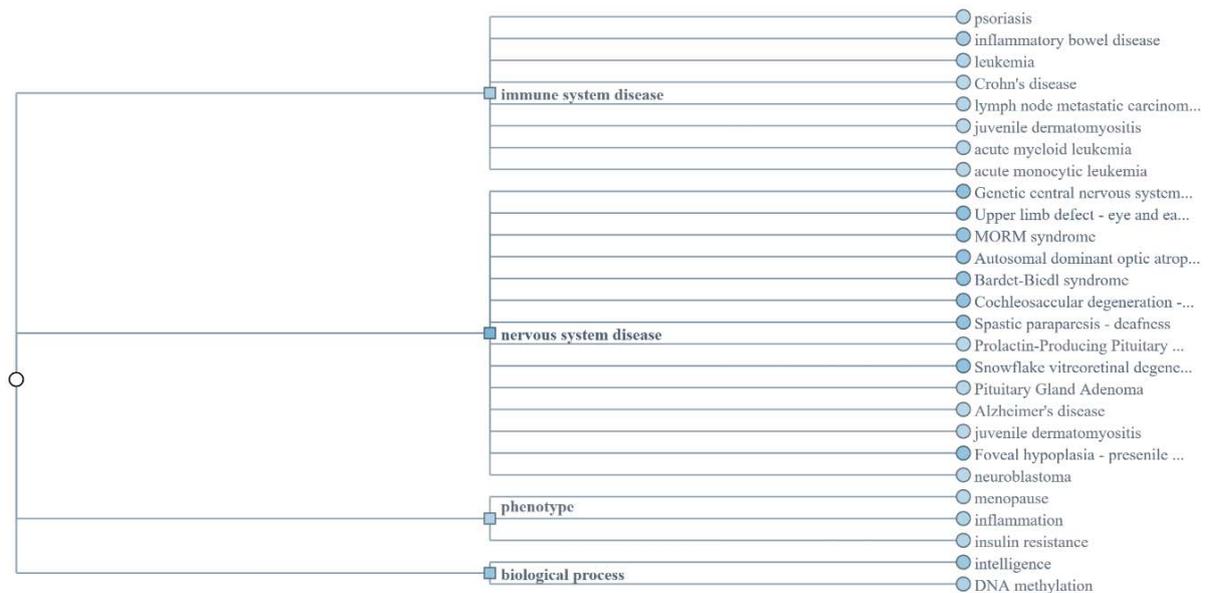


Figure 3. Diseases, phenotypes and biological processes associated with MACROD1

of these proteins hold significant roles in host interferon (IFN)-mediated antiviral defence and DNA repair. Specifically, PARP9 and PARP14 play two opposing roles in IFN γ -induced macrophage activation, where PARP9 promotes IFN γ responses, whereas PARP14 suppresses them by preventing the phosphorylation of STAT1. Additionally, MACROD1 is notably a promiscuous mitochondrial protein that regulates mitochondrial function and plays a role in DNA repair. Although knowledge on the function of MACROD1 remains incomplete, it is highly enriched in tissues which are energetically demanding, such as the heart or the musculoskeletal system. Furthermore, recent studies have correlated MACROD1 and MACROD2 knockouts with neurological dysfunction and cancer. Querying PARP14, PARP9 and MACROD1 in the OpenTargets platform revealed a strong association with a wide spectrum of human diseases spanning across multiple organ systems, a great number

of which possess an autoimmune component in their aetiology (**Figures 1–3**).

Potential B cell epitopes

In order to further verify the hypothesis that molecular mimics play a role in COVID-19 associated autoimmunity, we analysed identified homologous sequences for the presence of structural and linear B cell epitopes, which, in turn, measure their ability to stimulate the auto-reactive antibodies production. Interestingly, out of all the proteins containing homologous regions, only NSP3 – a replication/transcription SARS-Cov-2 protein – contained a tripeptide (*LKH*) homologous with the human proteome on a conformational level, at two different regions. Moreover, a search for linear epitopes using the aforementioned three algorithms, did not provide significant results in terms of antibody production potential for the specific conserved sequences (data not shown) (**Figures 4–5**).

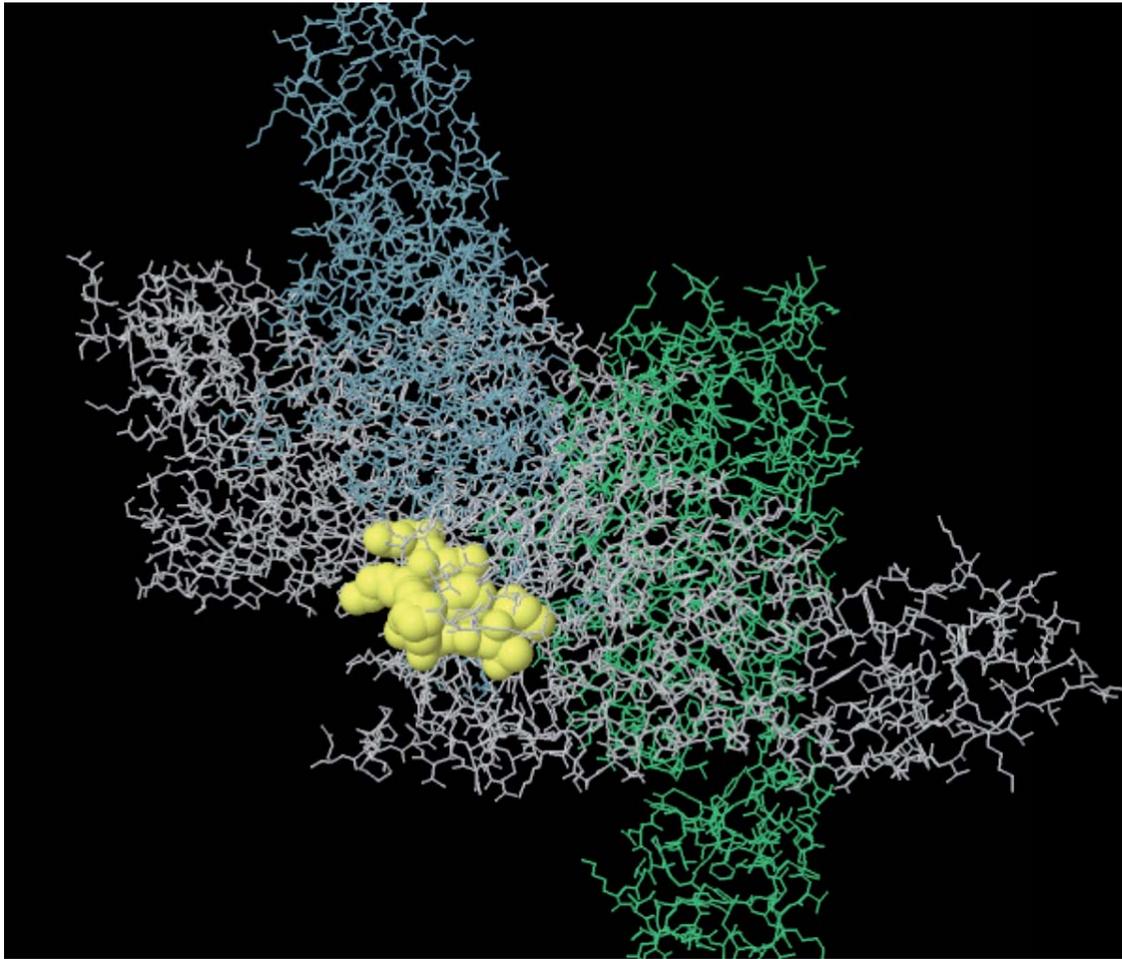


Figure 4. Three-dimensional render of the region within the NSP3 SARS-CoV-2 protein which contains the conformational LKH epitope (highlighted in yellow). The image was rendered using the ElliPro online server

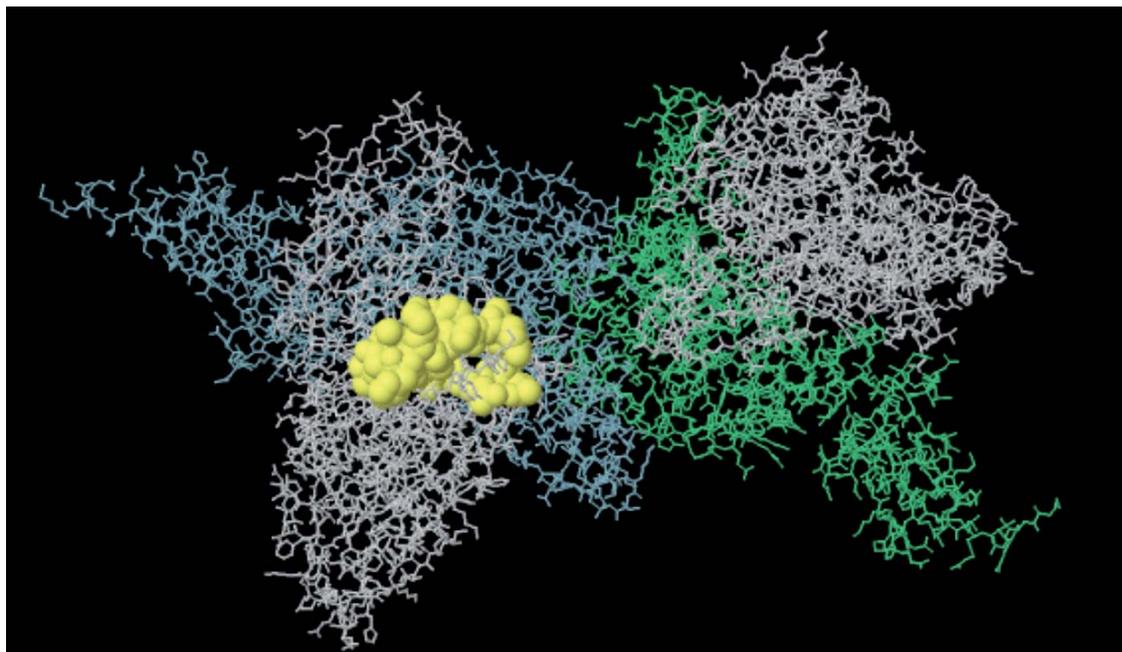


Figure 5. Three-dimensional render of the region within the NSP3 SARS-CoV-2 protein which contains the conformational LKH epitope (highlighted in yellow). The image was rendered using the ElliPro online server

Homologous peptides may act as HLA-II binding motifs

In terms of molecular mimicry, in order for breakdown of immunological tolerance towards self-peptides to occur, the intensity of the immune response elicited by human mimic peptides should presumably be sufficiently similar to that of viral peptides. Therefore, the HLA class II encoded HLA-DR-peptide and HLA-DQ-peptide

complex structures were evaluated for their ability to bind both viral-derived and human-derived peptides within the binding groove of HLA. The HLA molecules selected in this study predominantly present exogenous antigens to CD4+ T helper cells (T_h), and their correlation with a broad spectrum of autoimmune disease has been well established [52–58]. In order to determine whether any of the homologous sequences retrieved by

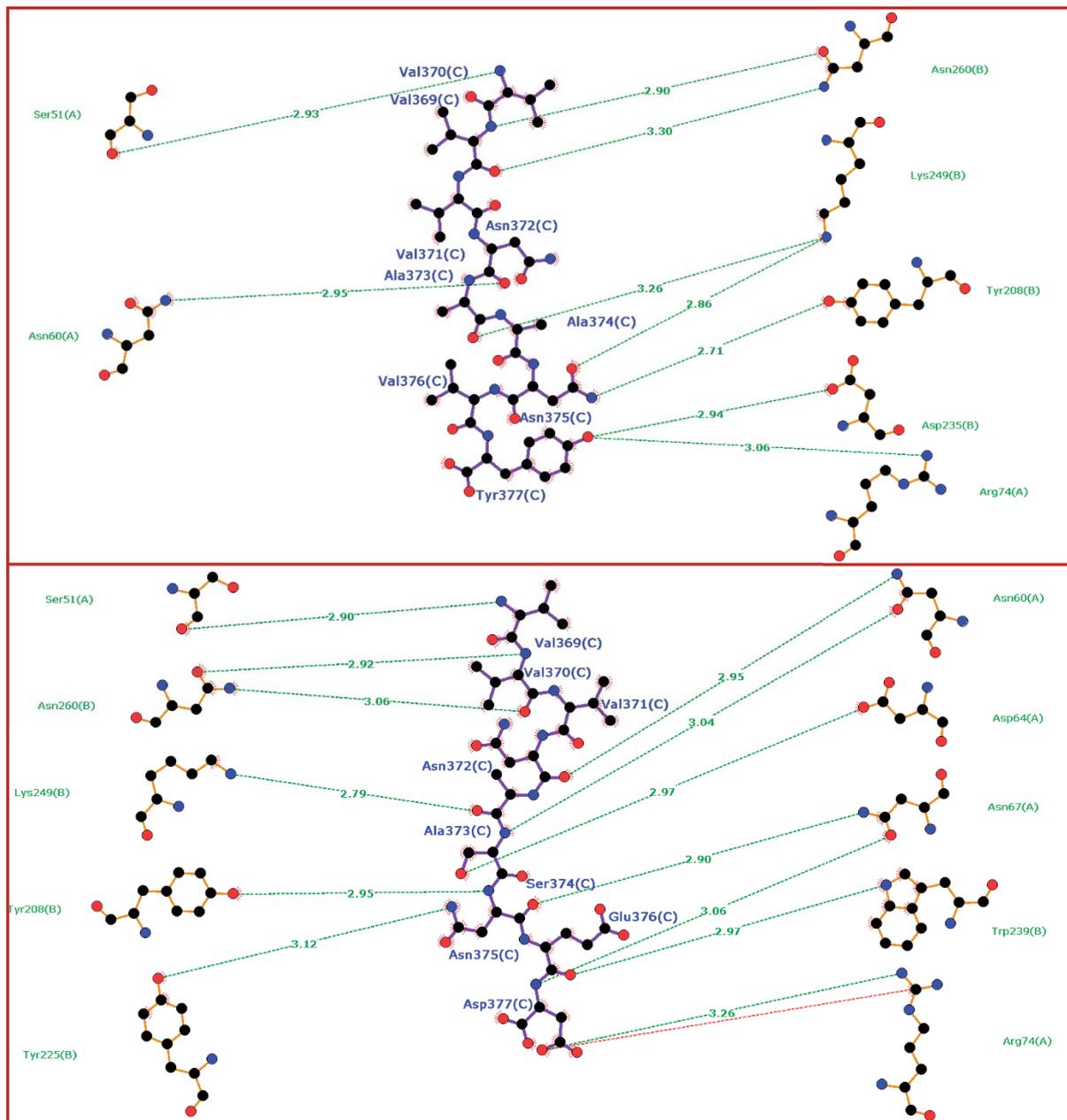


Figure 6. LigPlot-generated diagram of homologous viral (top) and human (bottom) peptides docked with the HLA-DRB1 binding groove. The viral peptide (VVVNAANVY) stems from ORF1a, while the human homologue (VVVNASNED) stems from MACROD1. Carbon atoms are coloured black, oxygen atoms are coloured red, nitrogen atoms are coloured blue. The bonds between C atoms coloured orange belong to HLA-DRB1 residues, whereas the bonds between C atoms belonging to the ligand peptide are coloured blue. Hydrogen bonds are represented with green lines. The red lines represent salt bridges

the BLASTp query could act as T cell epitopes, potential binders were generated after analysing the sequences using the IEDB analysis resource SMM-Align tool. The SMM-Align tool analysed the sequences for their ability to bind to 12 HLA-DR and 12 HLA-DQ alleles, the results of which are summarized in **Table 2**. Although the SMM-Align method automatically calculates binding ener-

gy and thus offers considerable insight into the immunogenicity of the peptide in the context of MHC-II, LigPlot diagrams were generated as to visualize the key residues which would play a part in these MHC-II-peptide dockings. SMM-Align generates both lone core sequences and core sequences with flanking residues; however, the binding affinity of flanked core sequences is sig-

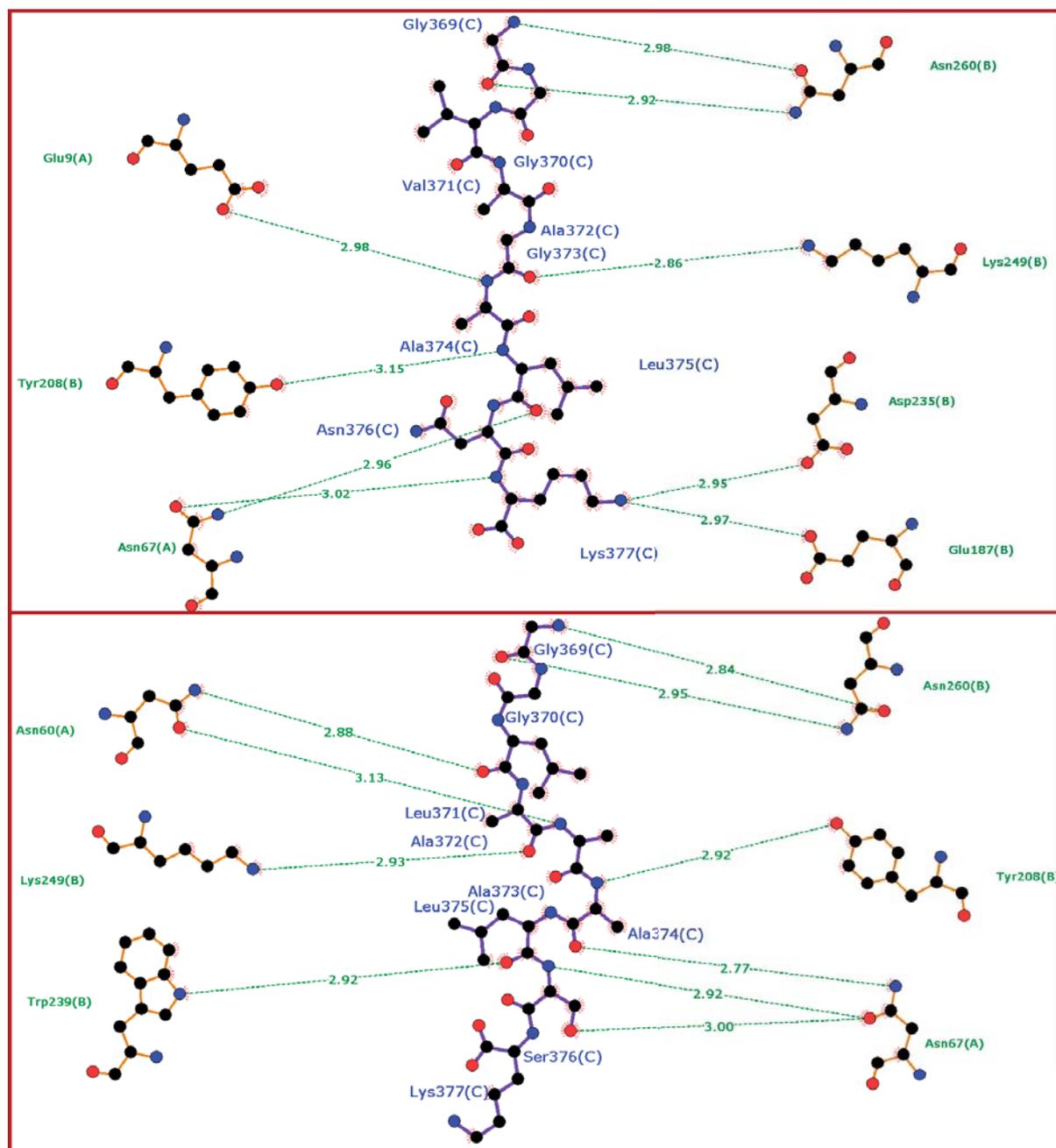


Figure 7. LigPlot-generated diagram of homologous viral (top) and human (bottom) peptides docked with the HLA-DRB1 binding groove. The viral peptide (GGVAGALNK) stems from ORF1a, while the human homologue (GGLAAALSK) stems from PARP9. Carbon atoms are coloured black, oxygen atoms are coloured red, nitrogen atoms are coloured blue. The bonds between C atoms coloured orange belong to HLA-DRB1 residues, whereas the bonds between C atoms belonging to the ligand peptide are coloured blue. Hydrogen bonds are represented with green lines

nificantly influenced by the core itself (59,60). Hence, both core and flanked core sequences with low IC50 values were docked with the HLA binding groove. Docking simulations with flanked cores can be found as PDB files in **Supplementary File 1**, with the SMM-Align results being available in **Supplementary File 2**. The length of hydrogen bonds between residues in the HLA binding

pockets, as well as the peptides themselves were taken as indicators of peptide stability within the pocket binding groove. A distance of ≤ 3.5 Angstroms (\AA) was considered indicative of good peptide stability.

The viral peptide VVVNAANVY and its human counterpart VVVNASNED, docked with HLA-DRB1, display high stability within the binding groove,

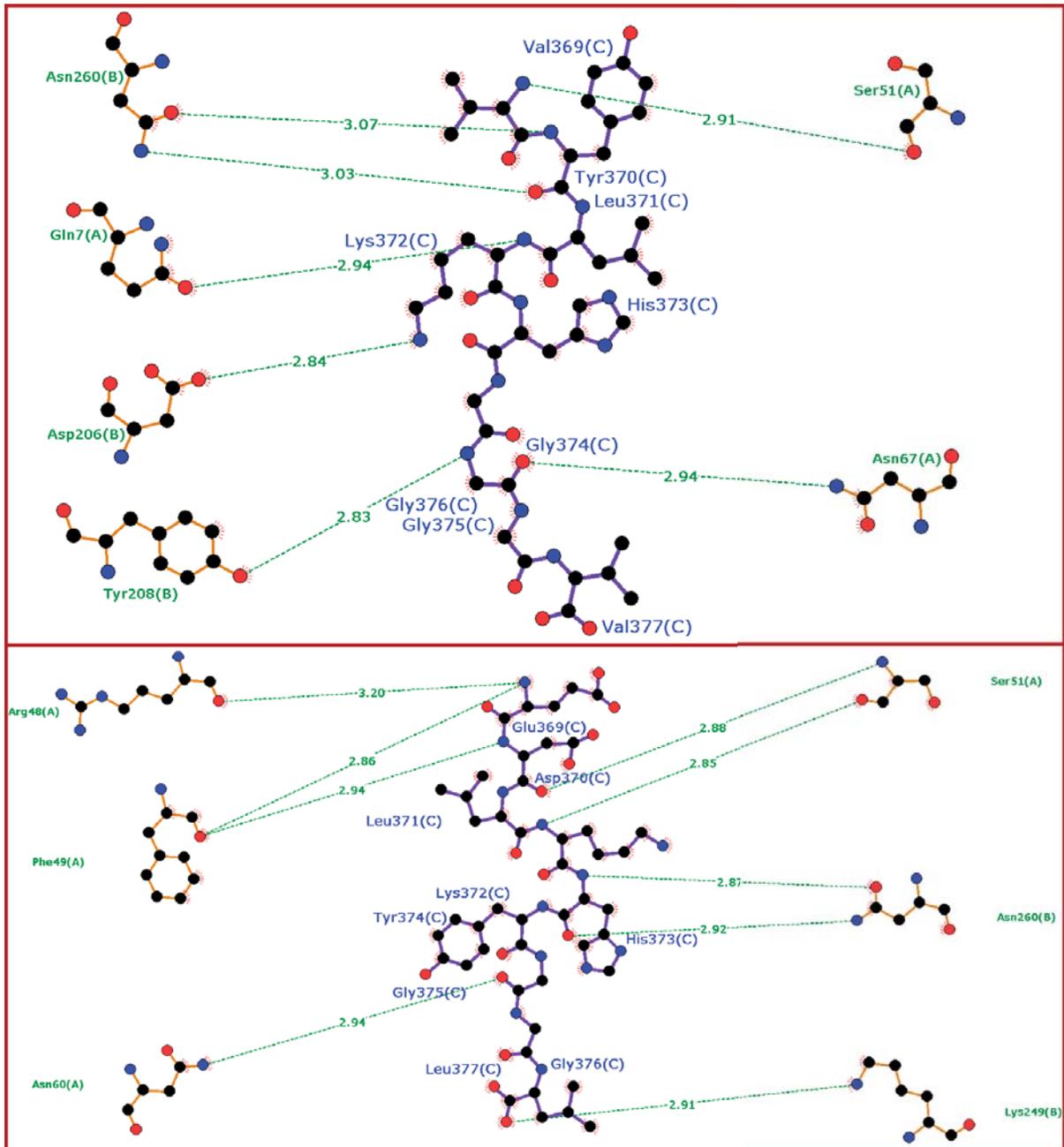


Figure 8. LigPlot-generated diagram of homologous viral (top) and human (bottom) peptides docked with the HLA-DRB1 binding groove. The viral peptide (VYLKHGGGV) stems from ORF1a, while the human homologue (EDLKHYGGL) stems from PARP14. Carbon atoms are coloured black, oxygen atoms are coloured red, nitrogen atoms are coloured blue. The bonds between C atoms coloured orange belong to HLA-DRB1 residues, whereas the bonds between C atoms belonging to the ligand peptide are coloured blue. Hydrogen bonds are represented with green lines

with a notable number of identical HLA residues (Ser51, Asn60, Asn260, Lys249, Tyr208, Arg74) interacting with both peptides at similar hydrogen bond lengths, within the 3.5 Å threshold (**Figure 6**). Furthermore, the peptides GGVAGALNK (viral derived) and GGLAAALSK (human derived)

demonstrate high stability within the HLA-DRB1 binding groove, similarly interacting with several identical binding groove residues (Asn260, Asn67, Lys249, Tyr208) (**Figure 7**). **Figure 8** presents the docked viral (VYLKHGGGV) and human (EDLKHYGGL) peptides interact with fewer identi-

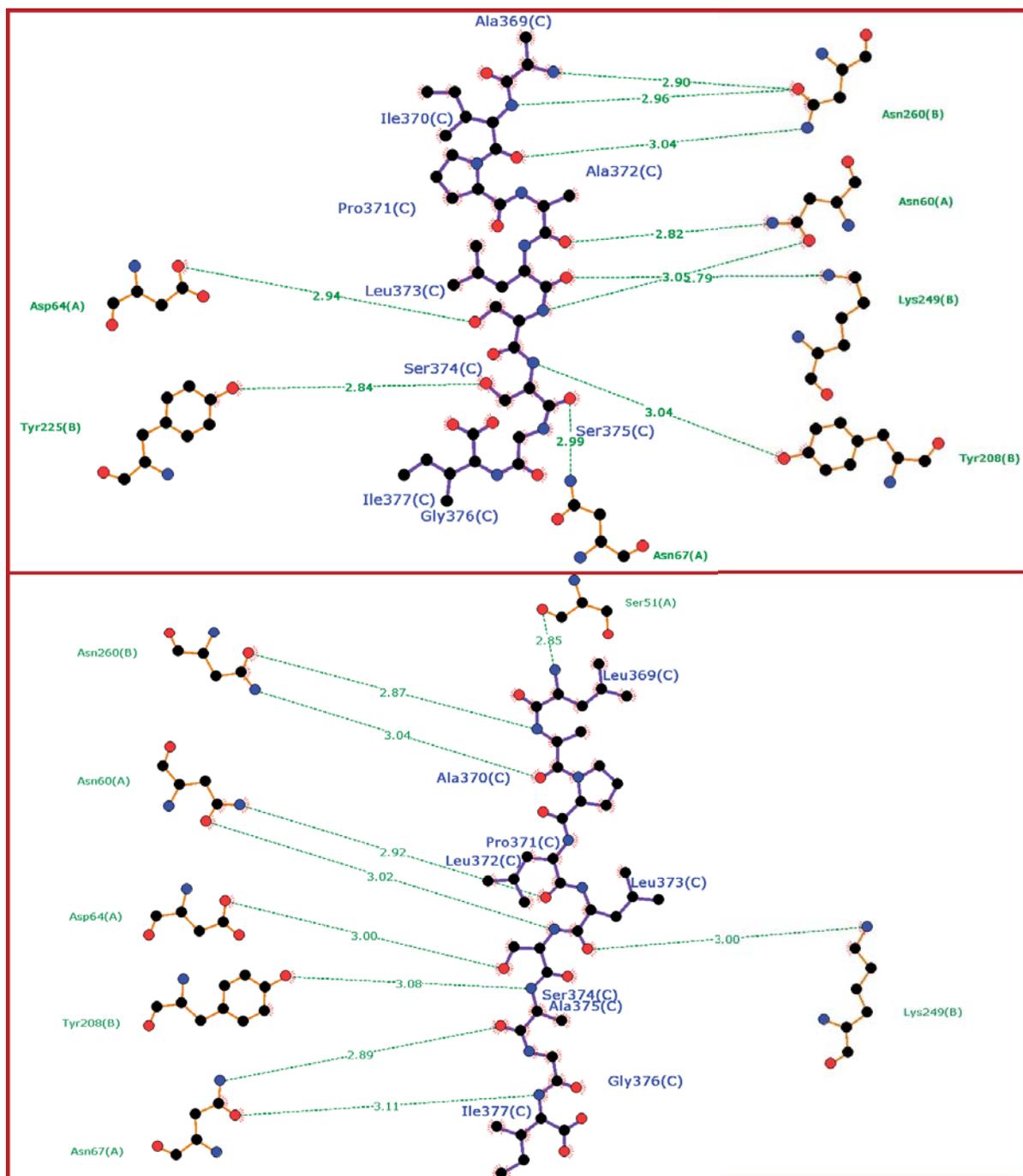


Figure 9. LigPlot-generated diagram of homologous viral (top) and human (bottom) peptides docked with the HLA-DRB1 binding groove. The viral peptide (LAPLLSAGI) stems from ORF1a, while the human homologue (AIPALSSGI) stems from PARP14. Carbon atoms are coloured black, oxygen atoms are coloured red, nitrogen atoms are coloured blue. The bonds between C atoms coloured orange belong to HLA-DRB1 residues, whereas the bonds between C atoms belonging to the ligand peptide are coloured blue. Hydrogen bonds are represented with green lines

cal residues, namely Asn260 and Ser51; however, the valine present in the viral-derived sequence and the leucine present in the human-derived sequence are functionally equivalent. In contrast, the viral peptide *LAPLLSAGI* and its human homologue *AIPALSSGI* demonstrate much higher binding homogeneity, with both peptides similarly interacting between identical residues (Asp64, Asn260, Asn60, Lys249, Tyr208, Asn67) at acceptable hydrogen bond lengths. In spite of the evident differences in amino acids which constitute these peptides, they share a high percentage of functionally equivalent amino acids (**Figure 9**).

Discussion

Viral infections have been intensely investigated for their potential to trigger a breakdown of immunological tolerance through the phenomenon of molecular mimicry, where viral protein sequences homologous with sequences of the human proteome may elicit T cell or B cell responses through cross-reactivity. Prior to correlating this process with SARS-CoV-2-mediated autoimmunity, several crucial factors of autoreactivity must be considered. Firstly, autoreactive antibodies are a natural part of the human immunoglobulin repertoire, although their binding affinity towards self-antigens is relatively weak, thus failing to contribute to phenotypes characteristic of autoimmunity. In fact, autoreactive antibodies appear to contribute to numerous homeostatic repair mechanisms, which is probably possible due to their weak binding affinity towards self-antigens. Moreover, autoreactive T cells are equally prominent in healthy individuals and their homeostatic maintenance is conferred through various tolerogenic mechanisms [61, 62]. In spite of the fact that majority of these mechanisms are not completely understood, the correlation between pathogen-induced inflammatory responses and the breakdown of immunological tolerance has been well documented for a number of virological agents. Nevertheless, the breakdown of immunological tolerance implies the dysregulation of the existing auto-reactive T cells (and B cells – for that matter), stimulating the production and subsequent inclusion of the novel auto-reactive T cells into the T cell repertoire. This process may be triggered during viral infections through inter-

molecular and intramolecular epitope spreading; with molecular mimicry as a prelude to this process. It is vital to note that the same mechanism may apply for B cells that produce autoantibodies, particularly when discussing the role of autoreactive CD4⁺ cells and their role in autoimmunity overall. However, in either case, arguably the most elegant and definitive examples of epitope spreading stem from research on demyelinating autoimmune conditions, such as multiple sclerosis, and revolve around interactions between professional antigen-presenting cells (APCs), autoreactive CD4⁺, CD8⁺ T cells and autoreactive B cells, as key mediators of sustained demyelination [63]. In fact, these key mechanisms appear to be similar in other autoimmune conditions.

The mechanisms by which SARS-CoV-2 molecular mimics may initiate post-infection autoimmunity are multiple, and primarily rooted in the concept of epitope spreading. Based on findings from the previous research on this phenomenon, it may be suggested that local antigen APCs initiate this process when they come to contact with autoreactive T and B cells which had migrated to the inflammation site [63, 64]. This, however, does not exclude dendritic cell (DC)-mediated priming of autoreactive lymphocytes within lymphoid organs. Thus, we suggest that, upon coming to contact with professional APCs presenting SARS-CoV-2 molecular mimics, autoreactive T and B cell subpopulations may promote three distinct and non-mutually exclusive immunopathologic continua, i.e.:

- 1) the lymphocytes may promote extensive organ damage at the site of infection and immune dysregulation (**Figure 10**) [65];
- 2) creation of a “fertile field” as a prelude to post-COVID-19 autoimmunity [42, 66–68];
- 3) initiation of intermolecular and intramolecular epitope spreading (**Figure 11**) [43–45, 64, 69, 70].

The first scenario implies the absorption of SARS-CoV-2, or its fragments, particularly these belonging to the ORF1a polyprotein, from the apoptotic infected cells by resident APCs, such as macrophages and DCs, which possibly depends on TCR specificity [71, 72]. CD4⁺ cells expressing TCRs which promiscuously interact with self-peptides and their presumed molecular mimics, may immunomodulate macrophages and natural killer (NK) cells to destroy tissues expressing self-pro-

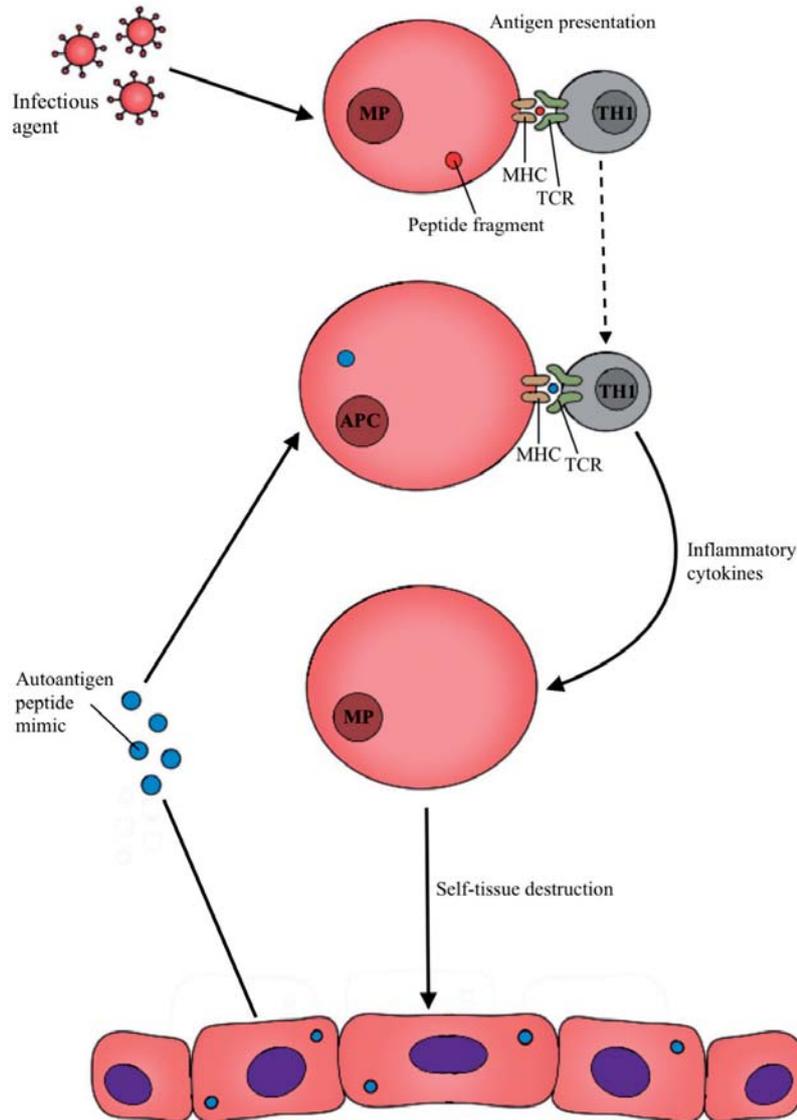


Figure 10. This illustration shows the case of acute self-tissue destruction mediated by the phenomenon of molecular mimicry, during the viral stage of COVID-19. Once a macrophage (MP) has processed the infectious agent, it presents the pathogen peptides containing the molecular mimic, to autoreactive CD4⁺ T helper cells (TH1) via the major histocompatibility complex (MHC) II. This may be followed by a subsequent interaction between the same CD4⁺ T helper cell and another antigen-presenting cell (APC) presenting the human-derived peptide mimic. Consequently, this cross-reactivity may prime the autoreactive lymphocyte to modulate self-destructive activities of macrophages via cytokine secretion

teins containing mimic regions; in this case - MACROD1, PARP14 and PARP9 [73–75]. Alternatively, one lymphocyte may express two TCRs which promiscuously interact with two or more molecular mimics, thus, increasing the chance of self-oriented immunopathology and further epitope spreading [61, 76, 77]. Alternatively, a single T cell may interact with a macrophage which presents a human peptide fragment containing

the mimic, followed by a subsequent interaction with an APC which presents the viral-derived mimic peptide [78]. Haematological findings from COVID-19 patients support the first scenario, as macrophages and NK cells have been substantially correlated with the extensive immunopathology documented in severe cases, where macrophages comprise for the key mediators of severe cytokine storm and overall pathogenesis

enhancement [79, 80]. Although this process is well within the viral phase of infection rather than within the scope of the post-infection autoimmunity, it adequately complements the current clinical data regarding organ damage associated with COVID-19 patients, and maintains the mimicry hypothesis.

Secondly, cross-reactivity with molecular mimics does not necessarily have to induce an immediate autoimmune response directed towards the tissues which abundantly express MACROD1, PARP14 and PARP9. Alternatively, much as other autoimmunity-associated viruses, SARS-CoV-2 could create a “fertile field” – a term which encompasses molecular mimicry, epitope spreading and viral persistence [81]. This would be induced by the substantial immune dysregulation caused by SARS-CoV-2, where the optimal conditions for autoreactive CD4⁺ and CD8⁺ T cells and B cells to become primed against regions within PARP14, MACROD1, PARP9, and potentially other self-proteins, would be created. Nonetheless, whether these primed cells initiate an immune response during the viral stage of COVID-19, likely depends on the level of immunopathology in the course of infection. For instance, an increased pro-inflammatory cytokine expression has been reported as a prerequisite for the creation of the “fertile field” in certain virus-mediated autoimmunity models, and cytokine dysregulation is certainly considered a hallmark of COVID-19 [9, 67, 82, 83]. Once the “fertile field” has been established, subsequent infections with the unrelated pathogens – viral or bacterial – may trigger a profound autoimmune response mediated by the primed autoreactive lymphocytes.

The third scenario, which represents an integrative component of the aforementioned two, entails not only cross-reactivity with PARP9, MACROD1 and PARP14, but also the potential to extend the epitopes containing molecular mimics beyond the ones we have identified for these proteins (intermolecular spreading). In addition, a formation of an immune response against PARP14, MACROD1 and PARP9, may trigger epitope spreading directed towards other proteins, including those belonging to the PARP protein family, or MACROD2 (intramolecular spreading). Consequently, this results in T and B cell epitope diversification, which may account for the fact that a variety of autoimmune diseases have

been reported in patients who have recovered from COVID-19 [2, 4, 21, 26, 27, 29, 30, 32, 33, 84].

Concluding, we have identified several homologous regions between the SARS-CoV-2 proteome and the human proteins PARP14, PARP9 and MACROD1, which may potentially behave as molecular mimics. The results obtained from molecular docking simulations between MHC-II and the identified regions, both viral and their

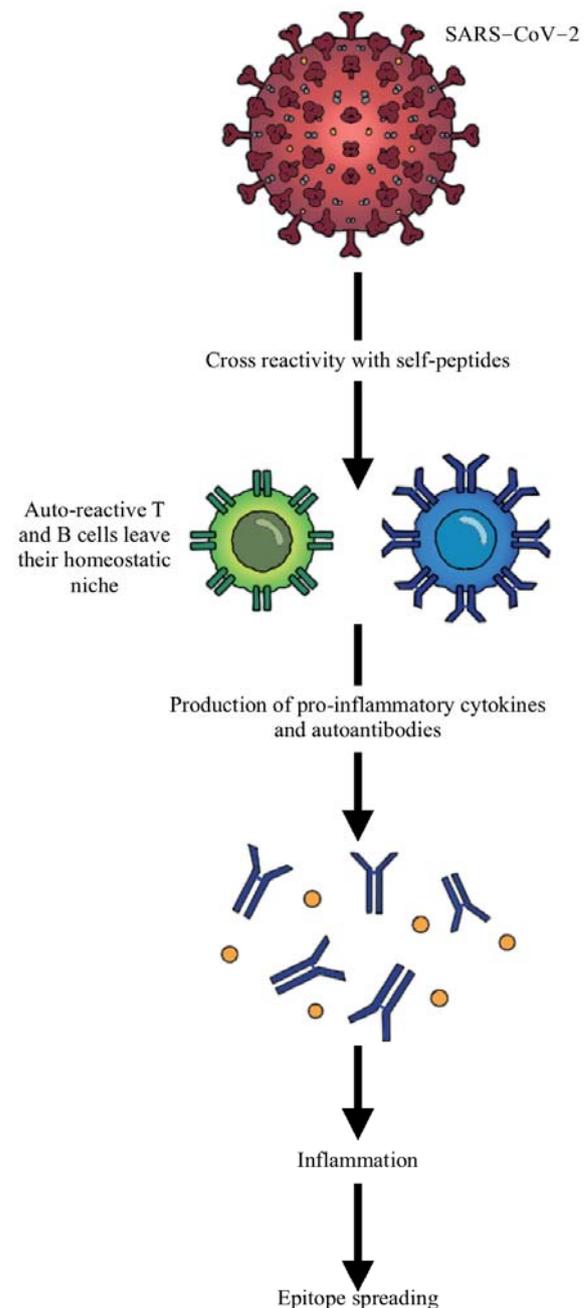


Figure 11. A schematic representation of the process of molecular mimicry-initiated process of epitope spreading, without an immediate initiation of self-tissue destruction

counterparts, prompted the conclusion that these potential mimics may be presented to autoreactive CD8⁺ and CD4⁺ T cells, in an identical or similar way to their human homologues, thus priming this lymphocyte subset towards an autoimmune response. Moreover, a prerequisite for these naturally-occurring T cells to leave their homeostatic niche, would be the creation of a “fertile field” by SARS-CoV-2-induced immune dysregulation; particularly in the context of cytokine dysregulation. This may result in either immediate and severe tissue destruction of cells containing the mimic peptides by autoreactive T cell-mediated macrophages, or in priming of the autoreactive repertoire of T cells in the absence of autoimmunity. In this case, these inert, yet primed, lymphocytes, may be prompted to leave their homeostatic niche and direct self-tissue destruction where PARP14, PARP9 and MACROD1 are abundant. In terms of the autoantibodies, although the number of linear and conformational B cell epitopes identified in our research is scarce, the results suggest the possibility that these homologous regions may prompt autoreactive B cells to produce antibodies for these epitopes, particularly the *LKH* tripeptide. Therefore, we were able to develop a plausible case – based on *in silico* analysis - where molecular mimicry potentially constitutes an aetiological mechanism of SARS-CoV-2-mediated autoimmunity, whether in a multi-organ damage context, or outside the viral phase of infection. This and other studies further support the notion that COVID-19 survivors may be prone to the elicitation or exacerbation of autoimmune conditions, due to the extensive immune dysregulation caused by the virus. Epidemiologically, this may be evaluated by means of a broader epidemiological surveillance of COVID-19 survivors, supplemented by serological cohort studies aimed at detecting autoantibodies and the presence of potential IFN I and IFN III gene mutations which could contribute to post-COVID-19 autoimmunity. The aforementioned findings would be indispensable in understanding the role of SARS-CoV-2 as a potential trigger of autoimmunity. Interestingly, this is the first time that the peptide sequence of MACROD1 has been implicated in COVID-19 autoimmunity. We also confirm that PARP9 and PARP14 may be involved, which is consistent with the previously published findings concerning the effects of these two proteins

in COVID-19-related autoimmunity [8]. Moreover, our findings corroborate those of the previous study of homologous sequences between PARP14 and SARS-CoV-2 ADP ribose 1'-phosphate. In fact, our approach focused on structural biology which further enhanced the aforementioned and other bioinformatic studies pertaining to this topic. An encouraging finding of this study is the lack of significant BLASTp results related to the homology between the human proteome and the SARS-CoV-2 S protein, particularly since the S protein sequence forms the antigen employed in the currently-approved COVID-19 vaccines. Furthermore, more extensive *in vivo* research should be conducted with regard to these peptide sequences, in order to determine their relevance for autoimmunity to address the limitations of *in silico* analysis, which been thoroughly discussed in recent years, particularly with regard to the immunoinformatic approaches. Nevertheless, the limitations of this study need to be taken into account, and the study should be regarded as indicative rather than definitive. As more data are collected to complement the existing immunoinformatic algorithms, along with improvements in molecular docking technology, the precision of such studies shall undoubtedly increase. Presently, the tools used to conduct this study, as well as the results obtained by their use, are of sufficiently high quality to supplement and clarify the hypothesis presented in this paper. However, *in vivo* studies are indispensable to confirm any results obtained within *in silico* research.

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Conflict of interest statement

The authors declare no conflict of interest.

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