Myosin 16 mimicries may explain post- COVID-19 related neurological consequences

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May 19, 2023

Abstract

Autoimmune diseases arise from an immune response against self-antigens, but their pathophysiology is not fully understood. One of the proposed mechanisms is molecular mimicry, where infectious agents share similar antigens with host proteins leading to cross-reactivity. Our study aimed to investigate the presence of molecular mimicry between SARS-CoV-2 and human proteome using bioinformatics techniques. To accomplish this, we constructed sequences of 8 consecutive amino acids for structural proteins of SARS-CoV-2, such as spike, nucleocapsid, membrane, and envelope proteins. Next, we evaluated the mimicry of these sequences with the human proteome and analyzed their antigenicity, allergenicity, toxicity, TAP affinity, and IFNγ and IL-10 induction. We also calculated the affinity of the amino acid sequence DEDDSEPV, which showed molecular mimicry, to HLA receptors and found that it had a good binding energy. Based on our in silico analysis, we found that DEDDSEPV amino acid sequence might trigger autoimmunity due to its similarity with myosin-16 protein. Our study provides evidence for the possibility of SARS-CoV-2 inducing autoimmunity via molecular mimicry. Our findings can have significant implications for understanding the pathophysiology of autoimmune diseases and may contribute to the development of potential therapeutic strategies.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a viral infectious agent that broke out in Wuhan, China, and rapidly spread from China to the world [1,2]. SARS-CoV-2 causes Coronavirus disease...
2019 (COVID-19) and postinfectious syndromes [3]. On 11 March 2020, the World Health Organization (WHO) declared the COVID-19 pandemic [4]. Flu-like symptoms, such as fever, fatigue, dry cough, headache, as well as myalgia, sore throat, nausea, and diarrhea, were reported and considered as clinical presentations of COVID-19 [5]. The SARS-CoV-2 virus belongs to the beta-coronaviridae genus [6]. It is a single-stranded RNA virus and has a spherical morphology. Also, it has four structural and sixteen non-structural proteins [7]. SARS-CoV-2 infects cells via splitting the spikes protein into S1 and S2 by TMPRSS8. S1 binds to the ACE-2 receptor, and S2 ensures the fusion of the virus into the cell [8]. When the virus enters the cell, it activates pattern recognition receptors (PRRs), which results in the release of proinflammatory cytokines from the infected cell, leading to macrophage activation [9]. Activated macrophages secrete cytokines and chemokines, leading to vasodilation and increased capillary permeability [10,11]. The alveoli are compressed by the passage of plasma into the interstitial space. This process also disrupts surfactant synthesis in alveolar type 2 cells in the lung. As a result of these pathological processes, alveolar collapse develops in the lung, and gas exchange is impaired [12]. IL-1, IL-6, and TNF, which are pro-inflammatory cytokines released into the blood, reach the hypothalamus via the blood and cause fever [13]. In several cases of COVID-19, the shortness of breath caused hypoxemia [14,15]. Critical patients develop septic shock and hypoxemia-related multi-organ dysfunction [16]. Hypoxia, which occurs with the development of pneumonia, causes tachycardia by activating the sympathetic system. The abnormal immune response can cause septic shock and death [17]. In short, due to pneumonia, vasodilation that decreases effective blood volume (BV) and peripheral resistance (PR) can lead to hypotension, reduced perfusion rate of the heart, and multi-organ failure [18]. In COVID cases, severe and critical patients show immune dysregulation [19], elevated inflammatory markers [20], low number regulatory immune cells [21], high-level inflammatory Th-17 cells [21], and neutrophils [22]. Medications used in autoimmune diseases, such as tocilizumab [23] and tofacitinib, are also used in COVID-19 patients [24]. Some autoantibodies were detected in COVID-19 patients [25]. It is thought that the immune dysregulation and hyperinflammatory state in COVID-19 can cause autoimmunity [26]. Viruses can cause Type II hypersensitivity reactions, including tissue damage caused by autoantibodies secondary to viral infections and inflammation. The aim of our study is to identify molecular mimicry between SARS-CoV-2 and the human proteome. We will also determine the immunological properties of sequences with molecular mimicry using immunoinformatics techniques.

**Molecular mimicries and viral infections**

Diseases that recognize host antigens as foreign and develop an immune response against our own antigens are called autoimmune diseases [27]. The etiopathogenesis of autoimmune diseases is still not fully explained, but environmental and genetic basis play a serious role in the clinical course of autoimmune diseases and the formation of the disease [28]. While the host’s genetic background may play a role in triggering an immune response against self-antigens, current evidence suggests that immune dysregulation caused by infectious agents is the primary environmental factor responsible for initiating autoimmune diseases, as supported by both epidemiological and molecular research [29]. Prolonged immune response to infections and/or uncontrollable inflammation may lead to the emergence of autoimmune diseases and worsen the clinical course of autoimmune patients [30]. Although viral and bacterial infections have been linked to the development of autoimmune diseases, it is unlikely that exposure to these infectious agents alone will trigger an autoimmune response [31]. The onset of autoimmunity also requires the patient’s genetic predisposition and/or environmental factors [32]. The production of autoantibodies is a hallmark feature of autoimmune diseases, but the pathophysiology underlying their development is still unclear [33]. Molecular mimicry with viruses and bacteria, first described over 30 years ago, has been shown to cause and/or exacerbate autoimmune diseases [34]. Viruses infect humans have the ability to mimic molecules present in the human body, which can lead to the development of autoimmunity [35]. Therefore, the dysregulated immune response to viral pathogens can disturb immune tolerance [29, 36]. Viral epitopes may be similar to human proteins, resulting in the production of cross-reactive antibodies [37]. These findings emphasize the critical role of infectious agents in the pathogenesis of autoimmune diseases and highlight the importance of considering both genetic and environmental factors in disease development. Further research is needed to fully elucidate the complex interplay between environmental and genetic factors in autoimmune diseases.
Epstein-Barr Virus (EBV), the causative agent of infectious mononucleosis, is thought to be the primary trigger for the development of many autoimmune diseases, including Multiple Sclerosis, Sjögren’s Disease, and Systemic Lupus Erythematosus [38, 39]. One potential mechanism behind this link is molecular mimicry between the amino acid range 627-641 of the EBV DNA polymerase protein, which plays a role in the replication of Epstein Barr virus, exhibits molecular mimicry with the myelin basic protein amino acids between 85-99 [40]. The myelin basic protein is a protein that regulates the myelin sheath along with oligodendrocytes and Schwann cells [41]. The resulting neuro-autoimmunity, driven by the molecular mimicry between EBV DNA polymerase and myelin basic protein, leads to the development of Multiple Sclerosis [42].

Infection with the Hepatitis B virus (HBV) can cause hepatocyte destruction and inflammation, resulting in liver damage [43]. The severity of liver damage and the likelihood of liver failure vary depending on the strain of HBV involved [44]. HBV polymerase, a protein involved in viral replication, has been shown to mimic myelin proteins, which can result in neuro-autoimmune events, and this cross-reactivity may contribute to the development of neurologic complications in some HBV-infected individuals [45].

Inflammation of the myocardium, the mechanically functional part of cardiac tissue, is primarily caused by viral infections [46,47]. While many viruses can lead to myocarditis, SARS-CoV-2 is a recent addition to this list [48]. However, it is necessary to note that most viral infections responsible for myocarditis do not lead to autoimmune myocarditis [49]. Exceptions to this include Coxsackievirus and murine cytomegalovirus, which have been linked to autoimmune myocarditis [50].

β cells, responsible for the major endocrine function of the pancreas, play a critical role in regulating important metabolic processes by secreting insulin [51]. Type 1 diabetes can develop due to pancreatic cell destruction, resulting in insufficient insulin production [52]. Coxsackievirus is one of the viruses thought to be involved in the pathogenesis of type 1 diabetes [53]. The autoimmune diabetes is thought to be caused by the molecular mimicry of the P2-C protein, which is involved in Coxsackievirus replication, and the glutamate decarboxylase (GAD65) enzyme in pancreatic cells [54]. This molecular mimicry can trigger an immune response that attacks and damages cells, resulting in decreased insulin secretion [54, 55].

Systemic sclerosis is a disease in which the working system of the immune system is impaired and has systemic involvement [56]. In systemic sclerosis, there are deformities in vascular structures and abnormal fibroblast activation due to autoimmune involvement. Skin, lung, heart, musculoskeletal, renal, and gastrointestinal systems are affected by systemic sclerosis [56, 57]. Raynaud phenomenon is characteristic, especially in the fingers [56, 58]. Tetraspan novel antigen-2 (NAG-2) is a protein expressed on endothelial cells and fibroblasts [59]. Systemic sclerosis may develop due to the molecular mimicry between the UL-94 protein of the human cytomegalovirus and the NAG-2 [59, 60]. In studies with anti-UL94 antibodies, it has been proven by laboratory studies that anti-UL94 antibodies bind to NAG-2 proteins [59, 60]. It has been shown that anti-UL94 antibodies can cause systemic sclerosis by inducing endothelial cell activation and apoptosis, as well as by activating fibroblasts [59, 60].

Stiff-person syndrome is a neuroendocrine autoimmune disease in which neurons and pancreatic β cells are affected [61]. In Stiff-person syndrome, autoimmune reactions develop due to the molecular mimicry between the UL-57 protein of human cytomegalovirus and glutamate decarboxylase (GAD-65) [62]. The 674 to 687 amino acids of the UL-57 protein and the amino acids between 339 to 352 position of the GAD-65 protein constitute molecular mimicry [62]. With the targeting of GAD-65 protein by the immune system, pancreatic β cells are affected, and type-1 diabetes develops [62, 63].

In Sjögren’s syndrome, an autoimmunity affecting the salivary and lacrimal glands, Coxsackie virus RNA was detected in the biopsy examinations of the salivary glands [64]. The role of molecular mimicry in the development of Sjögren’s syndrome, on the other hand, has 87% sequence homology between the 31-47 amino acid sequence range of the Coxsackie virus 2B protein and the 222-229 amino acid of the Ro60 autoantigen [65]. The presence of autoantibodies against the Ro60 antigen is important in the diagnosis of Sjögren’s disease [66].

Molecular Mimicry and Bacteria
As with viral infections, bacterial infections can trigger autoimmune diseases [67]. In the immune reaction against bacterial infections, lipid, protein and nucleic materials of bacteria are recognized by Toll-like receptors (TLR) expressed in various cells [68]. Toll-like receptors are classified according to their distribution in cell compartments. TLR-1, TLR-2, TLR-4, TLR-5, TLR-6 and TLR-11 are located in the endolysosomes, TLR-3, TLR-7, TLR-8 and TLR-9, bound to the plasma membrane [69]. Toll-like receptor ligands of bacteria are triacyl lipopeptide, lipoprotein, peptidoglycan, lipoteichoic acid, lipopolysaccharide, flagellin and CPG containing DNA [70]. Cytokines, chemokines, interferons and enzymes are produced through bacterial toll-like receptors, which control the immune response against bacteria [70, 71]. Cytokines, chemokines, interferons and enzymes produced due to Toll-like receptor stimulation can trigger autoimmune events by causing immune dysregulation if they are not produced regularly [72].

Streptococcal infections can also cause autoimmune reactions originating from molecular mimicry [73]. In addition, rheumatic fever, one of the most classic examples of molecular mimicry, is one of the autoimmune diseases originating from post-streptococcal molecular mimicry [74]. Some patients infected with *Streptococcus pyogenes* may develop rheumatic fever [75]. The M protein in the membrane of *Streptococcus pyogenes* bacteria is present in order to protect it from the immune system [76]. The M protein is a potent anti-phagocytic protein and is the major virulence factor of group-A streptococci [76, 77]. It binds to factor H via the *Streptococcus pyogenes* M protein and inhibits C3-convertase, which is one of the most important elements of the complement system. With the inhibition of C3 convertase, the formation of C3a and C3b, which is formed by the cleavage of C3, is prevented [78]. Due to the inability to form C3b, opsonization and phagocytosis of *Streptococcus pyogenes* are inhibited. In the immune reaction against *Streptococcus pyogenes*, an immune reaction also develops against the M protein [79]. During the humoral immune response developed against the M protein, autoantibodies are formed by the deterioration of the immune tolerance of some B cells [80]. While the autoantibodies formed react against the M protein, they also self-react against the myosin protein, which is present in the cardiac tissue and undertakes the mechanical function of the cardiac cycle and causes cardiac damage [81]. Chronic rheumatoid carditis, valvular heart diseases, cardiomyopathy, heart failure, atrial arrhythmias, and pulmonary and systemic embolisms can develop as complications of cardiac damage due to rheumatic fever [82]. In addition, autoantibodies can be formed against other cardiac muscle proteins such as vimentin, collagen, and troponymosin by the epitope spreading mechanism [80]. Another manifestation of rheumatic fever is the Sydenham chorea [83]. The formation mechanism of Sydenham chorea is also based on molecular mimicry [80]. The relationship of Sydenham chorea with molecular mimicry was understood by the cross-reaction of antibodies against N-acetyl-β-D-glucosamine found in group-A streptococci and lysoganglioside found in neurons in the central nervous system [80, 84]. In addition, some of the autoantibodies formed cause excitation by induction of Calcium calmodulin-dependent (CaM) protein kinase II [84]. Since the neurotransmitter that causes excitation is dopamine, post-synaptic Dopamine-2 (D2) receptor inhibitor haloperidol is used in the treatment of Sydenham chorea [85, 86]. The formation of musculoskeletal events due to rheumatic fever is similar to cardiac autoimmunity [80]. The mode of development of musculoskeletal events is the development of autoimmunity due to the similarity of musculoskeletal proteins and cardiac proteins [80]. The primary goal in the treatment of rheumatic fever is to reduce the burden of antigens that cause autoantibody production [87]. Therefore, it is aimed to reduce the number of bacteria carrying M protein by using penicillin [87]. In addition, most patients infected with *Streptococcus pyogenes* do not develop rheumatic fever [82]. Various risk factors are required for the development of rheumatic fever [88]. Risk factors for rheumatic fever are family history, infection with rheumatic fever streptococcal strains, and environmental factors [88].

It is caused by the molecular mimicry of Guillain-Barre syndrome (GBS) caused by *Campylobacter jejuni* infection [89]. Autoantibodies against gangliosides are produced due to infection by *Campylobacter jejuni* bacteria [89,90]. Autoantibodies (anti-ganglioside) formed against gangliosides cause destruction of gangliosides [90]. Motor neuropathies and motor nerve disorders may occur due to damage to gangliosides [91]. Following *C. jejuni* infection, Yuki et al. found that bacterial lipopolysaccharides (LPS) of *C. jejuni* resembled motor axolena molecules (eg, GM1, GM1b and GD1a), and that sequence similarity between *C. jejuni* LPS and host ganglioside molecules resulted in pathogenic autoantibody production and GBS.
development [92]. In Guillain-Barre syndrome, autoantibodies can develop against various ganglioside types such as GM1, GD1a, GT1a, GQ1b, GM1b [92,93]. In order to benefit from its anti-inflammatory effects in the treatment of Guillain barre syndrome, intravenous immunoglobulin obtained from a pool formed from healthy patients is administered [93]. Another treatment alternative is the application of plasmapheresis to remove autoantibodies from the blood [93,94].

Heat shock proteins (HSP) are involved in many physiological events [95]. One of the most important tasks of HSPs is the maturation, degradation, and re-folding of proteins [96]. In addition, HSPs are responsible for the regulation of the immune system by taking part in situations where human physiology is stressed [97]. Pseudomonas aeruginosa colonies in the lungs in cystic fibrosis, a systemic disease that affects almost all tissues [98]. Cystic fibrosis has complications affecting all systems [99]. Gastro-oesophageal reflux disease, exocrine pancreatic insufficiency, increased risk of appendicitis, meconium ileus, sinusitis, nasal polyps, persistent coughs, infertility, osteoporosis, and arthritis are complications of cystic fibrosis [99,100]. There is a correlation between anti-HSP27 autoantibodies and \textit{P. aeruginosa} colonization in the arthritic complication of cystic fibrosis [101]. Autoimmune arthritis develops due to mimicry between pseudomonal proteins and HSP proteins [101].

Autoantibodies are antibodies produced by autoreactive B cells that can penetrate a wide variety of threats [104]. Autoantibodies can be produced against DNA, RNA, lipids, proteins, centromeres, chromatin and ribosomal properties [104, 105]. Loss of B cell tolerance, defective apoptosis, loss of regulatory cells and cells may cause offspring of autoantibodies [104]. Autoantibodies can be seen in IgM and IgG isotypes [104,105]. The place of autoantibodies is indispensable to recognizing and treating autoimmune clearance [106]. In organ-specific autoimmune diseases, autoantibodies are present against those with organ antigens, whereas, in systemic autoimmune diseases, no autoantibodies are present against antigens found in most tissues [104]. In autoimmune thyroiditis and Graves’ disease, which are organ-specific autoimmune diseases, autoantibodies can develop against the thyroid antigens thyroglobulin, thyroperoxidase, and thyroid stimulating hormone stores [104,107]. In myasthenia gravis, another neuro-autoimmune disease, autoantibodies against the acetylcholine receptor can be formed by B cells [107]. In systemic autoimmune diseases, it consists of autoantibodies against common antigens found in most tissues [108]. For example, in systemic lupus erythematosus, anti-nuclear antibodies against nuclear material consist of anti-dsDNA against DNA and anti-histone autoantibodies against histone proteins [109]. However, auto-antibodies detected in some departments controls may not spread with autoimmune diseases [110]. Viral infections can also cause autoimmune diseases in various ways [111]. Viral infections can cause autoimmune disease by mimicry, bystander activation, by adjuvant rearrangements, and by epitope spreading [112].

Autoantibodies were detected in the COVID-19 and post-COVID-19 patients [103]. It has been shown that patients with positive autoantibodies have a more severe course of COVID-19 than other cases [113]. As a result of the study of Pascolini et al. on 33 COVID-19 patients, antinuclear antibodies, anti-cyttoplasmic neutrophil antibodies (ANCA), and anti-antiphospholipid antibodies (APL) showed that these autoantibodies develop due to COVID-19 [114]. Severe and critical COVID-19 patients have a higher frequency of APL autoantibody and the presence of APL antibody positivity is associated with extremely high-level ferritin, CRP, IL-6, and pulmonary thromboembolism [115]. This explains the hypercoagulable state in severe and critical COVID-19 cases and indicates that SARS-CoV-2 can induce autoimmune responses [114,116]. COVID-19 patients have a higher risk of lupus anticoagulant positivity [117]. Lupus anticoagulant-positive patients have a higher risk of thrombosis [118]. Several case reports demonstrated autoantibodies against RBC antigens which they can contribute to hemolytic anemia and are related to the severity of anemia in COVID-19 [119,120]. Some COVID-19 patients have neurologic symptoms. These patient’s case reports have been shown to develop autoantibodies against contactin-associated protein 2 (anti-Caspr2), ganglioside...
GD1b (anti-GD1b), and myelin oligodendrocyte glycoprotein (anti-MOG) [121,122]. COVID-19 patients can develop the hematologic system autoimmunity [123].

Material-Method

Data Collection

Structural proteins of SARS-CoV-2 (Spike, Nucleocapsid, Membrane, Envelope) were obtained from the NCBI database. The structural proteins aminoacid sequences of SARS-CoV-2 were fragmented into 8 amino acid-long peptide fragments.

Peptide Matching

The peptide-match server is an online tool to reveal the amino acid sequence similarity between amino acid sequences and desired organism [124]. Fragmented eight amino acid length peptides uploaded peptide-match server. This server predicts similarity between 8mers and human sequences [124].

Antigenicity prediction

In order to generate immune responses based on molecular mimicry, the 8mers that we constructed from SARS-CoV-2 structural proteins must be antigenic [125]. Predicted similar epitopes assessed for antigenicity in Vaxijen v2.0 antigenicity prediction server [125].

Prediction of binding affinity of peptides to TAP

The compatibility of 8mers with the TAP protein must be met for them to be presented as epitopes. Similar epitopes were analyzed for TAP affinity. For the analysis, we use the TAPreg tool [126].

Allergenicity prediction

To elicit an autoimmune response through molecular mimicry, 8mers must not possess allergenicity as this would result in the activation of an IgE-mediated response. The predicted similar epitopes were analyzed for allergenicity. For analyses of allergenicity, we use the AllerTOP v2.0 tool [127].

Toxicity prediction

Similar epitopes were analyzed for toxicity, the toxicity prediction we use the ToxinPred tool [128].

IL-10, and IFN-gamma inducing prediction

Similar epitopes were analyzed for IL-10, and IFN-gamma induction. The prediction of IL-10 [129] inducing predicted in IL-10pred, and prediction of IFN-gamma inducing predicted in IFNepitope[130] server.

HLA docking calculation

Matched best peptide (identical, antigenic, non-allergenic, non-toxic, IL-4 inducer, and IFN-gamma inducer, non-IL-10 inducer, and high affinity to TAP) docked to Class-I and Class-II MHC molecules. Class-I HLA docking simulated in CABS-dock server [131]. Structures of HLA-I molecules were retrieved from the RCSB-PDB server (4NQV, 4UQ3, 3RL2, 1X7Q, 5HGA, 5EO1, 3SPV, 1OGT, 3LKN, 1E27, 5INC). The docked models were analyzed for ΔG (kcal mol-1) and Kd (M) at 37.0 °C values. For this analysis, the PRODIGY server was used [132,133]. The Epidock server was used for DEDDSEPV peptide docking to the MHC-II receptor.
Results

Peptide Matching

Fragmented peptides imported to the peptide match server. We found six identical amino acid sequences (VNSVLLFL, VFLLVTLA, KKDKKKKA, SRSSSRSR, RRARVSVAS, DEDDSEPV) to the human proteome. Table-1 shows the UniProt codes and protein names of similar proteins with identical amino acid sequences.

Table-1
List of the identified 8mers of SARS-CoV-2 that have mimicry with human proteome.

<table>
<thead>
<tr>
<th>Amino acid sequence</th>
<th>UniProt ID</th>
<th>Protein Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>VNSVLLFL</td>
<td>O60518</td>
<td>Ran-binding protein 6</td>
</tr>
<tr>
<td>VFLLVTLA</td>
<td>Q6P4F1</td>
<td>Alpha-(1,3)-fucosyltransferase 10</td>
</tr>
<tr>
<td>KKDKKKKA</td>
<td>Q7RTP6</td>
<td>[F-actin]-monooxygenase MICAL3</td>
</tr>
<tr>
<td>SRSSSRSR</td>
<td>Q8N2M8</td>
<td>CLK4-associating serine/arginine-rich protein</td>
</tr>
<tr>
<td></td>
<td>Q96HJ9</td>
<td>Protein FMC1 homolog</td>
</tr>
<tr>
<td></td>
<td>Q96S94</td>
<td>Cyclin-L2</td>
</tr>
<tr>
<td></td>
<td>Q9Y383</td>
<td>Putative RNA-binding protein Luc7-like 2</td>
</tr>
<tr>
<td>RRARVSVAS</td>
<td>P37088</td>
<td>Amiloride-sensitive sodium channel subunit alpha</td>
</tr>
<tr>
<td>DEDDSEPV</td>
<td>Q9Y6X6</td>
<td>myosin-XVI</td>
</tr>
</tbody>
</table>

Antigenicity

Identical sequences must be antigenic because non-antigenic sequences cannot induce cross reactivity. Table-2 shows antigenicity and calculated antigenicity scores of identical peptides.

Table-2
Results of the predicted antigenicity scores and the antigenicity evaluation for the 8mer sequences possessing molecular mimicry.

<table>
<thead>
<tr>
<th>Amino acid sequence</th>
<th>Antigenicity Score</th>
<th>Antigenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>VNSVLLFL</td>
<td>0.1356</td>
<td>Probable NON-ANTIGEN</td>
</tr>
<tr>
<td>VFLLVTLA</td>
<td>0.8407</td>
<td>ANTIGEN</td>
</tr>
<tr>
<td>KKDKKKKA</td>
<td>-0.5109</td>
<td>Probable NON-ANTIGEN</td>
</tr>
<tr>
<td>SRSSSRSR</td>
<td>1.1071</td>
<td>ANTIGEN</td>
</tr>
<tr>
<td>RRARVSVAS</td>
<td>0.8045</td>
<td>ANTIGEN</td>
</tr>
<tr>
<td>DEDDSEPV</td>
<td>0.6515</td>
<td>ANTIGEN</td>
</tr>
</tbody>
</table>

Prediction of binding affinity of peptides to TAP

Peptides must be transported to the endoplasmic reticulum before they can be presented to CD4+ cells by MHC-I. TAP protein carries out the transport process, so the TAP affinities of the eight amino acid length peptides were evaluated and shown in Table-3.

Allergenicity prediction

The identical peptides allergenicity predicted in AllerTOP v2.0 bioinformatic tool and shown in Table-3. Identical epitopes causing autoimmunity should not necessarily be allergic.
Toxicity prediction

The identical peptides must be non-toxic to the human body. For prediction of toxicity, the ToxinPred tool used for toxicity prediction results is shown in Table-3.

Table-3

Evaluation of the transport efficiency through to TAP protein complex involved in antigen presentation, allergenicity, and toxicity of 8mer sequences possessing molecular mimicry.

<table>
<thead>
<tr>
<th>Amino acid sequence</th>
<th>TAP AFFINITY IC50 (nM)</th>
<th>Allergenicity</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>VNSVLLFL</td>
<td>8432.63</td>
<td>Non-allergen</td>
<td>Non-Toxin</td>
</tr>
<tr>
<td>VFLLVTGL</td>
<td>9921.22</td>
<td>Non-allergen</td>
<td>Non-Toxin</td>
</tr>
<tr>
<td>KKDKKKKA</td>
<td>22820.40</td>
<td>Non-allergen</td>
<td>Non-Toxin</td>
</tr>
<tr>
<td>SRSSRRSR</td>
<td>2944.01</td>
<td>Allergen</td>
<td>Non-Toxin</td>
</tr>
<tr>
<td>RRARSVAS</td>
<td>13850.83</td>
<td>Non-allergen</td>
<td>Non-Toxin</td>
</tr>
<tr>
<td>DEDDSEPV</td>
<td>55920.29</td>
<td>Non-allergen</td>
<td>Non-Toxin</td>
</tr>
</tbody>
</table>

IL-10, and IFN-gamma inducing peptides prediction

IFN-gamma cytokine levels increased and IL-10 levels decreased in autoimmunity. Table-4 shows IFN-gamma and IL-10 induction prediction.

Table-4

Prediction of interferon-gamma and IL-10 production induction capability of 8mer fragments of SARS-CoV-2 proteins featuring molecular mimicry between and the human proteome.

<table>
<thead>
<tr>
<th>Amino acid sequence</th>
<th>IFN-gamma prediction</th>
<th>IL-10 prediction</th>
</tr>
</thead>
<tbody>
<tr>
<td>VNSVLLFL</td>
<td>IFN-gamma inducer</td>
<td>Non-IL-10 inducer</td>
</tr>
<tr>
<td>VFLLVTGL</td>
<td>Non-IFN-gamma inducer</td>
<td>Non-IL-10 inducer</td>
</tr>
<tr>
<td>KKDKKKKA</td>
<td>Non-IFN-gamma inducer</td>
<td>Non-IL-10 inducer</td>
</tr>
<tr>
<td>SRSSRRSR</td>
<td>IFN-gamma inducer</td>
<td>Non-IL-10 inducer</td>
</tr>
<tr>
<td>RRARSVAS</td>
<td>Non-IFN-gamma inducer</td>
<td>Non-IL-10 inducer</td>
</tr>
<tr>
<td>DEDDSEPV</td>
<td>IFN-gamma inducer</td>
<td>Non-IL-10 inducer</td>
</tr>
</tbody>
</table>

HLA docking results

Matched best peptide (identical, antigenic, non-allergenic, non-toxic, IL-4 inducer, and IFN-gamma inducer, non-IL-10 inducer, and high affinity to TAP) docked for affinity to Class-I and Class-II MHC molecules. The MHC-I docking results show DEDDSEPV sequence has a high affinity to HLA-A*0101, HLA-A*0201, HLA-A*2402, HLA-B*0702, HLA-B*2705, HLA-B*5101, and HLA-B*5801. Table-5 shows Gibbs free energy calculation and Kd values of docking results. Figure-1 shows docked models of DEDDSEPV and HLA subtypes.

Table-5

Results of the calculation of ΔG and Kd values of DEDDSEPV sequence affinities to common HLA subtypes.

<table>
<thead>
<tr>
<th>HLA subtypes</th>
<th>ΔG calculation</th>
<th>Kd (M) at 37.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-A*0101</td>
<td>-9.6</td>
<td>1.6*10^-7</td>
</tr>
<tr>
<td>HLA-A*0201</td>
<td>-9.4</td>
<td>2.2*10^-7</td>
</tr>
<tr>
<td>HLA-A*0301</td>
<td>-8.3</td>
<td>1.3*10^-6</td>
</tr>
<tr>
<td>HLA-A*1011</td>
<td>-7.8</td>
<td>3.3*10^-6</td>
</tr>
<tr>
<td>HLA-A*2402</td>
<td>-9.3</td>
<td>2.6 \times 10^{-7}</td>
</tr>
<tr>
<td>------------</td>
<td>------</td>
<td>----------------</td>
</tr>
<tr>
<td>HLA-B*0702</td>
<td>-9.2</td>
<td>3.4 \times 10^{-7}</td>
</tr>
<tr>
<td>HLA-B*0801</td>
<td>-7.9</td>
<td>2.8 \times 10^{-6}</td>
</tr>
<tr>
<td>HLA-B*2705</td>
<td>-9.4</td>
<td>2.5 \times 10^{-7}</td>
</tr>
<tr>
<td>HLA-B*3501</td>
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<tr>
<td>HLA-B*5101</td>
<td>-9.8</td>
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</tr>
<tr>
<td>HLA-B*5801</td>
<td>-10.3</td>
<td>3.3 \times 10^{-8}</td>
</tr>
</tbody>
</table>

Figure-1

These models depict the docked structures of DEDDSEPV with MHC-I receptors. The top three models represent the HLA-A*0101, HLA-A*0201, and HLA-A*2402, while the bottom four models represent the HLA-B*0702, HLA-B*2705, HLA-B*5101, and HLA-B*5801 receptors, respectively.

Discussion

COVID-19 is a novel pandemic that has had significant global health consequences. The clinical course of COVID-19 is similar to systemic autoimmune diseases in some patients. Biological and chemical anti-inflammatory medications are used in the treatment of autoimmunity in severe and critical patients with COVID-19 [134,135]. In some cases, the immune response to COVID-19 resembles the immune response that develops in patients with systemic autoimmunity [136]. This situation can cause multi-organ to represent presentation in both COVID-19 and autoimmune patients [137]. The SARS-CoV-2 virus can disturb the self-tolerance of host antigens at least in part through molecular mimicry. Indeed, the development of autoantibodies and sometimes organ-specific (e.g., GBS) or systemic (e.g., SLE-like disease) autoimmunity has been observed in COVID-19. We used immunoinformatics and proteomics tools to explain the autoimmune conditions that may develop during and after COVID-19 infection and the previously unknown pathophysiology of neurological events in COVID-19. In vitro and in vivo studies have been carried out in previous studies in the literature to determine the molecular mimicry between organisms that can cause autoimmune diseases and the human proteome. With the development of machine learning and artificial
intelligence-based bioinformatics tools, molecular mimicries between organisms and the human proteome can be detected using the methodology we use. As a limitation of our study, like every algorithm, bioinformatics tools have a margin of error. Therefore, the DEDDSEPV amino acid sequence, which we determined as a potential molecular mimicry with 100% amino acid sequence similarity in our study, may need to be confirmed in vitro or in vivo. In the study of Churilov et al., using in silico techniques, spike protein and autoantigens of type-1 diabetes-related and Addison autoimmunities were defined as molecular mimicry [138]. Nunez-Castilla et al. described potential molecular mimicry using structural bioinformatics techniques in their study [139]. In their study, Nunez-Castilla et al. contributed to the elucidation of the pathophysiology of autoimmune thyroiditis due to SARS-CoV-2 infection by identifying the presence of molecular mimicry between the TQLPP amino acid sequence in the Spike protein and human thyroid peroxidase [139]. In our study, we defined the molecular mimicry between SARS-CoV-2 and the human proteome using immunoinformatic techniques. Obviously, by using both techniques together, more accurate results can be obtained and it is easy to understand the autoimmunities between which organisms and the human proteome.

Conclusion

SARS-CoV-2 eight amino acid length peptides matched the human proteome and found six identical peptides. The peptide sequence with the highest probability of cross-reactivity was selected and analyzed. Selected peptide docked MHC-I and MHC-II receptors, these docking results show DEDDSEPV sequence can cause cross-reactivity to human myosin-16. Myosin-16 is a motor protein and is highly expressed in the central nervous system. Myosin-16 protein is also highly expressed in endocrine tissues (especially the pituitary gland), liver, and gallbladder. In our study, we proved by using in-silico methods that the DEDDSEPV peptide sequence can cause cross-reactivity. Auto-antibodies that may develop against myosin-16 may explain the neurological symptoms and diseases that may develop due to COVID-19. This could explain the pathophysiologic symptoms and diseases that may develop due to COVID-19 of the endocrine system, which affects the entire physiology of the human body, such as the immune system. Liver function tests are high in critical and severe COVID-19 patients. Since myosin-16 is expressed at a high level in the liver tissue, it can explain the irregularity in liver function tests. Our study is the first in the literature to try to define the molecular mimicry between SARS-CoV-2 and the human proteome using immunoinformatic techniques. We think that in the future it will illuminate the similarity between other organisms and the human proteome using the methodology in our study. It may contribute to elucidating the pathophysiology of autoimmune diseases by elucidating the similarity between other organisms and the human proteome. When molecular mimicries are illuminated and which organism triggers which autoimmunity, removal of the triggering organism or reduction of its antigenic load can open a new era in the treatment of autoimmune diseases.

Funding

The authors declare that they did not obtain funding for this work

Declaration of Competing Interest

A.S. and H.B. are members of the management board, while F.H.K and C.B.Ş are researchers at silicosome Biotechnology (Konya, Turkey).

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