



# Human molecular chaperones share with SARS-CoV-2 antigenic epitopes potentially capable of eliciting autoimmunity against endothelial cells: possible role of molecular mimicry in COVID-19

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## Abstract

Severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), the cause of COVID-19 disease, has the potential to elicit autoimmunity because mimicry of human molecular chaperones by viral proteins. We compared viral proteins with human molecular chaperones, many of which are heat shock proteins, to determine if they share amino acid-sequence segments with immunogenic-antigenic potential, which can elicit cross-reactive antibodies and effector immune cells with the capacity to damage-destroy human cells by a mechanism of autoimmunity. We identified the chaperones that can putatively participate in molecular mimicry phenomena after SARS-CoV-2 infection, focusing on those for which endothelial cell plasma-cell membrane localization has already been demonstrated. We also postulate that post-translational modifications, induced by physical (shear) and chemical (metabolic) stress caused respectively by the risk factors hypertension and diabetes, might have a role in determining plasma-cell membrane localization and, in turn, autoimmune-induced endothelial damage.

**Keywords** Severe acute respiratory syndrome coronavirus 2 · COVID-19 · Molecular chaperones · Molecular mimicry · Autoimmunity · Endothelialitis

## Introduction

Severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) causes COVID-19, a disease manifested with a wide spectrum of signs and symptoms, from a paucisymptomatic flu-like syndrome to a devastating multiorgan failure (MOF) (Wynants et al. 2020).

Histopathological lesions of the lungs were the first to be reported, but soon after similar morphological damages (mainly diffuse microthrombosis and disseminated intravascular coagulation or DIC) were found also in other organs, including liver, kidney, and brain (Sessa et al. 2020). Virtually all organs present these histological features that may have a common mechanism: endothelialitis due to an

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autoimmune attack against endothelial cells of vessels (Ackermann et al. 2020).

Many clinical reports (including those concerning putative efficacious therapies in COVID-19 patients) support the autoimmune theory. However, only a few have suggested that molecular mimicry may be at the basis of immunological cross-reactivity between viral and human molecules, thereby playing an active role in generating autoimmunity in COVID-19 (Cappello 2020a, b; Sedaghat and Karimi 2020; Cappello et al. 2020; Angileri et al. 2020a, b; Lucchese and Flöel 2020).

We postulate that molecular chaperones (many of which are heat shock proteins) must be considered among the main suspects of molecular mimicry phenomena for various reasons: (1) they are evolutionary ancient and highly conserved (Feder and Hofmann 1999; Cappello et al. 2019). Consequently, they share epitopes not only between different species but also between them and other proteins; (2) their canonical localization is intracellular, but they may also occur in the plasma-cell membrane and extracellularly, which allows their encountering the immune system provoking an immune reaction, especially if they have undergone post-translational modifications (PTM) (Balogi et al. 2019; Caruso Bavisotto et al. 2020); and (3) autoimmunity generated by antigenic epitopes cross-reactive between human molecular chaperones and microbial molecules have already been described in various diseases, and the autoimmune reaction involves also endothelial cells (Lamb et al. 2003; Cappello et al. 2009).

The above findings and considerations encouraged us to search for SARS-CoV-2 protein molecular mimicry of human molecular chaperones that could generate immunological cross-reactivity in COVID-19.

We compared the amino acid sequences of all the SARS-CoV-2 proteins with the sequences of human chaperones to determine if they share segments with immunogenic-antigenic potential that might be causing autoimmunity. Particularly, we focused on molecular chaperones that have already been shown to be present in endothelial cells.

## Materials and methods

We performed an exhaustive search of all contiguous segments of SARS-CoV-2 proteins with an exact identity to human protein segments. We implemented a sliding window approach to systematically compare all segments of viral and human proteins (Polimeno et al. 2008; Lucchese 2019). Human and SARS-Cov-2 protein sequence files were downloaded from UniProt database. Only segments with a length of six amino acids or more were considered.

Further analyses were performed using the Immune Epitope Database and analysis resource (IEDB, <https://www.iedb.org/>), a database of experimentally validated epitopes

and a tool to predict T cell and B cell epitopes. We used the BebiPred 2.0 (Jespersen et al. 2017) and the Kolaskar and Tongaonkar Antigenicity scale (Kolaskar and Tongaonkar 1990), both algorithms embedded in the B cell prediction analysis tool available in IEDB (Zhang et al. 2008). For CD4 T and CD8 T cell epitope prediction, we applied previously described algorithms developed to predict dominant HLA class I and dominant HLA class II epitopes (Paul et al. 2013, 2015).

## Results

Sequence analysis of 20,365 human proteins showed that 3781 share peptides of at least six amino acids ( $\geq 6$  mer) with SARS-CoV-2 proteins, and 17 of them are molecular chaperones. Notably, all the shared peptides between chaperones and viral proteins are part of immunogenic epitopes predicted using IEDB for either B or T lymphocytes (Table 1).

## Discussion

COVID-19 is a disease that, in some subjects, can be lethal (Lippi et al. 2020). The main risk factors associated with a poor prognosis are hypertension and diabetes, which can generate, respectively, physical (shear) or chemical (metabolic) stress to endothelial cells. And we know that stress can induce molecular chaperones to migrate to the plasma-cell membrane and to exit the cell, probably after being modified (Caruso Bavisotto et al. 2020) and, consequently, can be met by the immune system and recognized as foreign antigens. This would elicit an autoimmune response. The substantial number of DNAJ family proteins that we found (Table 1) could be a clue of this hypothesis, because of its cellular location (i.e., on plasma membrane) and its pathways to exposure to the immune response (Kotlarz et al. 2013).

The results obtained by the bioinformatics prediction tool for immunogenic epitopes showed that the shared peptides between 17 human chaperones and viral proteins have a high likelihood of being recognized by the human immune system, triggering an autoimmune reaction. As reported in the supplemental material (Table S1), these chaperones are widely expressed in human tissues and some of them are on the plasma-cell membrane under normal conditions. We hypothesize that cell stress (shear and/or metabolic) associated with COVID-19 triggers the translocation of chaperone molecules from their intracellular location to the plasma-cell membrane and extracellular space and, thus, creates the conditions for autoimmunity. In this brief report, we decided to focus on endothelial cells because COVID-19 characteristics point to an autoimmune attack against these cells, which substantiates our hypothesis.

**Table 1** Peptides of immunologic relevance shared between SARS-CoV-2 and human chaperones

Shared peptide ( $\geq 6$ amino acids)	SARS-CoV-2 protein (Uniprot ID)	Human chaperone (name, Uniprot ID)	Putative epitope/IIEDB prediction
<b>TILGSA</b>	Replicase polyprotein 1ab [P0DTD1]	Heat shock 70 kDa protein 13 [P48723]	<b>TILGSA</b> LEDEFTEPF/CD4 T Lymphocytes
<b>LPYDPP</b>	Replicase polyprotein 1ab [P0DTD1]	Heat shock 70 kDa protein 4 [P34932]	<b>DDYVYLPYDPP</b> FSRI/B Lymphocytes
<b>GTVYEK</b>	Replicase polyprotein 1ab [P0DTD1]	Heat shock 70 kDa protein 4 L [O95757]	<b>NIFGTVYEK</b> //CD8 T Lymphocytes
<b>EIPKEE</b>	Replicase polyprotein 1ab [P0DTD1]	Heat shock 60 kDa protein (HSP60) [P10809]	<b>AEIPKEE</b> VKPFITESKFSVEQRKQDDKK/B Lymphocytes
<b>EEKFKE</b>	Replicase polyprotein 1ab [P0DTD1]	DnaJ homolog subfamily B member 1 (Hsp40) [P25685]; DnaJ homolog subfamily B member 4 [Q9UDY4]; DnaJ homolog subfamily B member 5 (Hsc40) [O75953]	<b>EEKFKE</b> GVVE/CD8 T Lymphocytes
<b>LLAPLL</b>	Replicase polyprotein 1ab [P0DTD1]	DnaJ homolog subfamily C member 25 [Q9H1X3]	<b>HEVLLAPLL</b> SAG/B Lymphocytes
<b>GLTGTG</b>	Spike glycoprotein [P0DTC2]	DnaJ homolog subfamily B member 2 [P25686]	<b>GLTGTG</b> VLTESNKKFLPFQGF/B Lymphocytes
<b>VLSDRE</b>	Replicase polyprotein 1ab [P0DTD1]	DnaJ homolog subfamily C member 9 [Q8WXX5]	<b>VREVLSDRE</b> LHLSWE/CD4 T Lymphocytes
<b>DFSRVS</b>	Replicase polyprotein 1ab [P0DTD1]	DnaJ homolog subfamily C member 14 [Q6Y2X3]	<b>PNNTDFSRVS</b> AKPPP/CD4 T Lymphocytes
<b>DDFVEI</b>	Replicase polyprotein 1ab [P0DTD1]	Alpha-crystallin A chain [P02489]	<b>SKCVCSVIDLL</b> LLDDFVEII/B Lymphocytes; CSVIDLLDDDFVEII/CD4 T Lymphocytes
<b>KDKKKK</b>	Nucleocapsid phosphoprotein [P0DTC9]	Heat shock protein HSP 90-beta (HSP90) [P08238]; Putative heat shock protein HSP 90-beta 2 (HSP90Bb) [Q58FF8]	<b>KTFPPTEPKKDKKKK</b> KADETAQALPQRQKQK/B Lymphocytes
<b>LKELLQN</b>	Replicase polyprotein 1ab [P0DTD1]	Sacsin [Q9NZJ4]	<b>AVLDMCASL</b> KELLQN/CD4 T Lymphocytes
<b>LVAELEG</b>	Replicase polyprotein 1ab [P0DTD1]	FK506-binding protein-like [Q9UJM3]	<b>MVELVAE</b> LEGIQY/CD8 T Lymphocytes
<b>LGSPLSL</b>	ORF9b [P0DTD2]	Stress-responsive DNAJB4-interacting membrane protein 1 (SDIM1) [Q6ZPB5]	<b>PHILRLGSP</b> LSLNMMA/CD4 T Lymphocytes

Literature data support this hypothesis. It has been demonstrated that stress agents induce HSP90 localization on the surface of primary human endothelial cells (Profumo et al. 2018), and HSP60 is present on the surface of arterial endothelial cells, initiating atherosclerosis by the recognition of atherogenic HSP60 epitopes (Almanzar et al. 2012). Moreover, the cell surface presence of HSP70 is modulated by shear stress in cultured endothelial cells and aorta endothelium, potentially restricting thromboresistance and supporting thrombosis/inflammation in stress situations (Thaís et al. 2019).

Reports about efficacy of some therapies in COVID-19 patients also tend to support the autoimmune hypothesis (Saghazadeh and Rezaei 2020; Prete et al. 2020; Picchianti Diamanti et al. 2020). Advances in the elucidation of the role of autoimmunity, as hypothesized here, will be made as more autopsies are carried out on COVID-19 victims. We have remarked on the need of information that can only be obtained by autopsy, which in some countries such as Italy is scarce (Pomara et al. 2020). This fact delayed the realization that COVID-19 was not just a severe pneumonia but a systemic disease, and resulted in a lack of material to study, e.g., by immunohistochemistry and immunomorphological techniques, the histopathological manifestation of the disease.

Availability of tissues from victims of COVID-19 will allow dissection of the cellular and molecular patterns and mechanisms underpinning the damage of epithelia and other structures that ultimately cause death. It will, thus, be possible to assess the validity of our hypothesis, which emphasizes the role of autoimmunity due to molecular mimicry in the pathogenicity of COVID-19. Finally, studies on molecular mimicry phenomena will also help in directing experiments and clinical trials for producing safe and efficacious vaccines, as already indicated by others (Lucchese 2020).

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