

# Are superantigens the cause of cytokine storm and viral sepsis in severe COVID-19? Observations and hypothesis

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## Abstract

Right now the world is going through an unprecedented pandemic caused by a novel coronavirus. Recent papers pointed out the fatal outcome in most of the severe cases, in which a cytokine storm has been proven to be the cause of a systemic shock, acute respiratory syndrome, multiorgan failure and consequently death. Several explanations have been proposed trying to explain the pathophysiology of the cytokine storm, but viral proteins with a possible superantigen activity as a cause of immune dysregulation have not been addressed. If this hypothesis is proven, a different treatment approach might change the outcome in severe cases.

## 1 | INTRODUCTION

In December 2019, a new disease, now known as COVID-19, caused by a novel coronavirus, emerged in several local hospitals of Wuhan, Hubei Province, China.<sup>1</sup> The International Committee on Taxonomy of Viruses (ICTV) named this novel coronavirus as SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). To date, there have been 7 690 708 confirmed cases of COVID-19, including 427 630 deaths, reported to World Health Organization.<sup>2</sup>

SARS-CoV-2 is a member of  $\beta$ -CoVs, a class of enveloped, positive-sense single-stranded RNA viruses.<sup>3</sup> Coronavirus particles consist of a helical nucleocapsid structure, formed by the association between nucleocapsid (N) phosphoproteins and the viral genomic RNA, which is surrounded by a lipid bilayer where structural proteins are inserted: the spike (S), the membrane (M) and the envelope (E) proteins. The virus enters target cells through an endosomal pathway. The spikes of SARS-CoV are composed of S protein that forms homotrimers protruding from the viral surface. S protein comprises two functional subunits

responsible for binding to the host cell receptor (S<sub>1</sub> subunit) and fusion of the viral and cellular membranes (S<sub>2</sub> subunit). The first contact to the cell is through the S<sub>1</sub> subunit, that recognizes and binds to host receptor angiotensin-converting enzyme 2, and subsequent conformational changes in S<sub>2</sub> facilitate fusion between the viral envelope and the host cell membrane. Then, this complex is translocated to endosomes. Inside the endosome, the S protein is cleaved and the viral genome is released. Once sufficient amounts of new genomic RNA and structural proteins have been produced, assembly of particles occurs. Assembly and release of virions are the last stages of the virus life cycle.<sup>4</sup>

This disease was considered at first as virus-induced pneumonia. Patients most frequently presented with fever, a non-productive cough, sore throat and myalgia. The vast majority of patients only have a common, mild form of illness. In more severely ill patients, dyspnoea and hypoxia became more prominent. Some patient's respiratory status continued to deteriorate and they developed sepsis, respiratory failure and acute respiratory distress syndrome (ARDS).<sup>5</sup>

SARS-CoV-2 infection can cause five different outcomes: asymptotically infected persons (1.2%); mild cases (80.9%); severe cases (13.8%); critical cases (4.7%); and death (2.3% in all reported cases).<sup>6</sup>

Most of the individuals infected with SARS-CoV spontaneously recovered without clinical intervention. However, little is known about the patients that progress to an aggressive systemic response and ARSD.<sup>7</sup>

Recent publications are accumulating evidence suggesting that a subgroup of patients with severe COVID-19 might have a cytokine storm syndrome.<sup>8,9</sup>

Hyperinflammation in COVID-19 may cause cytopenias, coagulopathy, tissue damage, liver dysfunction and macrophage activation, having features of reactive hemophagocytic lymphohistiocytosis. The overwhelming production of inflammatory cytokines is responsible for organ dysfunction and eventually death.

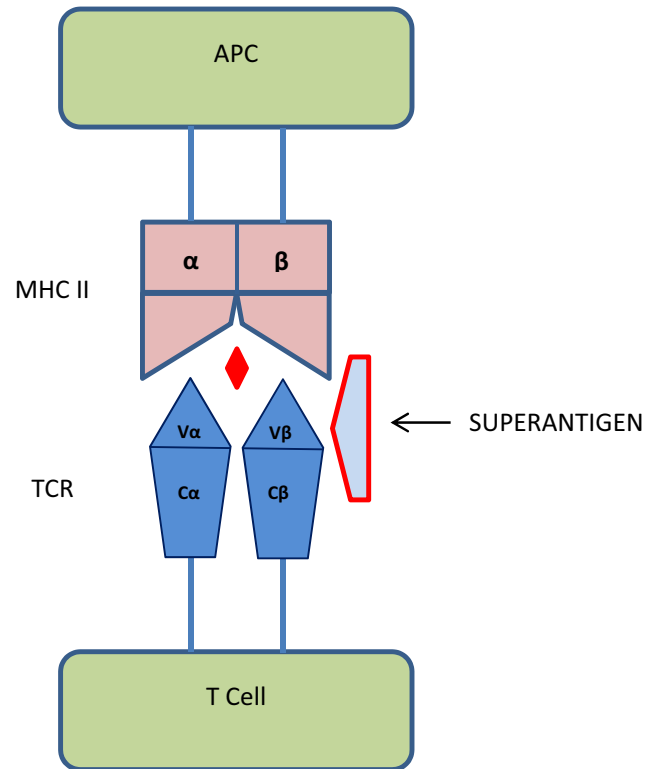
In vitro SARS-CoV-2 cell experiments reveal that the cells secrete low levels of the antiviral factors interferons (IFNs) and high levels of proinflammatory cytokines (interleukin [IL]-1 $\beta$ , IL-6, and tumour necrosis factor [TNF]) and chemokines (CCL-2, CCL-3 and CCL-5).<sup>8</sup>

Knowledge about COVID-19 is still incomplete regarding its pathogenicity. In a recently published paper, Chen et al revealed that serum cytokines levels of interleukin 2R (IL-2R), IL-6, IL-10 and TNF- $\alpha$  were markedly higher in severe cases than in moderate cases. They also demonstrated that absolute numbers of total T lymphocytes, CD4+ T cells and CD8+ T cells were reduced in the vast majority of patients with either severe or moderate COVID-19, but more profoundly in severe cases. In contrast, the proportion of B cells was significantly higher in severe cases than in moderate cases. Regulatory T cells (Tregs) were markedly lower in severe cases. Males are more susceptible to SARS-CoV-2 infection than females. People over 50 years old, particularly those with underlying comorbidities, may be more likely to develop severe COVID-19. Common complications observed in severe cases included shock, ARDS and respiratory failure.<sup>10</sup>

## 2 | ANTIGEN PRESENTATION

Antigen-presenting cells (APCs) interact with T cells to link innate and adaptive immune responses, displaying antigens on their surface via major histocompatibility complexes (MHC), also referred as human leucocyte antigen (HLA), MHC class I to display endogenous antigens to activate CD8+ cells and MHC class II, to display exogenous antigens and activate CD4+ cells. Only APCs produce MHC-II receptors to display exogenous antigens and activate CD4+ cells.<sup>11</sup>

Cytotoxic and helper T cells utilize membrane-bound T cell receptors (TCRs) to bind MHC receptors. T cell activation is initiated when the TCR recognizes a peptide presented in the MHC on an APC, and soon after it initiates proliferation and differentiation of antigen-specific effector cell



**FIGURE 1** Schematic representation of interactions between superantigen, MCH class II and TCR

subtypes characterized by the expression of different surface markers and cytokine production abilities, depending of the antigen nature.<sup>12</sup>

T cell receptor comprises of two peptide chains, either  $\alpha/\beta$  or  $\gamma/\delta$ , but 90% of peripheral blood T cells have  $\alpha/\beta$  peptide chain.  $\alpha$  Peptide chain contains three regions V (variable), J (junctional) and C (constant), while  $\beta$  peptide chain in addition has a fourth region D (diversity). TCR genes are rearranged as lymphocytes mature in the thymus. Mature T cells, which are released from the thymus, are irreversibly committed to recognize one specific antigenic epitope presented in the MHC molecule.<sup>12</sup>

In classical response, after antigen processing by APC, an epitope from a protein antigen acts as a bridge between the HLA complex of APC and TCR. Only a small proportion of T cells become activated (<0.01%) particularly after a co-stimulatory signal is produced by the APC. Response is highly regulated in order to limit harmful effects.<sup>13</sup>

## 3 | VIRAL SUPERANTIGEN HYPOTHESIS

Superantigens (SAGs) are microbial or viral proteins that act as extremely potent polyclonal T cell mitogens. They bind

**TABLE 1** Similarities between severe SARS-CoV-2 infection and superantigen-mediated diseases

Summary of most important features described in severe COVID-19 and their correlation with superantigen effects	
COVID-19	Superantigen effects
Over-production of proinflammatory cytokines	Massive T cell activation and release of cytokines, (TNF- $\alpha$ , IL-2, IL-6, INF- $\gamma$ ) in large amount, results in capillary leak and systemic shock <sup>26</sup>
CD4 cell and NK cell cytopenias	T Cells undergo superantigen-induced apoptosis and upregulate Fas and Fas ligand <sup>19</sup>
Hypercoagulable state	Superantigens induce procoagulant activity and tissue factor expression in human monocytes via release IL-1 $\beta$ <sup>17</sup>
Shock	Massive release of cytokines results in capillary leak and systemic shock <sup>14</sup>
Adult respiratory distress syndrome	Capillary leak syndrome generates hypotension and acute respiratory distress syndrome <sup>14</sup>

MHC class II molecules without any prior processing and stimulate large number of T cells.

Superantigens bind directly to TCR and MHC-II receptor outside the conventional antigen-binding site, thus bypassing the restrictive feature of conventional antigen processing (Figure 1).

Superantigens can activate up to 25% of an individual's T cells, in contrast with less than 0.01% in classical response. Because the number of T cells that share V domains is high, large numbers of T cells (regardless of antigen specificity) may be activated by superantigens. There is an initial T cell derived initial peak of IL-2, INF $\gamma$  and TNF- $\alpha$  followed by a second peak from macrophage-derived cytokines such as IL-1, IL-6, IL-8 and TNF- $\alpha$ , along with chemokines, leukotrienes, prostaglandins and complement, that in severe cases may result in vasodilatation associated with vascular leakage of fluid, leading to tissue hypoperfusion, shock and multiorgan failure.<sup>14</sup>

Most of the microbial superantigens appear to share a common MHC class II binding site (referred to as the generic site). However, differences in amino acid sequence dictate that each superantigen has an affinity for specific MHC class II alleles (eg some bacterial SAGs prefer HLA-DQ rather than HLA-DR).<sup>15</sup>

There is increasing data suggesting that cytopenias and hypercoagulable state are frequent in patients with severe COVID-19.<sup>16,17</sup> Coagulation is activated when tissue factor is exposed to blood. Inflammatory mediators promote coagulation. There is high fibrinogen levels, low anti-thrombin levels, increases in prothrombin and activated partial thromboplastin times.<sup>18</sup> The increase in D-dimers is one of the commonest laboratory findings in COVID-19 patients requiring hospitalization, and its elevation has been described as a predictor of mortality. The International Society of Thrombosis and Haemostasis has published an interim guidance and

recommend monitoring prothrombin time, D-dimer, platelet count and fibrinogen to determining prognosis in COVID-19 patients requiring hospital admission. There is evidence that COVID-19 can produce disseminated intravascular coagulation with a predominantly thrombotic phenotype and also a high rate of pulmonary embolism.<sup>16</sup>

In animal models, after stimulation with SAGs, peripheral T cells undergo clonal expansion followed by apoptosis.<sup>19</sup> In a recent study of immune dysregulation in patients with severe COVID-19, CD4 Cell and NK Cell cytopenias were characteristics of Infection by SARS-CoV-2, with over-production of proinflammatory cytokines by monocytes and dysregulation of lymphocytes characterized by CD4 lymphopenia and subsequently B cell lymphopenia,<sup>20</sup> but the presence viral superantigens were not assessed.

The latest publication describing hyperinflammation and shock is by Riphagen et al in *The Lancet* recently.<sup>21</sup> They reported a cluster of eight children, all of them progressed to vasoplegic shock, with multiorgan involvement similar to Kawasaki disease and toxic shock syndrome. Although only one patient tested positive for SARS-CoV-2 post-mortem, four of them had COVID-19 exposure from a close relative. Studies have shown that Kawasaki disease may be caused by a superantigen and evidence suggests that genetic factors are involved in the susceptibility and outcome of the disease.<sup>22</sup> In Table 1, we summarized the main features of severe COVID-19 and superantigen effects.

#### 4 | IT IS POSSIBLE TO DEMONSTRATE THIS HYPOTHESIS?

SAGs structure can be recognized by X-ray crystallographic techniques.<sup>23</sup>

It is a challenge to study systemic effects of SAGs due to a lack of suitable animal models. Animal models used to study superantigen-mediated diseases include rabbits, mice, pigs and ferrets. The most study disease is by far the toxic shock syndrome. The animal disease should mimic the human disease. The route of infection, pathophysiology and severity must be similar.<sup>24</sup> In contrast with humans, there is not a strong binding of SAGs to murine MHC class II molecules. A possible solution to this is to develop transgenic mice to express human MHC class II (HLA-DR3) molecules. To identify a potential superantigen, a lymphocyte mitogenicity assay can be performed using peripheral blood mononuclear cells incubated with increasing concentrations of the potential superantigen.

Assessing V $\beta$  TCR skewing in peripheral blood or tissues of patients has been used to identify causative factors in human diseases that are thought to be caused by superantigens.<sup>25</sup> In patients with severe COVID-19, the presence of skewing T cell V $\beta$  repertoire can be analysed by flow cytometry, using protocols for analysis of V $\beta$  repertoires V $\beta$  restricted activation and compare the results with a control group.

## 5 | CONCLUSIONS

After the publication of several recent papers of patients with severe SARS-CoV-2 infection describing alterations that are comparable with the ones described in SAGs in vitro and in vivo studies, we hypothesize that severe cases of COVID-19 might be due to the presence of viral superantigens. Several alterations in patients with severe infection resemble a toxic shock. Important questions need to be answered in the actual context of a new type of disease, with high mortality rate: It is possible that SARS-CoV-2 proteins act as superantigens? It is possible that viral superantigens are responsible for viral sepsis, shock and cytokine storm in severe COVID-19?

Basic research in COVID-19 should provide leads for the development early therapeutic strategies that allow severely ill patients to overcome this unprecedented disease.

## CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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