INTRODUCTION

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), like SARS-CoV and the Middle East respiratory syndrome (MERS)-CoV display very similar characteristics, showing a multi-level complexity and acting like “moving targets” for the immune system. Like all other corona viruses, SARS-CoV-2 seems to cause acute lung injury, followed by peripheral blood lymphopenia and eosinopenia (Nassar et al., 2018), and at final stages multi-organ collapse. The symptoms that have already been described for MERS-CoV for extra-pulmonary sites also include abdominal pain, nausea and vomiting, diarrhea and acute renal failure (Nassar et al., 2018).

In adaptive immunity, viruses will preferentially stimulate cell-mediated immunity by generating cytotoxic CD8-positive T cells (Tc), which by destroying virus-infected cells clear the organism from the infective pathogen, while also generating memory cells for future protection of the host. Depending on the viral load, a humoral response will be sequentially developed leading to specific antibody production, which in most cases provides additional mechanisms for pathogen clearance.

Basic lessons in immunology dictate that the antigen presenting cells (APCs) present the antigenic epitopes of the pathogenic proteins as complexes with major histocompatibility complex class II (MHCII) molecules to helper CD4-positive T cells (T4), which will then specifically activate either Tc cells for cell killing or B cells for antibody production. The Tc cells will recognize the complex of major histocompatibility complex class I (MHC1) with the antigenic epitope on target cells, which will be eliminated by apoptosis. On the other hand, B cell differentiation will lead to the production of sequential antibodies starting from a primary response, represented by the IgM isotype, followed by a secondary response elaborating different IgG isotypes (IgG1 to 4) with differential structure and function. In order to ensure survival, CoV seems to follow multilevel strategies of attack, including misleading of the immune system.

Structural features of SARS-CoV-2

Like other corona viruses SARS-CoV-2 includes four structural proteins: the spike protein (S), which interacts with host cells, the envelope protein (E) and the membrane protein (M), which along with protein S form the viral envelop, and finally the nucleocapsid protein (N), which supports the RNA genome (Wu et al., 2020).

Attention has mainly been drawn to the S protein, which is used for viral infectivity. Recent data suggest that in SARS-CoV-2, S protein trimers simulataneous bind an angiotensins converting enzyme 2 (ACE2) protein homodimer (Yan et al.,...
Recently, basigin, also known as extracellular matrix metalloproteinase inducer or CD147 has been identified as another ligand for viral entry to the host’s cells (Wang et al., 2020). Additional virulence factors include the non-structural proteins Nsp1, Nsp3e and ORF7a, which interfere with the innate immunity of the host, assisting thus viral immune escape (Wu et al., 2020).

**SARS-CoV-2 virulence through binding to ACE2**

Recently SARS-CoV-2 has been shown to infect primarily alveolar epithelial, endothelial cells and macrophages, mainly through the ACE2 protein (Li et al., 2003; Hamming et al., 2004). Surface expression of ACE2 has been found in various human organs. The protein is highly expressed on lung alveolar epithelial cells and enterocytes of the small intestine, but it has also been detected in arterial and venous endothelial cells, as well as arterial smooth muscle cells of oral and nasal mucosa, nasopharynx, stomach, colon, skin, lymph nodes, thymus, bone marrow, spleen, liver, kidney, and brain (Zhu, 2004). Such wide distribution of ACE2 explains the wide spectrum of symptoms involved in SARS-CoV infection.

ACE2 is a type 1 integral membrane glycoprotein that mainly degrades angiotensin I-7 to oppose the actions of angiotensin II, maintaining thus the balance of the renin-angiotensin system to ensure homeostasis to the vascular function. Its role in atherosclerosis, hypertension, heart failure, chronic kidney disease and lung dysfunction has been well documented (Tikellis & Thomas, 2011). In the lung, ACE2 regulates the balance of circulating angiotensin II/ angiotensin 1-7 levels. Thus, increased levels of angiotensin II induce pulmonary vasoconstriction and enhance vascular permeability facilitating pulmonary edema. Therefore, a first level of SARS-CoV-2 virulence is being manifested through hepatocyte deprivation from the physiological ACE2 function, since ACE2 has been shown to protect from acute lung failure (Imai et al., 2005). Additional studies indicate that treatment of sepsis-induced acquired protein deficiency reverses ACE2 inhibition and decreases pulmonary inflammatory response (Richardson et al., 2008).

**SARS-CoV-2 virulence through binding to CD147**

In a preprint manuscript, Wang et al., (2020) demonstrate that SARS-CoV-2 can also invade host cells through binding of S protein to CD147.

CD147 is a determinant of the Ok blood group system and has been identified as an essential receptor for *plasmodium falciparum*, the malaria parasite, on red blood cells. It is also widely expressed in many epithelial, neuronal, lymphoid and myeloid cell types, while it is over-expressed in a variety of cancers and plays important roles during developmental processes, wound healing, nutrient transport, inflammation, including acute lung inflammation, atherosclerosis, arthritis and microbial pathologies (Grass & Toole, 2016). Although not yet defined, CD147 might be related to SARS-CoV-2 virulence.

**SARS-CoV-2 virulence through non-structural proteins**

In SARS-CoV, among the various non-structural proteins, Nsp1 interacts with the 40S ribosomal subunit inducing host’s mRNA degradation, while also inhibiting type I interferon (IFN) production. In lineage C beta coronaviruses, Nsp3 binds to hosts’s ADP-ribose and the virus becomes resistant to the host’s innate immunity. Furthermore, SARS-CoV ORF7a binds to the bone marrow matrix antigen 2 (BST-2), which normally inhibits the release of newly synthesized viruses from the cell, and blocks its anti-viral activity (Wu et al., 2020). Although such information is not yet available for SARS-CoV-2, similar functions are expected.

**SARS-CoV-2 manipulates the immune system of the host**

As postulated by Fu et al., (2020), SARS-CoV-2 activates primary inflammatory responses that include cytokine/chemokine release, expression of anti-viral factors, pulmonary cell infiltration, lymphopenia and renin-angiotensin system dysfunction by causing apoptosis, pyrolysis, ACE2 downregulation and shedding. Thereafter, viral infection initiates secondary inflammatory responses, which begin along with the development of adaptive immunity and the generation of neutralizing antibodies. Thus, virus/neutralizing antibody complexes may skew macrophage responses, abrogate wound healing, induce chemokine production, acute lung injury and cellular damages by Fc receptor (FcR)-mediated activation, complement system activation and antibody-dependent cellular cytotoxicity reactions (Fig. 1).

**Host immune system**

The viral entry to the cells initiates innate and acquired immunity. If innate immunity succeeds to eliminate the infection 6 to 96 hours after invasion, through generalized, non-specific anti-viral mechanisms, the host might present only light symptoms and remain healthy thereafter. Otherwise, adaptive immunity takes action towards the development of cell-mediated and humoral immunity. In both cases, the antigen presentation process leading to the generation of MHCII/antigen complexes for recognition by the T cell receptor (TCR)αβ of CD4-positive T Henri cells and MHCII/antigen complexes for recognition by CD8-positive Tc cells is required (Fig. 1).

Since the generation of virus-specific T Henri cells requires recognition of the MHCII/antigen complex, this implies that the antigenic epitopes of SARS-CoV proteins (Zhu, 2004) will have to bind to MHCII molecules of the host. If this reaction is not achieved the host cannot mount either types of immune mechanisms against the virus. However, only a few HLA-DR polymorphisms with questionable repetitiveness have been correlated to susceptibility to SARS-CoV (Ng et al., 2010).

**Two phases in SARS-CoV-2 infection process**
Figure 1: During the primary inflammatory response the virus upon binding to ACE2 initiates mild inflammatory responses pushing immunity towards the production of antibodies. Although Tc should have a protective role against the virus, it seems that they are down-regulated. During the secondary inflammatory response the host’s antibody production through binding to FcRs leads to severe inflammatory responses. Numbers in squares indicate the possible interventions points for infection avoidance or treatment (1: interfere with antigen recognition by Tc, 2.3: fortify Tc activity, 4: block Ah/FcR binding). ACE2: angiotensin converting enzyme 2; MHC: major histocompatibility complex; PLC: protein loading complex; TCR: T cell receptor, ag: antigen; FcR: Fc receptor.

The acquisition of virus-specific T_{H} provides the green light for the generation of Tc and later B cells. In order for effector CD8-positive cells to destroy virus-infected cells, they need to recognize the complex MHC-I/antigen to the surface of infected cells. If MHC-I/antigen complexes fail to be generated, CD8-positive cells are unable to fulfill target cell killing (Fig. 1). Indeed, several HLA-A and B polymorphisms have been correlated to susceptibility to SARS-CoV infection (Oh et al., 2012), yet this remains to be verified. Recent information on SARS-CoV-2 defined HLA-B*46:01 as the genotype with the fewest predicted binding peptides for SARS-CoV-2, while HLA-B*15:03 showed the greatest capacity to present highly conserved SARS-CoV-2 peptides (Nguyen et al., 2020).

Virus-specific CD8-Tc cells provide substantial protection against lethal SARS-CoV infection (Channappanavar et al., 2014). Memory CD4- and CD8-positive cells specific for SARS-CoV structural proteins could be identified in recovered SARS patients four years after infection (Fan et al., 2009). Memory CD4 cells were producing IL-2, TNFa and IFN-γ and their frequency was higher in patients with severe SARS infection than in cases with moderately severe infection, while CD8 cells produced mainly IFN-γ (Li et al., 2008). However, only limited information on CD8-positive Tc cells have been described in case report patients with SARS-CoV-2 infection (Thevarajan et al., 2020). Similar to SARS-CoV infection, SARS-CoV-2 patients that do not need intensive care show increased levels of IL-1b, IFN-γ, IP-10, MCP-1, IL-4, and IL-10, while more severe cases (intensive care unit –ICU-patients) show high levels of IL-2, IL-7, IL-10, GCSF, IP-10, MCP-1, MIP-1A, and TNF-a, suggesting an acute cytokine reaction associated with disease severity (Huang et al., 2020). It is interesting to note that IFN-γ that is a potent inducer of immune molecules involved in the antigen presentation process, especially for MHC-I molecules in cellular immunity, is missing from the list of highly produced cytokines in ICU patients at least at the periphery.

Antigen specific CD4 T cells will also stimulate the generation of B cells to produce specific antibodies. In general, antiviral neutralizing antibodies play an important role in viral clearance during primary viral infections and re-infection prevention. The antibody-mediated reactions include interaction with surface viral epitopes preventing binding to target cells, complement activation, Fc receptor binding leading to opsonisation, antibody dependent cellular cytotoxicity, all of which result in acute inflammatory reactions. However, in the case of SARS-CoV the production of neutralizing antibodies has not always been shown to be protective (Fu et al., 2020).

Studies using experimental models have shown that anti-S protein-neutralizing antibodies produced in response to SARS-CoV infection could cause severe lung injury by altering inflammatory responses (Liu et al., 2019). Additionally, it has been shown that SARS-CoV patients who developed anti-S-neutralizing antibody faster, had a higher chance of dying from the disease. According to Zhang et al. (2006), it took an average of only 14.7 days for the deceased patients to reach their peak levels of neutralizing antibody activities, as opposed to 20 days for the recovered patients. Therefore, it seems that the evolution of SARS-CoV has evoked mechanisms to deceive host’s defense and push humoral immune responses to mechanisms that will prolong and expand infectivity, also leading to acute inflammatory reactions and organ failure (Fig. 1).

Perspectives and future directions against SARS-CoV-2

The lessons from SARS-CoV show that the important cell populations that have to be protected and propagated are CD8-positive Tc cells. This is apparently one of the weaknesses that the virus tries to avoid, pushing infectivity towards B cells and antibody production. To this extend, two types of rapidly manageable approaches could be proposed. The first consists of exogenously increasing the levels of IFN-γ, which induces immune molecules involved in antigen presentation, especially for class I antigen loading, including immune proteasome β units (LPM2, LMP10, LMP7, MECL1) and PA28 activator, TAP1, 2 and the heavy MHC-I chain itself. Like all cytokines, IFN-γ is short-lived, is approved for use (IMUKIN®, IMMUKIN®, IMMUKINE®) and could be provided during phase I of infection, before the beginning of the virus-induced secondary inflammatory process.

Another even more manageable way to improve CD8 functions is through metabolic regulation. It has been shown that SARS-CoV M protein may function as a cytosolic PAMP to stimulate IFN-β production by activating a TLR-related signal pathway (Wnag & Liu, 2016), which can thereafter reprogram metabolic pathways also activating de novo fatty acid synthesis, which is required for membrane production and organelle expansion (Everts et al., 2014). Excess of neutral lipids has been shown to induce apoptosis to T cells (Al-Saffar et al., 2002). It has been suggested that enhancement of fatty acid catabolism may synergize with TLR4 activation to boost T cell priming (Nicoli et al., 2018). In addition, previous studies have shown that L-carnitine, which serves for the transport of long chain fatty acids in mitochondria for catabolism, in the presence of arachidonic acid could increase production of prostaglandin E2 (PGE2) from T and B lymphocytes (Athanasakis et al. 2003). Following up these thoughts, it could be suggested that the short-term administration of L-carnitine could mobilize fatty acid reserves against virus’s needs, while also inducing PGE2 production, which through its anti-inflammatory activity could be proved very useful at the beginning and during phase II of the response (Fig. 2).
Another step of intervention lies on the neutralizing antibody production and FcR binding during the secondary inflammatory response (Fig. 2). Could SARS-CoV interfere with the immunoglobulin class switch mechanisms (Yu & Lieber, 2019), or the induction of inflammatory FcR in the population? It seems very important to define the IgG isotype induced by SARS-CoV as well as the type of FcR used thereafter to mediate the inflammatory reaction (Vidarsson et al., 2014). Indeed, IgG1 and IgG3 interact efficiently with most FCyRs, while IgG2/IgG4 show reduced affinity to a number of FCyRs (Bruhns et al., 2009). In addition monomeric IgG3 binds more efficiently than monomeric IgG1 to FcγRIIa, FcγRIIib, and FcγRIIIb, while binding efficiency of complexed IgG3 to all FcRns (protect IgGs from degradation) exceeds that of IgG1 (Bruhns et al., 2009). Binding to FcγRI induces phagocytosis, cell activation and microbe killing, binding to FcγRIIA induces phagocytosis and degranulation, binding to FcγIIIB1 does not induce phagocytosis and inhibits cell activity, binding to FcγRIIB2 induces phagocytosis but inhibits cell activity, binding to FcγRIIIA induces ADCC and cytokine production, while binding to FcγRIIB1 induces microbe killing (Nimmerjahn et al., 2015). So far there is no evidence as to whether SARS-CoV favors the expansion of a specific type of FcγR or whether this is part of a regulatory mechanism of the host’s immune system. In contrast to the inflammatory/stimulatory effects initiated by IgG binding to most FcRs, recombinant IgG2aCH2 domain has been shown to alter M1 and M2 macrophage function towards inflammatory and anti-inflammatory signals respectively (unpublished observations, BSc thesis Nikos Boutacoglou, Department of Biology, University of Crete, 2019). Therefore, understanding in depth the type of FcR involved in SARS-CoV infection could allow the development of FcR neutralizing tools for therapy.

The development of vaccine could indeed prevent the population from SARS-CoV infection, but the vaccine should mainly trigger CD8 cells and controlled antibody production. To this extend, the previously described personalized implantable vaccine technology could be applied. According to this technology, subcutaneous implantation of 3-dimensional (3D) laser micro-textured Si scaffolds loaded with host’s antigen presenting cells naturally seeded with antigen induce specific memory CD4 and CD8 cells and further activation of the immune response in vivo (Zerva et al., 2015; Zerva et al., 2019). Although this could provide a solution in vaccination, it cannot be applied in the middle of a pandemic crisis. However, such technology could be applicable in selected sensitive populations to isolate infection in restricted areas. Obviously, the development of classical vaccines leading to antibody production could be protective, once we thoroughly understand which IgG isotype will be able to eradicate the virus.

Finally, attention has to be given on the timing of interventions. During the virus-induced primary inflammatory responses, one should fortify CD8 mediated defenses, which inevitably at a smaller degree will also drift antibody production. Once antibody production levels reach a critical point (which needs to be defined), mild anti-inflammatory treatments should be envisaged. Close observation of the patients’ symptoms (antibody production, cytokine production, infection spreading, etc) should be able to identify the correct moment of treatment, with the otherwise contradictory medication.

CONCLUSION

Although much of knowledge has been gathered within a few months on SARS-CoV-2 structure and biology, this could not be used so far to definitely fight the virus. Taking a step back helps to image the greater picture and join the pieces of the puzzle together. Knowing the weaknesses of the virus, while understanding the immune mechanisms evoked by recovered patients, would allow protecting susceptible organisms and avoiding fatal situations.

References


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