Immune response to COVID-19 infection: a double-edged sword

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ABSTRACT
COVID-19 global pandemic has not ceased to spread worldwide since December 2019. Today, scientists and healthcare workers are urgently working to stop this viral invasion and protect the world community. Deciphering the specific cellular and molecular immune response to the new coronavirus 2019 is an essential step in order to develop effective treatment and vaccine. Recovery from COVID-19 infection was linked to appropriate immune responses. However, disease severity was correlated to impaired immune reactions. This review summarized the latest research findings on the role of immune system in fighting and also in the pathogenesis of COVID-19. In addition, it highlighted the immunological basis for the new coronavirus 2019 prevention, therapy and diagnosis.

1. Introduction
The world health organization (WHO) has declared the novel Coronavirus disease COVID-19 as global pandemic. It became the number one cause of morbidity and mortality across the world. SARS-CoV-2, an RNA coronavirus coated with several pathogenic membrane proteins (spike glycoprotein (S), membrane (M) protein, envelope (E) protein, and nucleocapsid (N) protein), was at the origin of this disease. It first appeared in Wuhan, China on December 2019. After that, clusters of cases were rapidly emerged in all over the world. At the end of this summer, the number of COVID-19 confirmed cases had reached 25,118,689 including 844,312 deaths (WHO). Patients are characterized by being either asymptomatic, having mild, moderate or severe symptoms that may result in death [1,2]. Appropriate immune response would be protective from SARS-CoV-2 viral replication and tissues injuries [3,4]. However, alterations in innate and adaptive immunity were associated with COVID-19 pathogenesis and disease severity [5]. Therefore, today researchers around the world were urgently mobilized to find answers to the following crucial questions, how does the immune system protect the host from the virus? How does the immune response contribute to the severity of the disease? The present review summarized the latest findings of research studies related to these two research questions. In addition, it covered the different preventive, therapeutic and diagnostic strategies that had been developed from immunological perspective since the emergence of COVID-19 outbreak.

2. Initiation of an immune response against SARS-CoV-2
Viruses interact with specific receptors in order to gain entry into target cells. SARS-CoV-2 interacts with Angiotensin-converting enzyme 2 (ACE2) receptor [6–8] and in concert with the type II transmembrane serine protease TMPRSS2 enters the host cells by endocytosis [9]. The c-terminal domain of the S1 subunit of SARS-CoV-2 S spike ensures a very high affinity to ACE2 receptor [10–12]. Among the ACE2+ cells, the epithelial cells in bronchioles and alveoli were considered the main targets of SARS-CoV-2 [13,14]. Similar to other pathogens, this viral invasion triggers inflammation, activation of professional antigen-presenting cells (APCs) that present the viral peptides to CD4 and CD8 T cells and direct stimulation of B cells. The production of immunoglobulins in infected patients follows the classical pattern, where IgM are produced in the acute phase then IgG appear later [2,15]. In addition, the secretory IgA (sIgA) responsible for mucosal immunity appears very early (day 5) in respiratory tract [16]. The viral load in tissues affects the immune response, where the reaction was more effective and rapid at low doses of SARS-CoV-2 than high exposure [1].

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3. A pathogenic inflammation in COVID-19 disease

Inflammation is placed on the first line of innate immunity defense. Today, the recent studies had unequivocally confirmed that COVID-19 severity was positively correlated with the degree of inflammation [17–21]. Zhang et al. [22] found that severe COVID-19 patients had a hyper inflammation signature compared to non-severe cases. Moreover, children with COVID-19 known for their mild disease case had less inflammatory profile than adults [23]. SARS-CoV-2 enters the target cell along with the ACE2 receptor, which decreases the expression of ACE2 on the cell surface and increases the inflammation and tissues destruction [3,6]. ACE2 plays an anti-inflammatory role by converting Angiotensin II into Ang (1–7) [24] that reduces vaso-permeability, edema and neutrophils infiltration to the lungs [25]. Controversially, Ziegler et al. [14] data suggested that SARS-CoV-2 might upregulate ACE2 expression to enhance the infection further.

3.1. Inflammasome activation

Once present in the host cell, the SarS-CoV-2 activates the NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome. This contributes to the release of the pro-inflammatory cytokines, interleukin (IL)-1β and IL-18 [20]. The downstream NF-κB pathway was also activated upon the interaction of the viral RNA with toll-like receptor (TLR) 3, TLR7, TLR8, and TLR9, which enhances pro-inflammatory cytokines production too. Consequently, the inflammation starts and ignites the release of high number of cytokines from activated immune cells.

The Endoplasmic Reticulum (ER) stress caused by SARS-CoV-2 is considered the main possible mechanism underlying NLRP3 activation. The ER stress markers had been detected in infected cells such as lactate dehydrogenase (LDH) [26–32]. The rapid viral replication causes accumulation of unfolded proteins in the ER, which induces a stress that the unfolded protein response (UPR) is unable to control. Hence, NLRP3 is activated and the infected cell dies by pyroptosis [33,34]. Unfortunately, the correct folding of functional proteins like surfactant proteins in pneumocyte II and GPCR olfactory receptors proteins is suppressed because of the hijack of the ER by the virus. Importantly, the viroporins ion channels E, open reading frame 3a (ORF3a) and ORF8a of SARS-CoV-2 can trigger also NLRP3 activation signaling pathway [35–37]. For instance, viroporin E can induce a channel pore in ER and subsequently increases outflow of Ca2+ to cytoplasm, which in turn activates NLRP3 [38,39]. In parallel, viroporin E and ORF3a can together directly stimulate NF-κB signaling pathway that upregulates pro-inflammatory molecules expression [40]. Altogether, it is clear that NLRP3 inflammasome activation driven by ER stress plays a major role in the pathogenesis of COVID-19 (Figure 1) [41–43].

3.2. Neutrophils and NETosis

Neutrophils are the first effector molecules recruited to the site of infection in response to cytokines. Severe COVID-19 cases showed a very high number of infiltrating neutrophils to the lungs especially in Intensive Care Unit patients [17,44]. Hence, elevated neutrophil-lymphocyte ratio could be considered as a biomarker for COVID-19 infections [18]. In addition to phagocytosis, neutrophils build up neutrophil extracellular traps (NETs) by releasing their DNA, granules such as proteinase 3 and danger-associated molecular patterns (DAMPs) in order to kill pathogens. DAMPs in turn activate immune and non-immune cells, which triggers more cytokines and chemokines release from cells [45]. However, the sustained NET formation observed in COVID-19 created an auto-amplification loop of necro-inflammation and dominated the defense functions of neutrophils. NET-specific markers such as myeloperoxidase DNA and citrullinated histone H3 were identified in high amounts in severe cases of COVID-19 patients [46].

3.3. Complements, key players in hyper-inflammation

Complements are functional proteins of the innate immune response that increase inflammation, activate leukocytes and clear viruses. Once NETs are established, neutrophils release CFP (Complement Factor Properdin), C3, and CFB (Complement Factor B) complements that stabilize the alternative complement pathway (AP) [47]. Interestingly, AP pathway results in anaphylatoxins production, such as C5a that stimulates neutrophils and together with C3a induce inflammation and tissues damages linked to acute respiratory distress syndrome (ARDS). Compared to non-hypoxic COVID-19 patients, a higher level of complement receptor (CR 3) was observed in neutrophils, mast cells, monocytes/macrophages, basophils, eosinophils, T cells, and B cells in hypoxic COVID-19 patients [48].
3.4. Cytokine storm syndrome

The uncontrolled release of cytokines, also called ‘Cytokine storm syndrome’ is plausibly the major factor underlying COVID-19 immuno-pathogenicity [49]. This storm was at the origin of tissues damages, hyper inflammation [50] and even mortality cases [19]. It was triggered by a hyper activation of immune cells, which led to a boom of cytokines release [51]. The over expression of Induced Protein-10, hepatocyte growth factor, monokine-inducd gamma IFN, monocyte chemotactic protein-3 and macrophage inflammatory protein 1 alpha were highly associated with the disease severity classes [52]. Interestingly, the upregulation of IL-6 got a special attention in COVID-19 pathogenicity since it plays a major role in recruiting neutrophils to lungs [53] and consequently inducing hyper inflammation, neutrophilia and NETosis. In addition, Mazzoni et al. [54] showed that IL-6 was responsible for impaired cytotoxicity properties of immune cells in severe cases of COVID-19. However, IL-6 level in COVID-19 stays lower than in cytokine release syndrome (CRS) [53,55]. Surprisingly, infected patients displayed reduced levels of anti-viral effectors especially interferon types I and III cytokines [56] and eosinophils [57,58]. This decline in anti-antiviral agents would certainly weaken the innate immune reaction and facilitate viral replication.

4. How much does the adaptive immunity defend against SARS-CoV-2?

4.1. T lymphocytes

Given the high number of CD8\(^+\) infiltrating cells (80%) recruited to the site of infection, it was admitted that the cellular immunity had the major role to protect against SARS-CoV-2 [59]. Unfortunately, studies showed that CD8\(^+\) infiltrating cells had a T cell exhaustion profile [60] and non-exhausted T cells count were reduced in severe cases [61]. The main reason for exhaustion might be linked to the overexpression of Natural Killer group 2 member A (NKG2A) inhibitor receptor. This hypothesis is supported by the fact that a high level of NKG2A on CD8\(^+\) cells was noted in COVID-19
patients compared to healthy group [62] and a reduction in NGK2A expression was observed in recovered patients [63].

In addition to exhausted T cells, lymphopenia is another aspect of cellular immunity in COVID-19 infection [53]. T cells count becomes low and highly reduced in severe cases, where a percentage less than 5% was an indicator of high mortality rate [64,65]. Eighty-five percent of COVID-19 patients with pneumonia showed lymphopenia [50]. Lymphocytes percentage might now be used as biomarker to classify severity of disease or recovery [66]. Along with CD8^+ lymphopenia, Natural Killer cytotoxic immune cells amount had also been reduced in COVID-19 patients, which also exacerbated the severity of infection.

Some researchers had suggested that first the virus over-activates CD8^+ cells then the T cells become exhausted. This over activation induces an over-cytotoxicity response that contributes partially to the tissues damage too [67]. In addition to lymphocytes exhaustion and lymphopenia, the SARS-CoV-2 were able to infect T cells through the binding of S viral spike with CD26 or CD147 on T cells and caused their depletion [68]. Moreover, some cytokines such as IFN-α/β or cytokines derived from mononuclear macrophages killed T cells by apoptosis [69]. Regulatory T cells (Treg), known for their important role in limiting respiratory infections [70], were also affected in COVID-19 lymphopenia [64]. Finally, T helper cells Th1/Th17 were also hyper activated in order to stimulate B lymphocytes production of specific antibodies anti-SARS-CoV-2 [62,71].

4.2. B lymphocytes and immunoglobulins

During COVID-19 infection, the humoral immune response was activated through the direct interaction with the virus or upon stimulation by CD4^+ T helper [15]. However, not all patients showed the same pattern of immunoglobulins production during infection. The study of To et al. [72] revealed that a high proportion of COVID-19 patients had developed IgG earlier than IgM in serum. This might be explained by the presence of IgG released from memory B cells due to previous exposure to other coronaviruses with similar epitopes, which gives the disease an antibody-dependent enhancement (ADE) characteristic. Sixteen percent of B-cells specific for SARS-CoV are able to recognize SARS-CoV-2 [73]. Given that secretory IgA (sIgA) mission is to protect mucosal respiratory tract from pathogens, it should normally be considered the most important immunoglobulin to neutralize SARS-CoV-2. In COVID-19 patients, IgA appeared very early [16], persisted longer than IgM [74] and stimulated pro-inflammatory cytokines release like IL-6 and monocyte chemoattractant protein (MCP)-1 [75]. A group of IgM seronegative COVID-19 patients in Florence, Italy had developed IgA (5–7 days post symptoms), with an average of IgA level much higher than IgG two and three weeks later [76]. Importantly, IgA anti-SARS-CoV-2 were also detected in saliva and could remain for three months or longer after symptoms [77]. High IgG and IgA levels were correlated with COVID-19 severity [26]. Interestingly, a seronegative patient does not mean there is no immunity, IgA and IgG, for example, were detected in breast milk of an infected patient and not in other body fluid [78].

5. Preventive, therapeutic and diagnostic strategies from immunological perspective

5.1. Prevention

In order to control COVID-19 pandemic with the absence of an effective treatment, preventive steps are urgently needed. Deciphering the immunopathogenesis had provided scientists with interesting clues to set up preventive practices. The development of SARS-CoV-2 vaccines is still under development. These may include RNA, viral vector, virus-like particles, inactivated or live-attenuated viruses [71]. A mRNA-1273 vaccine, a novel lipid nanoparticle (LNP)-encapsulated mRNA-based vaccine which encodes the spike protein (S protein) of SARS-CoV-2 was able to enhance antibodies production against the viral spike protein following the second dose during phase I trial [79]. A vaccine vector under preparation consists of a newly synthesized avian ortho-avulavirus 1 (AOaV-1) carrying the spike glycoprotein and hemagglutinin-neuraminidase HG genes which has promising results [80]. In Canada, a series of authorized clinical trials are under investigations including recombinant BCG vaccine VPM1002 that is undergoing phase 3 clinical trial, coronavirus-like particle CoVLP vaccine in phase 1 and IMM-101 in phase 3 trial [81]. A study by Ahmed et al. [73], determined B and T cells epitopes derived from S and N proteins of SARS-CoV-2 and suggested the use of immune vaccination against these epitopes. Oral β-glucan vaccine has also been proposed as it has anti-viral effects and can boost the immune response by inducing trained immunity (TRIM). BCG vaccine was also found to stimulate a specific immune response against the SARS-CoV-2 envelope [82]. Herd immunity, when enough people in a population (70–90%) become immune to the virus, might also be protective [83,84]. However, WHO mentioned that currently, only around 10% of the global population has anti-
SARS-CoV-2 antibodies in their blood and it is still not known if they can be considered immuno-protected. Finally, the natural antibodies anti-A and anti-B developed by blood type O might have a protective function against COVID-19 infection [85,86].

5.2. Therapy

Since the emergence of the pandemic, laboratories worldwide had put their full power in order to find out an effective cure for COVID-19. Unfortunately, a specific anti-viral substance to kill SARS-CoV-2 was not yet discovered. Pharmacologically, some had successfully tried remdesivir drug that had limited viral replication through inhibiting viral RNA polymerases [87,88]. Fortunately, others had used Angiotensin-converting enzyme ACE2 inhibitors that prevented the binding of the virus to its specific receptor [89,90] and subsequently protected cells from infection (Figure 1). Lipid mediators like Elovanoid (ELV)-N32 or Resolvin D6-isomer (RvD6i) were able to reduce ACE2 expression which resulted in preventing viral attachment to its target and could be used to stop the infection [91].

Today, researchers are trying to understand the immunological behavior of infected patients in order to set up better immunotherapies for COVID-19. Given that, the pathogenesis is highly correlated with an explosive inflammatory reaction, it would be essential to block this excessive immune response. Thus, researchers are now targeting cellular and molecular effectors of inflammation. Several trials are testing colchicine to inhibit neutrophils recruitment and inflammation [91] while others are assessing the impact of NETS inhibitors (e.g., PAD4). Interestingly, Shu et al. [92] recommended the use of mesenchymal stem cells derived from human umbilical cord to reduce cytokines and C-reactive proteins (CRP) in COVID-19 patients. CR3 antagonists (Simvastatin) use can also help in preventing and treating COVID-19 pneumonia [48]. Immunosuppressors like corticosteroids were highly used to treat severe cases by reducing hyper-inflammation and cytokine storm syndrome (Figure 1). Given the crucial role of NLRP3 inflammasome in increasing infection severity, it is important to look for strategies that inhibit NLRP3 pathways activation [91].

On the other hand, a very low immune response increases severity of COVID-19. Therefore, it would be essential to improve immunity in patients with immunodeficiency. Immunomodulatory molecules that enhance lymphocytes proliferation and activation could be used to rescue the fall in lymphocytes count due to lymphopenia [93]. Baricitinib, Janus Kinas (JAK) inhibitor, can enhance anti-SARS-CoV-2 spike antibodies production [94]. Given their anti-viral properties, Interferon therapy might also be beneficial since interferon I and III levels are reduced in infected patients.

A large panoply of monoclonal antibodies was recommended for COVID-19 treatment. Meblazumab, anti-CD147 receptor antibody may protect T cells from being infected by SARS-CoV-2 [95] since the virus was able to bind to CD147 and enter the cell. Interestingly, blocking activity of IL-6, the major cytokine related to COVID-19 pathogenicity would be very effective. Tocilizumab, IL-6 receptor antagonist is a potential candidate that prevents inflammation, tissue damages and restore cytotoxicity properties of immune cells [54,96]. The use of complement inhibitors in COVID-19 such as eculizumab anti-C5 antibody is under trials. Interestingly, the T lymphocytes depletion could be rescued by blocking NGK2A receptor that plays a major role in exhaustion of T cells. Fortunately, Monalizumab, antibody anti-NGK2A receptor may prevent CD8 T cells and NK from dysfunction [62]. Canakinumab, new release from Novartis may calm down cytokine storm syndrome, a main aspect of COVID-19 pathogenesis.

Given the important role of immunoglobulins in fighting SARS-CoV-2, passive immunity through immunoglobulins transfusion was considered effective for treatment. Despite the controversies about the use of recovered patient’s serum as treatment, patients who had received convalescent serum therapy presented good outcomes [97]. Infected patients who have pneumonia in addition to COVID-19 should take IgG4 treatment and no other IgGs for more safety [98]. The use of IgA anti-SARS-CoV-2 has also good promises for therapy [99,100]. Interestingly, IgA monoclonal antibody MAb362, that binds to S spike proteins of SARS-CoV-2 prevents the viral attachment to ACE2 and provides patient with mucosal immunity [101]. Finally, the administration of mammalian target of rapamycin (mTOR) inhibitors was effective to prevent ADE that causes uncontrolled immunoglobuline heavy chains (IgH) release [102].

5.3. Serological tests

Three diagnostic test types are used in COVID-19 diagnosis: real-time polymerase chain reaction RT-PCR, antigen detecting tests and antibody detecting tests. A study by Brooks and Das compared these three types. The percent positive agreement (PPA) also known as sensitivity is higher in molecular (86.14%) than antibody (68.44%) and antigen (61.70%). However, the specificity or percent negative agreement (PNA) is high in all types: 98.26% in
antigen type and almost 95% for others [103]. Another study conducted by Miller et al. compared PCR and serology based on enzyme-linked immunosorbent assay ELISA for antibodies IgM, IgG and IgA anti-SARS-CoV-2 targeting its receptor binding domain RBD in the S protein. The sensitivity of ELISA was higher with days after the onset of symptoms with >50% seropositive by one isotype at least, at day 7 and 100% at day 21 [104]. These results were also demonstrated by Peterhoff et al. who additionally found the absence of cross-reactivity between antibodies resulted from previous corona-like seasonal viruses and anti-SARS-CoV-2 antibodies. These antibodies were detected by RBD-based ELISA showing high specificity 99.3% and sensitivity: 98% for IgM, 96% for IgG and 92% for IgA, 10 days after PCR-confirmed infection [105].

Moreover, a study tested several in-house and commercial serological tests against nucleocapsid and spike proteins. Results showed sensitivities and specificities respectively for in-house ELISA tests against anti-trimer spike: IgA 90%/100%, IgG 90%/99.3% and anti-nucleocapsid IgG 89%/98.3%. However, sensitivities/specificities for commercial EDT\textsuperscript{TM} Novel Coronavirus COVID-19 ELISA for anti-nucleocapsid antibodies showed 84.5%/95.1% for IgG and 73.7%/100% for IgM. In addition to other sensitivities/specificities for commercial tests like Euroimmun ELISA 95%/93.7% (S1 IgA), 82.8%/99.7% (S1 IgG), and lateral flow assays by Chembio Diagnostics Systems revealing 82.0%/91.7% (nucleocapsid IgM) and 92%/93.3% (nucleocapsid IgG) [106].

In addition, a comparison of the sensitivity and specificity of: anti-SARS-CoV-2 nucleocapsid IgG testing through a chemiluminescence microparticle immunoassay CMIA manufactured in Abbott laboratories, anti-SARS-CoV-2 nucleocapsid antibodies by a sandwich electro-chemiluminescent assay ECLIA from Roche diagnostics and IgG anti-S1/S2 domains of the SARS-CoV-2 by a chemiluminescence immunoassay CLIA from DiaSorin was performed by Perkmann et al. All three tests showed high specificities: 99.2%, 99.7% and 98.3% respectively. However, a significant problem of incompatibility was found at low seroprevalences, which imposes serious positive results confusions [107].

Other serological methods were also studied. The MosaiQ\textsuperscript{®} COVID-19 Antibody microarray was found to test anti-S1 IgG and IgM of the SARS-CoV-2 with 100% clinical specificity and 88% clinical sensitivity [108]. Furthermore, biolayer interferometry immunosorbent assay (BLI-ISA) is a rapid automated (dip-and-read) diagnostic method that can be used for the detection of anti-SARS-CoV-2 IgG antibodies [109]. A rapid gel card agglutination assay based on indirect ELISA, using red blood cells coated with SARS-CoV-2 peptide (RBD, spike proteins and nucleocapsid) can also detect IgG in the patients’ serum [110]. Similarly, NovaTec tested the NovaLisa SARS-CoV-2 IgG, IgA and IgM detection with 94.9%/96.2%, 89.7%/98.7% and 48.7%/98.7% as sensitivity and specificity respectively. Also, the BioRad has the Platelia SARS-CoV-2 total antibodies detection with 97.4% specificity and 94.9% sensitivity against the viral nucleocapsid [111].

6. Conclusion
As understanding the roles of immune responses in COVID-19 disease has evolved, a new vision to the immunity to SARS-CoV-2 has been revealed to the public health. The two opposite aspects of immune reactions during infection, the ‘good’ and ‘harmful’ patterns, are the keys to set up effective strategies in order to control this pandemic (Figure 2). Today, researchers are urged to explore more the mechanisms involved in COVID-19 immune-pathogenesis in order to bring out the essential elements.
Disclosure statement

The authors declare no conflict of interest.

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