FEATURES OF THE INNATE IMMUNE RESPONSE DURING THE SARS-COV-2 INFECTION

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First reports of the acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and the disease caused by the virus—coronavirus disease 2019 (COVID-19), were announced in late December 2019. Ever since, the disease has taken more than 6 million lives worldwide. COVID-19 is considered as dominantly respiratory and vascular disease which pathogenesis could be explained by hyperactivation of the immune response. Innate immunity receptors are responsible for the first contact with the virus and subsequent activation of transcription factors leading to the production of the high amounts of interferons (IFNs) and proinflammatory cytokines (IL-1β, IL-6, TNF, etc.). Such an inflammatory response limits viral replications. However, SARS-CoV-2 have developed several ways to avoid immune protection by the host. Dysregulated secretion of these cytokines may lead to cytokine storm and PANoptosis, a life-threatening condition.

This review article aims to describe the main characteristics of the innate immune response during the SARS-CoV-2 infection. *Acta Medica Medianae 2023;62(3):47-53.*

Key words*: SARS-CoV-2, COVID-19, inflammation, cytokines, receptors*

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Introduction

First reports on the specific pneumonia cases of unknown etiology were announced in December 2019 by Chinese Center for Disease Control and Prevention. Later, in January 2020, the causative agent was identified and labeled as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the disease caused by the virus as coronavirus disease 2019 (COVID-19). The disease had a devastating influence on demography in the world that resulted in more than 6 million deaths worldwide (1). After infecting the host cells, SARS-CoV-2 is subject to genetic mutations over time. Therefore, different variants of virus have been described: Alpha (B.1.1.7) - first appearance in the United Kingdom in 2020; Beta (B.1.351) – in South Africa since 2020; Gamma (P.1) - in Brazil since 2021; Delta (B.1.617.2) - in India since December 2020; Omicron (B.1.1.529) - in South Africa since 2021.

The main mode of SARS-CoV-2 transmission is through a respiratory system and viral droplets. Another way of infection is upon a contact with contaminated surfaces. There are epidemiological reports indicating the presence of SARS-CoV-2 on plastic and stainless steel for up to three days, millboard for up to a day (2, 3). Other studies showed the presence of live virus in feces of patients with SARS-CoV-2 infection indicating possible fecal-oral transmission (4).

Epidemiological data has shown that individuals of all ages can be infected with SARS-CoV-2 infection. However, elderly and patients with certain medical comorbidities (cardiovascular disease, chronic lung disease) are at great risk of developing severe disease (5).

COVID-19 is considered as dominantly respiratory and vascular disease since SARS-CoV-2 primarily affects the respiratory and vascular systems. However, function of other organs may be disturbed by the infection as well (Figure 1).

The pathogenesis of COVID-19 disease most likely can be explained by overactivation of the immune response. This review article aims to describe the characteristics of the innate immune response during the SARS-CoV-2 infection.
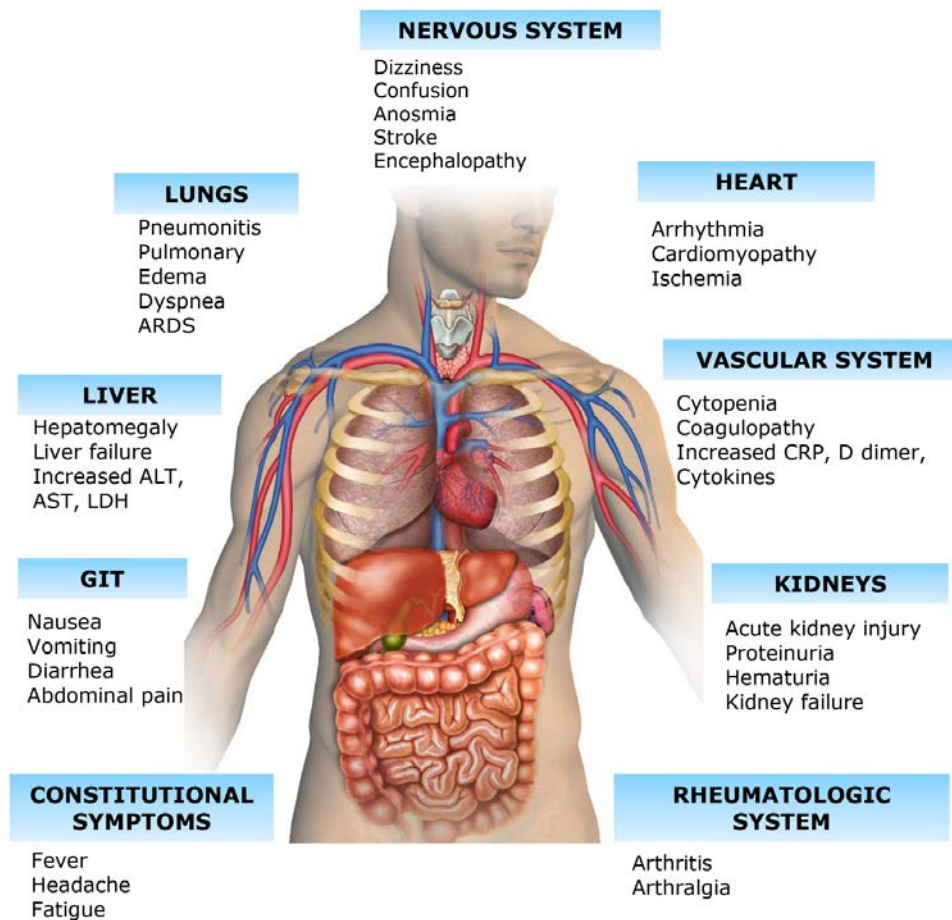
Figure 1. The most common clinical manifestations of COVID-19

COVID-19 is considered as dominantly respiratory and vascular disease. However, infection can also affect other major organ systems, such as nervous system, gastrointestinal tract (GIT), hepatobiliary, cardiovascular, renal, rheumatologic, etc.

ARDS, acute respiratory distress syndrome; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; CRP, C-reactive protein. *(Adapted from https://medlineplus.gov/anatomy.html)*

SARS-CoV-2 receptors

The SARS-CoV-2 virus consists of the positive single-stranded (ss) RNA genome and it is classified into the order *Nidovirales*, family *Coronaviridae,* subfamily *Coronavirinae*, and genus *Betacoronavirus* (6). The most important proteins for viral replication, structuring, pathogenicity and binding with cellular receptors are expressed on its surface. They are termed as spike (S), envelope (E), membrane (M), and nucleocapsid protein (N) (7). The virus also produces certain open reading frames (ORFs) responsible for encoding the accessory proteins significant in viral pathogenesis (8). The SARS-CoV-2 enters the host cells binding to specific receptors. Namely, as the most important protein mediating membrane fusion and viral penetration, S glycoprotein binds to angiotensin-converting enzyme 2 (ACE2), its' crucial receptor with the highest binding affinity.

S-protein forms trimmers on the surface of the virus, in the form of S1 and S2 subunits with receptor-binding protein (RBD), where RBD directly interacts with the ACE2 (9). After binding to the receptor, proteolysis of the S protein takes place upon which viral membrane and target cell merge.

During this process, S1 subunit recognizes and binds to the receptor, while S2 subunit mediates the fusion, after which the viral RNA enters the cell (10).

Recent studies have described several membrane proteins that function as ACE2 cofactors or alternative receptors. Most of them are expressed on plethora of the cells, such as epithelial cells, platelets, alveolar epithelium, dendritic cells, hepatocytes, etc. (11−16). The most notable ones are presented in Table 1.

Table 1. ACE2 cofactors or alternative receptors for SARS-CoV-2

Receptor	Cell expression
ACE2	Epithelial cells, macrophages, platelets, endothelial cells, smooth muscle cells, many other cells
Neuropilin 1 (NRP1, CD304)	Nerve cells of the brain and nasal cavity, endothelial cells
Chondroitin sulfate	Most of the cells
CD147 (Basigin)	Highly expressed on cells of the immune system
GRP78	Different cells
CD206	Macrophages, monocytes, dendritic cells
CD249	Epithelial cells, macrophages, platelets, endothelial cells, smooth muscle cells, many other cells

SARS-CoV-2 and PRRs

The main route of the SARS-CoV-2 entry into human organism is through the respiratory tract, but contact with an infected surface is important as well (17). Innate immunity receptors responsible for the first contact with the virus belong to the pattern recognition receptors (PRRs) -Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), NOD-like receptors (NLRs) and inflammasomes (18). In general, these receptors are mainly expressed in dendritic cells (DCs) and macrophages where they recognize microbial nucleic acids and upon engagement of myeloid differentiation protein 88 (MyD88) and TRIF adaptor molecules, activate the transcription of type I and type III interferons (IFNs) as well as nuclear factor kB (NF-kB)—dependent proinflammatory cytokines and chemokines with the consequent induction of death of the infected cell (19, 20).

TLRs are a family of transmembrane receptors consisting of ectodomains with multiple leucine-rich repeats (LRRs), linked by a transmembrane domain to a conserved cytosolic domain called the Toll/IL-1 receptor homology (TIR) domain (18). To date, 28 TLRs have been identified in vertebrates, of which humans possess only 10 (TLR1-10) (21). Bearing in mind the function and position in the host cell, there are two main groups of TLRs: [1] *Cell membrane* TLRs - expressed on the surface of the cell, and they include TLR1, 2, 4, 5, 6, and 10; and [2] *Intracellular* TLRs - expressed within the host cells on the organelle bio membranes like endoplasmic reticulum (ER), endosomes, and lysosomes, and they include TLR3, 7, 8, and 9 (22). These intracellular TLRs are responsible for viral recognition, i.e. their pathogen-associated molecular patterns (PAMPs) such as single strand (ss)-RNA, double strand (ds)-RNA or CpG-DNA (20). Regarding SARS-CoV-2 infection, to date there no data confirming direct involvement of any type of human TLRs. Namely, *in vivo* murine studies have described that the SARS-CoV-2 E protein recognition by macrophage TLR2 mounts inflammatory responses (23). *In silico* studies suggest that TLR1, TLR4 and TLR6 are the receptors with the highest affinity for SARS-CoV-2 S protein binding (24). Additionally, chromosomal TLR7 anomalies have been described among the young individuals with the severe forms of the disease. Such a finding indicates a protective role of TLR7 during the viral infection (25).

NLR family of proteins represent a group of PRRs responsible for the initiation of innate immune response during the cellular injury and stress (26). The best described member of the family, NLRP3 inflammasome, mediates caspase-1 activation and the secretion of proinflammatory cytokines IL-1β/IL-18 and cleavage of gasdermin D, which forms pores in the plasma membrane leading to pyroptotic cell death (27). Several studies have suggested that NLRP3 senses SARS-CoV2 infection (28, 29). Their common findings are reflected through the increased levels of IL-1β and IL-18 in plasma, which correlated with disease severity and mortality in patients with COVID-19.

SARS-CoV-2 and cytokines

Numerous evidence suggest that COVID-19 morbidity and mortality are related to high amounts of both IFNs and proinflammatory cytokines (30, 31). In general, their role is reflected through clearing the infection and maintaining cellular homeostasis. However, dysregulated production of proinflammatory cytokines may lead to a cytokine storm, a life-

threatening condition. In the context of SARS-CoV2 infection, this excessive production of cytokines may induce PANoptosis (32). This condition is defined as programmed cell death pathway dependent on PANoptosomes – a complex consisted of caspase(s) with or without inflammasome components (32). Synergism of IFN-γ and TNF induces a lethal shock syndrome in mice, similar to a cytokine storm detected in some patients with severe COVID-19 (33). PAMPs, DAMPs and pathogens may trigger PANoptosis and this very process is most probably responsible for multiorgan damage in COVID-19 patients.

Viral evasion strategies

One of the main functions of innate immunity is to induce an inflammatory response that will limit viral replications. However, SARS-CoV-2 has developed several ways to avoid such immune protection by the host. Namely, it may inhibit IFN production through the expression of several viral proteins that block IFN signaling pathways (34). The SARS-CoV-2 alters myeloid response with an excess of circulating immature monocytes, neutrophils and myeloid progenitors. This condition is known as emergency myelopoiesis and it is observed among patients with mild to severe COVID-19 (35). During this stage of the disease, myeloid cells produce high amounts of inflammatory cytokines which lead to vascular permeability and organ failure (36). Hypercoagulation, followed by arterial and venous embolism is also often detected among COVID-19 patients (37). It is presumed that the virus alters the vascular endothelium during inflammatory process and activates the cells included in the release of coagulation factors (von Willebrand factor, factor VIII) (38). In addition, severe COVID-19 is accompanied by a high titer of autoantibodies specific for nuclear antigen, T and B cell antigens, chemokines, and cytokines which all together activate mechanisms responsible for tissue damage and organ failure (39, 40).

Conclusion

The COVID-19 pandemic resulted in a loss of more than 6 million lives worldwide (1). Despite numerous preventive efforts and rapid advances in basic and translational science, the infection still remains a global threat.
Wide spectrum of receptors and effector molecules, with IFN signaling, cytokine production and cell death, makes the innate immune system the first line of defense against SARS-CoV-2 infection. However, the virus itself developed strategies to avoid these protection mechanisms, leading to hyperactivation of the innate immunity with consequential hyperinflammation, cytokine storm, severe diseases and mortality. Therefore, many treatment strategies targeting innate immune response have been introduced. Such a therapy balances between inflammation and immunomodulation preventing excessive pathological inflammation (32).

As the SARS-CoV-2 infection persists, there is a need for additional knowledge of the COVID-19 immunopathogenesis and hence development of new therapeutics.

Acknowledgements

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KARAKTERISTIKE UROĐENOG IMUNSKOG ODGOVORA TOKOM SARS-COV-2 INFEKCIJE

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Prva saznanja o infekciji akutnim respiratornim korona virusom (SARS-CoV-2) i bolesti izazvanoj virusom – COVID-19, objavljena su krajem decembra 2019 godine. Od tada je bolest uzela više od šest miliona života širom sveta. SARS-CoV-2 smatra se dominantno respiratornim i vaskularnim oboljenjem, čija se patogeneza može objasniti hiperaktivacijom imunskog odgovora. Receptori urođenog imuniteta odgovorni su za prvi kontakt sa virusom i naknadnu aktivaciju transkripcionih faktora, koji dovode do proizvodnje velikih količina interferona (IFN) i proinflamatornih citokina (IL-1β, IL-6, TNF itd.). Takav inflamatorni odgovor ograničava replikaciju virusa. Međutim, SARS-CoV-2 je razvio nekoliko načina da izbegne imunski odgovor domaćina. Neregulisano lučenje ovih citokina može dovesti do razvoja citokinske oluje i PANoptoze, stanja opasnih po život. Ovaj pregledni članak ima za cilj da opiše glavne karakteristike urođenog imunskog odgovora tokom infekcije izazvane SARS-CoV-2 virusom. *Acta Medica Medianae 2023;62(3):47-53.*

Ključne reči*: SARS-CoV-2, COVID-19, inflamacija, citokini, receptori*