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Review

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Insights into Innate Immune Response Against SARS-CoV-2 Infection

Adina Huțanu[1*], Anca Meda Georgescu[2], Akos Vince Andrejkovits[2], William Au[3,4], Minodora Dobreanu[1,5]

1. Department of Laboratory Medicine, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures, Romania

2. Department of Infectious Disease, Infectious Diseases Clinic, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures, Romania

3. Department of Preventive Medicine and Community Health, University of Texas Medical Branch, Galveston, TX, United States

4. Preventive Medicine, Center for Advanced Medical and Pharmaceutical Research, George Emil Palade University of Medicine, Pharmacy, Sciences, and Technology of Targu Mures, Târgu Mureș, Romania

5. Department of Immunology, Center for Advanced Medical and Pharmaceutical Research, George Emil Palade University of Medicine, Pharmacy, Sciences, and Technology of Targu Mures, Târgu Mureș, Romania

Abstract

The innate immune system is mandatory for the activation of antiviral host defense and eradication of the infection. In this regard, dendritic cells, natural killer cells, macrophages, neutrophils representing the cellular component, and cytokines, interferons, complement or Toll-Like Receptors, representing the mediators of unspecific response act together for both activation of the adaptive immune response and viral clearance. Of great importance is the proper functioning of the innate immune response from the very beginning. For instance, in the early stages of viral infection, the defective interferon response leads to uncontrolled viral replication and pathogen evasion, while hypersecretion during the later stages of infection generates hyperinflammation. This cascade activation of systemic inflammation culminates with cytokine storm syndrome and hypercoagulability state, due to a close interconnection between them. Thus an unbalanced reaction, either under- or over- stimulation of the innate immune system will

[*] **Corresponding author**: Adina Huțanu, Department of Laboratory Medicine, George Emil Palade University of Medicine, Pharmacy, Science, and Technology Targu Mures, Romania. E-mail: adina.hutanu@umfst.ro

lead to an uncoordinated response and unfavorable disease outcomes. Since both cellular and humoral factors are involved in the time-course of the innate immune response, in this review we aimed to address their gradual involvement in the antiviral response with emphasis on key steps in SARS-CoV-2 infection.

Keywords: *innate immunity, monocytes, acute phase reactants, COVID-19, antigen-presenting cell*

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Introduction

Among Coronaviruses (CoVs), SARS-CoV-2 was first identified during December 2019 in China (1) to be the cause of the COVID-19 disease and the infection was quickly labeled as a pandemic in March 2020 (2). By comparison with the previous SARS-CoV and MERS that caused loco-regional restricted epidemics (3), the SARS-CoV-2 was highly infectious and pathogenic, causing over 174,000,000 cases and over 3,758,000 deaths around the world by June 10th, 2021 (https://COVID19.who.int) (4).

For SARS-CoV-2 infected persons, symptoms vary having iceberg-type features, from asymptomatic carriers to severe acute respiratory syndrome (SARS), with mild/moderate symptoms between the two extremes (5). The virus can be detected using the RT-PCR procedure and the positive test is used to recommend urgent isolation or quarantine of the individuals. However, new variants potentially undetected by first-generation tests have emerged, indicating the need for monitoring by viral genome sequencing (5).

Virus entry into host cells is mediated by the ACE-2 receptor and transmembrane protease serine 2 (TMPRSS2). The primary target cells are in the lung, but various extrapulmonary cells (gastrointestinal tract, heart, kidney, liver, and nervous system) have also been found to be infected (6).

Upon infection with viruses, hosts usually mount a multifaceted immune response against the infection and the response can be divided but not separated into the innate and adaptive immune responses (5). However, SARS-CoV-2 infection has peculiar characteristics and outcomes, suggesting a unique immunopathogenic process. Indeed, the infections leading to COVID-19 are frequently triggered by profound dysregulation in the immune response, especially the cytokine storm which often increases the mortality rate (7). Despite the critical role of immune response to SARS-CoV-2 infection, reports on innate immune response are scarce. Therefore, this review was conducted to provide insights into involvement of the innate immune system as a critical host defense step against SARS-CoV-2 infection.

Players of the Innate Immunity

The innate response is the first line of host defense against pathogens and non-pathogens. It is an extensive response that involves different categories of activities: epithelial and mucosal barriers with their secretory products that serve as chemical barriers, cells of non-specific immunity (neutrophils, monocytes, macrophages, dendritic cells, eosinophils, NK cells), and various molecules and proteins such as Toll-like-receptors (TLRs), acute phase reactants and cytokines. However, there are individual differences in the response, e.g., genetic polymorphisms of cytokines and receptors, genetic susceptibilities related to HLA haplotypes (8), which may impact disease outcomes (9–11). Although, until recently, the general beliefs were that only adaptive immune system possesses immunological memory that facilitates an immediate enhanced immune response during the second encounter with the same pathogen, more and more studies argue that also the innate immune system is able

to mount the immunological memory (12). This seems to be achieved by increased accessibility of transcription factors to pro-inflammatory genes by shaping the epigenetic and metabolic environment of the cells (12).

Epithelial and mucosal barriers

Physical barriers are represented by epidermal and mucosal layers. The keratinocytes, the most predominant cells of the epidermis, originating from the basal layer constitute a major barrier for microbes and water, due to keratin secretion and other substances with antibacterial functions like defensin, cathelicidins, or lactic acid (13,14). In addition, the Langerhans' cells with the important function of antigen presentation express both MHC class I and MHC class II molecules with a key role in naïve T cells activation (14). Although there are several reports regarding skin manifestation in COVID-19 (15–17), it was hypothesized that wounded skin might be a reservoir for SARS-CoV-2 and can therefore enhance pathogenicity of COVID-19 (18), but this could only be speculation. As for the mucous membranes, especially the mucosa of the respiratory tract, they are loaded with cells that express the ACE-2 receptors which facilitate entry of the virus into cells (19). A single-cell RNA sequencing showed that the prevalent ACE-2 expressing cells are type II alveolar cells (AT-II) and to a lesser extent type I alveolar and endothelial cells, macrophages, and fibroblasts (20). The study also revealed a significant difference between ACE-2 expressing and non-expressing AT-II cells, in the first ones the viral-related processes linking to the viral life cycle and replication being over-expressed (20). Indeed, a minimally invasive lung biopsy on three patients who were infected with SARS-CoV-2 revealed the proliferation of AT-II with virus particles inside the cytoplasm, shedding of the virus, and cell necrosis, however, less severe than observed during the SARS infection (21). Besides the lung, other cell types in the digestive, renal, and nervous systems also express ACE-2, thus acting as additional reservoirs and gates for virus infection. MHC class I downregulation by ORF8 protein has been reported as an escape strategy of SARS CoV-2 which may affect the antigen presentation and assist the viruses to escape from immune surveillance (22). On the other hand, genetic variability across the major genes of MHC-I and -II were predicted to affect susceptibility to and severity of SARS CoV-2 infection; vulnerable, but also protective haplotypes were calculated *in silico* studies according to the binding affinity of 8-9 or 15-mer peptide derived from SARS CoV-2 peptidome (8,23).

Dendritic cells

Dendritic cells (DCs) are the professional antigen presentation cells (APC), having the major role to present various antigens in the context of MHC class I and MHC class II to the naïve lymphocytes into the area of the lymph nodes (14), thus activating and modulating the immune response. Dendritic cells are found in two forms: conventional and plasmacytoid, the latter ones are an important source of type-I interferon during the viral infection (24). The conventional DCs are present in the lymphoid organs (spleen, thymus, and lymph nodes) as resident and migratory DCs which are responsible for the antigen presentation to naïve T cells, ensuring the connection between innate and adaptive immune response (24).

After activation, DCs become a reservoir of many cytokines, including IFNs and TNF-α; however, the spectrum of cytokines produced vary depending on the nature of the stimuli (24). In relation to SARS-CoV-2 infection, the DCs from the respiratory airways are both guards and victims at the same time, since the interstitial pulmonary DCs express both ACE-2 receptor for virus entry and CD147, the novel described receptor for S protein (24,25). Aside from the

antigen presentation, these cells could serve also as a virus reservoir after the activation of viral escape mechanisms.

Furin, a proteolytic enzyme found in abundance in the lung was reported to enhance cleavage at the furin-like S2' site of SARS-CoV-2 (26). Consequently, it becomes a host factor that can enhance the pathogenesis of the SARS-CoV-2. Indeed, the virus up-regulates furin production in monocyte-derived DCs, despite the low expression of the entry receptors on these cells (24,27). In severe COVID-19 cases, the virus was reported to exert negative effects on DCs in several different ways: reduction of viability and functions of DCs (27), increased numbers of conventional DCs compared with plasmacytoid DC, impaired expression of co-stimulatory CD86 molecules (28), and reduced type I and III IFNs response in infected cells (27,29), leading to improper activation of the immune response.

Monocytes and macrophages

Monocytes and macrophages have been known as important inflammatory cells in the alveoli, along with neutrophils, generating exudate and interstitial fibrosis. Additionally, these inflammatory cells were found in the inflammatory infiltrate in the heart as well in the intestinal mucosa of severe COVID-19 (21). A study of the behavior of the monocyte/macrophage infected differentially with MERS and SARS-CoV revealed that only MERS is capable of replication in macrophages with attenuated antiviral IFN response, increased induction of pro-inflammatory mediators and cytokine storm (30). On the other hand, there was an increase in the expression of MHC class I and co-stimulatory molecules that were more prevalent in MERS than SARS-CoV infection and which mirrored the clinical outcome (30). The cytokine storm is produced after the uncontrolled release of pro-inflammatory cytokines from lymphoid and myeloid line cells (31). At the beginning of the infectious process, monocytes, a source of macrophages at the site of infection, were the main producers of IL-6 under TLRs stimulation (32). Macrophages possess CD 126 marker which is a receptor for IL-6, a cytokine with pleiotropic activities, mediated either by classic (via a membrane-bound receptor on different cell lines) or by trans-signaling (via soluble receptors generated by limited proteolysis) pathways (32). In addition, IL-6 is a potent pro-inflammatory cytokine with additional anti-inflammatory properties mediated by different signaling pathways. The anti-inflammatory actions were mediated via membrane-bound receptors while the pro-inflammatory responses were mediated via soluble IL-6R (32). Interestingly, IL-6 gene polymorphisms were found to generate greater shedding receptors under the action of proteases and a greater soluble form of IL-6R that act as a buffer for systemic effects of IL-6 (33,34). The review published by Martinez et al. depicted the role of the monocyte/macrophage cells in all stages of COVID-19, from pre-infection and early stage of infection when the comorbidities that alter the macrophage/monocyte dictate the disease outcome from the very beginning, until the recovery stage when these cells act as scavenger cells and promote tissue repair (35). The pro-resolving mediators produced under M2-phenotype actions had the main role to limit inflammation and restore homeostasis. Between the two extremities, there are several key points with the commitment of monocytes and macrophages to modulate immune host response and clearance of the virus. Chemokines produced by macrophages during the initial immune response attract more inflammatory cells (polymorphonuclears, monocytes) at the infection site, while IL-6 induces hepatic synthesis of acute-phase reactants (35). In the severe forms of COVID-19, there is a profound dysregulation of immune response with neutrophilia, lymphopenia, a decrease of non-classical/secretory

monocytes (CD14-CD16+) due to their recruiting in the lung (36) and altered production of mediators which were released by macrophages/ monocytes. The hyper-inflammatory syndrome was often accompanied by a hyper-coagulability state driven by procoagulants from macrophages (37). The infection of monocytes and macrophages with SARS-CoV-2 did not induce a cytopathic response, but up-regulated expression of IFNα in monocytes and expression of CD163+ with M2-phenotype switch in macrophages (38). These activities were associated with the induction of immune paralysis (38). Spleen and lymph node macrophages ACE-2+ and CD196+ were found to be infected with SARS-CoV-2, since the nucleocapsid materials were found from immunofluorescent staining (39), thus contributing to the spread of the virus (40).

Natural Killer cells

Natural killer (NK) cells which express both inhibitory and stimulatory receptors on their surfaces are important players in antiviral and antitumoral protection. Interestingly, integration of these opposite signals is crucial to the prevention of detrimental effects due to the self-activation of NK. Through interactions with IL-15/ IL-15R alfa which are expressed on DCs, NK cells would provide a pivotal role in modulating the adaptive immune response (41). NK cells also interacted with other non-specific immune cells like neutrophils or macrophages, or by soluble mediators, i.e., TNFα, to produce IFN-γ and IL-18, important cytokines for NK-Macrophage-DCs interaction (42). In COVID-19 severe patients CD56-CD16+ NK subsets were significantly higher and in asymptomatic patients CXCL2, CXCR4 and IFNγ were upregulated in CD56++CD16- NK cells (43). Aside from generating direct cytotoxic effects on virus-infected cells, NK cells were also involved in antibody-dependent cytotoxicity mediated by anti-S antibody, for instance (44). During the severe forms of SARS-CoV-2 infection, lymphopenia became evident and it was associated with a reduced number of cytotoxic T-cells as well as NK cells. The mechanisms were probably due to organ sequestration (45) and/or cell exhaustion (46). Consequently, NK lymphopenia was frequently associated with prolonged viral shedding and low survival rates (47).

Neutrophils

Neutrophils, cells from the myeloid lineage, are important for the innate immune response to infections. Against bacterial infections, neutrophils perform clearance of pathogens and debris using a variety of mechanisms i.e., phagocytosis, cytokine and chemokine production, oxidative burst, or neutrophil extracellular traps (NETs) formation (48,49). Against viral infections, the mechanisms are less clearly understood. However, they are involved in creating the NETs (50) after diverse TLRs stimulation (51) as an attempt to limit the infection. The NET structure made by activated neutrophils and mediated by reactive oxygen species (ROS) and mitogen-activated protein kinase (MAPK) have the main role of capturing bacteria, fungi, and viruses (51), thus limiting the pathogenic processes. In addition, NETs exert several antipathogenic factors, some of them acting for virus inactivation, like myeloperoxidase (MPO) or defensins (51). However, there is a double-edged sword effect, since the NET formation is a potential generator of proteolytic enzymes or autoantibodies, it may contribute to the development of autoimmune diseases (52). A recent investigation revealed a strong link between SARS-CoV-2 infection and exacerbation of viral-induced NET formation (53). The findings illustrate important contributions of NETosis dysregulation in the innate immune system towards the pathogenesis of severe COVID-19 and hyper-inflammation (49,54).

The overproduction of NETs in regard to virus clearance by hyperactivated neutrophils may

trigger mechanisms with harmful effects, as described in table 1.

The specialized innate immune receptors, pathogen recognition receptors (PPRs) which are found on the innate cells, including neutrophils, have the central role in host-pathogen interaction. After TLRs stimulation, massive production of pro-inflammatory cytokines and chemoattractant mediators occur increasing the influx of the immune cells at the site of infection in graded response to the severity of the infection (49). There are some assertions that neutrophils and other granulocytes act as APCs under different cytokine exposure (especially IFNγ) or via direct interactions with T cells. By enhancing the surface expressing or de novo synthesis of MHC class II, CD80, and CD86 molecules which are involved in antigen presentation (63), the neutrophils are the new arrival member in APCs class, even though their precise activities still need to be discovered. Moreover, these cells seem to have the ability to modulate the adaptive immune response including viral infections (64).

Eosinophils

Aside from their pro-inflammatory role, eosinophils also generate eosinophil extracellular traps (EETs) in reaction to the presence of infections or other inflammatory conditions in the respiratory tract (65). Eosinophils, like neutrophils, express various TLRs e.g TLR6, TLR7-10, with a lower expression on eosinophils except for TLR7 which was found as the main culprit for eosinophils activation after PAMP interactions (66). The up-regulation of TLR7 and TLR8 in eosinophils under the influence of interferon during viral infection could be involved in excessive inflammation (66). However, the eosinopenia that accompanied severe forms of COVID-19 is unlikely to be involved in disease outcome, instead might be a complementary fact (67). A recent review emphasizes the roles of eosinophils as APCs (63) and as virus-recognition cells, through several TLRs, especially TLR7, endosomal pathogen-recognition receptors that recognize RNA viruses, such as coronaviruses (67). A study on fatal cases of COVID-19 reveals that severe eosinopenia was a feature of the fatalities (68). On the other hand, the numbers of eosinophils and lymphocytes were positively correlated regardless of the disease severity (69). Although there was not enough data on the role of eosinophils in the pathogenicity

Table 1. Main harmful effects from over-production of neutrophil extracellular traps (NETs)

Mechanisms	Side effects	References
Procoagulant effects	Clot formation independent of XI, XII, or VII factors; increased clot density; resistance to lysis.	(55)
Prothrombotic effects	Increased plasma levels of nuclear DNA, MPO, and nucleosomes (derived from inflammatory leucocytes); platelet activation and fibrin formation propagation.	(56,57)
Disseminated intravascular coagulation (DIC)	DIC in septic shock patients possible through platelet traps and microvascular occlusions.	(58)
Inflammation	Cytokine production in different tissues regardless of the induction stimuli.	(59,60)
Auto-inflammatory processes	Stimulation of auto-antibody production against MPO, DNase, histone, neutrophil elastase, peptidyl-arginine deiminase type IV (PAD4).	(61,62)

of COVID-19, decreased number of eosinophils was a predictor of disease severity (70,71).

Molecules of the innate immune response

Besides innate immune system cells, there are molecules that also belong to the non-specific immune response. These molecules are important in the initial steps for the control of viral multiplication/replication, for maintenance of antiviral environment and enhancement of disease resolution.

Complement system

Complement activation is an important feature of pathogenesis and disease severity during infection with SARS-CoV-2, although the exact mechanisms of specific antiviral response remain to be explained. It is well known that the complement system together with PRRs are intimately engaged in immune response, especially in severe systemic inflammations. Their involvement was clearly demonstrated in C3-/- deficient mice which expressed severe lung inflammation and respiratory dysfunction compared with that in normal mice for the same viral load (72). Regarding the SARS-CoV-2 infection, over-activation of the complement is driven by N-protein throughout the lectin pathway which was activated by the mannan-binding and lectin-associated serine protease (MASP-2) (73). Like many other molecules involved in humoral immune response, the complement system has a dual role, one beneficial for protection against pathogens and one detrimental by hyper-inflammation. The detrimental effect is mainly promoted by the C3a and C5a fragments which are generated during the normal activation process. These anaphylatoxins activate immune cells having a chemoattractant effect on neutrophils and monocytes at the site of infection, thus aggravating the lesions and prompting the release of cytokines. There is a close link between complement activation and network extracellular traps which were generated during NET-osis (74) since the NET contains several complement factors together with free DNA, myeloperoxidase (MPO), and histones. Initially, the NET-osis acts as an adjuvant in host antipathogen defense. However, an excessive NETs formation drove the hyper-inflammation and hypercoagulability processes (75). There is a close relationship between complement activation and coagulopathy because the membrane attack complex (MAC) which was the final stage of the complement activation was an important factor for coagulation cascade activation via the Tissue Factor-activated pathway or contact activation (76). Excessive complement activation in lung tissues and high levels of serum C5a were observed in severe forms of SARS-CoV 2 infection (73). Uncontrolled complement activation also contributed to the induction of hypercoagulability status, apart from the disseminated intravascular coagulopathy. In these cases, D-dimers could be a reliable tool for patient evaluation and a cutoff of 2.0 μg/mL on admission predicts the in-hospital mortality of COVID-19 patients (77).

Toll-Like Receptors

Toll-Like Receptors (TLRs) belong to a family of membrane and endosomal molecules with receptor activities for sensing pathogens, named Pathogen Recognition receptors (PRRs). Pathogen Associated Molecular Patterns (PAMPs) and Danger Associated Molecular Patterns (DAMPs), mostly cellular debris generated after cell destruction, are some of the molecules recognized by TLR. In humans, there are ten members of TLRs which are expressed on various innate or adaptive immune cells. The main functions of TLRs are to recognize different pathogen patterns: bacterial lipopolysaccharides (LPS), flagella, cilia, bacterial unmethylated DNA, or viral structures like dsARN or ssARN (78). An *in silico* study revealed significant involvement of TLR1, TLR4, and TLR6 in bind-

ing to S-protein of SARS-CoV-2 (79), among them, TLR4 showed the most powerful interaction (78). After TLR7 and TLR8 activation by viral ssARN, several pathways were activated downstream, leading to impaired IFNs synthesis and increased production of pro-inflammatory cytokines and pulmonary lesions (80). Generating DNA network consecutive to NET-osis activation is dependent on TLR4 binding to viral proteins, while activation of TLR 2 and TLR 4 by DAMPs which serve as endogenous antigens like high-mobility group box 1 (HMGB1), induce the synthesis of pro-inflammatory cytokines and chemokines that consequently activate the immune response (81). Another viral sensing receptor important in COVID-19 pathology was shown to be TLR9, the activation and uncontrolled stimulation of which engaged gene transcription with excessive cytokine production and immune cell activation (82). All of them contribute to disease severity and unfavorable outcomes. In a nested case-control study, a more severe variant of COVID-19 was found in males with loss-of-function mutation of TLR7 with a reduction in the IFNs response (83). Sex differences in innate immune response could be explained by higher expression of TLRs in women due to the fact that TLR genes are located on X chromosomes (84).

Interferons (IFNs)

IFNs are a constellation of cytokines that belongs to the same family with slightly different actions, receptors, kinetics, and effects. A recent review indicates the importance of IFNs in both the antiviral defense and the exacerbated pro-inflammatory syndrome during the COVID-19 pathology (85). Type I IFN synthesis can be induced in many nucleated cells after the virus and their PAMPs are recognized by the host pattern recognition receptors (PRRs) or TLRs, especially TLR3, TLR7, and TLR9. These interactions would generate an increased pro-inflammatory response via robust production of IL-6, TNF-α, and IL1β. The proper production of type I IFN was reported to be essential for the antiviral response while impaired IFN I production (both IFN-α and β) was associated with high viral load and excessive inflammatory response, via increased TNF α and IL-6 production and signaling (86). The action of type III IFN was mainly limited to the protection of the epithelial barriers, without a systemic activation of the immune response (85). While the adequate IFN synthesis was important at the very beginning of the infection in order to modulate the host-virus interaction and to reduce the viral load, IFN deficit was followed by severe outcomes of the disease (87), probably due to high viral load and impaired virus clearance (88). Type-I IFN synthesis was significantly reduced in infected macrophages leading to impaired viral control and exacerbated pro-inflammatory response (89). However, viruses normally possess multiple mechanisms to evade immune response: from inadequate recognition by PRRs to inhibition of IFN synthesis or signaling suppression (90). In addition, several structural and non-structural SARS-CoV-2 proteins inhibit IFN synthesis by interacting with different signaling pathways (90). However, adequate modulation of IFNs secretion is a driving factor in the immune response. Therefore, their dampened secretion during the early stages of infection led to an ineffective clearance of the virus, while an excessive and extended IFNs production during a viral infection promoted the hyper-inflammatory syndrome (91), both situations being important contributors to serious disease outcomes. Bastard et al. found neutralizing IgG autoantibodies against type I IFN in 10% of the patients with life-threatening COVID-19 pneumonia (92).

Cytokines

The cytokine storm is a critical characteristic of COVID-19. The expression is due to the over-

production of pro-inflammatory cytokines by the immune cells in response to SARS-CoV-2 infection and is the main pathogenetic mechanism to cause multiorgan failures and severe evolution. While a reasonable production of the inflammatory mediators is required in order to restore immune homeostasis, an exacerbated response would cause serious dysfunctions and severe inflammatory syndrome. Interleukine 6 (IL-6), along with TNFα and IL-1, are considered the major pro-inflammatory cytokines involved in the pathogenesis of COVID-19 (93,94), the synthesis of which is rapidly induced after the virus is recognized by the host's PRRs.

In severe form of COVID-19, the expression of IL-1 along with IL-1R and associated signaling molecules are highly activated (95). Being a pleiotropic cytokine, IL-6 has dual functions. Protective effects are mediated by promoting tissue repair (96), phagocytic neutrophils survival (97), optimal T cell response, and prevention of severe lung damage during viral infections (98,99), while detrimental effects by modulating differentiation of Th2 and Th17 phenotype over Th1 (100), attracting neutrophils and pro-inflammatory macrophages towards sites of infection to aggravate tissue injury (101,102). In an extensive cohort study on dynamic measurement of serum IL-6 levels, the results indicate a correlation between IL-6 and severe evolution of the disease, and a concentration over 37.65 pg/ml was a highly specific predictor for in-hospital death (103). Early measurement of plasma IL-6 could serve as a potential predictor of hypoxemia which would require oxygen therapy (104) or mechanical ventilation (105). During disease recovery, serum IL-6 was found to be lower compared with the disease period with a significantly negative correlation between plasma IL-6 levels and the total number of T cells, CD4+, and CD8+, since IL-6 along with other pro-inflammatory cytokines interfere with cell proliferation and survival (106). Plasma levels of IL-6 and TNFα were increased in severe cases and were independent predictors of disease severity (107). Additionally, the TNF-alfa levels were higher in patients with different comorbidities, like hypertension, diabetes mellitus, or kidney disease. IL-6 and IL-8 but not TNFα were correlated with the necessity for ventilation (107). Apart from TNFα which was correlated with markers of tissue damage, other pro-inflammatory cytokines were in concordance with classical inflammatory markers and oxygen saturation (107).

Acute phase reactants

Under the influence of pro-inflammatory cytokines in COVID-19 some proteins denoted acute elevation of C-Reactive Protein (CRP), serum amyloid A (SAA), ferritin or complement factors (108). In addition, C3 and C4 levels declined as the disease evolve while others denoted a negative evolution as in the case of pre-albumin and albumin, representing the negative arm of acute-phase reactants (108). The induction or suppression of these proteins was under the influence of - cytokines, mainly IL-6 (109). The plasma levels of CRP and ferritin were positively correlated with each other and with D-dimers, a marker of secondary fibrinolysis (110). An early pandemic study revealed a strong correlation between CRP and albumin with virus-induced pulmonary lesions and disease severity (111). Ferritin, a primary marker for iron metabolism, has been well documented to be an important acute phase reactant in COVID-19 patients (112). The plasma levels of ferritin were a strong predictor of disease severity and independently associated with mortality (112,113). Similarly, the SSA is positively associated with disease severity and patient mortality (114).

In order to evaluate and monitor the evolution of infected patients, a panel of laboratory tests has been recommended (115). These tests consisted of biochemical markers to assess the cardiac (Creatine Kinase, Troponin, and NTpro-Brain

Natriuretic Peptide), hepatic (AST/ALT, LDH, bilirubin, Prothrombin Time), and renal function (creatinine, Blood Urea Nitrogen) as well some tests for inflammatory response estimation. Some of these inflammatory markers mirror the innate immune response and have IVD regulations, being used daily for monitoring. CRP and ferritin are valuable markers for the estimation of the inflammatory response, while procalcitonin (PCT) is a good predictor for sepsis during SARS-CoV-2 infection, D-dimers for DIC in septic shock. For IL-6 determination, there are some useful IVD tests for the "cytokine storm syndrome" evaluation and estimation of the utility of anti-IL-6 therapy, while for TNFα or other cytokines the IVD certified tests are limited.

Future directions and conclusions

The innate immune system needs proper functioning, as the first line of defense against infections, to ensure adequate prevention and rapid healing. The innate immune system has a dual function, it is necessary for the activation of antiviral host defense and eradication of the infection, yet hyperactivation during the later stages contributes to hyperinflammation. Thus, an unbalanced response either under- or over-stimulation of this system will lead to uncoordinated response, and unfavorable disease outcomes. A failure in the modulation of the initial innate immune response with hyperactivation and aggressive cytokine release may be one of the explanations for the appearance of severe forms of the disease in young people without comorbidities. The laboratory tools addressing the integrated investigations of innate arm of the immunity, from the neutrophils, lymphocytes subclasses including NK cells, the complement system, IFN response or acute phase reactants, and IL-6 monitoring, to the state-of-the-art gene sequencing will offer a better perspective on mechanisms involved in limiting viral pathogenicity and patient evolution during the SARS-CoV-2 infection. Consequently, therapeutic approaches targeting the very first immune response in COVID-19 or gradually in different compartments of the innate system, in order to maintain the balance of pro- and anti-inflammatory stimuli, are of vital importance.

Author's contribution

AH: study conception, data acquisition, manuscript writing, and editing; AMG: manuscript writing and revision; AVA: data acquisition; WA: manuscript revision; MD: writing, critically revised the manuscript.

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Competing interest

Nothing to declare.

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