COVID-19 and the immune system

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SHORT TITLE: Innate and adaptive immune responses in COVID-19 disease

SUMMARY:

A close interaction between the virus SARS-CoV-2 and the immune system of an individual results in a diverse clinical manifestation of the COVID-19 disease. While adaptive immune responses are essential for SARS-CoV-2 virus clearance, the innate immune cells, such as macrophages, may contribute, in some cases, to the disease progression. Macrophages have shown a significant production of IL-6 suggesting they may contribute to the excessive inflammation in COVID-19 disease. Macrophage Activation Syndrome may further explain the high serum levels of CRP, which are normally lacking in viral infections. In adaptive immune responses, it has been revealed that cytotoxic $CD8^+$ T cells exhibit functional exhaustion patterns, such as the expression of NKG2A, PD-1, and TIM-3. Since SARS-CoV-2 restrains antigen presentation by downregulating MHC class I and II molecules and, therefore, inhibits the T cell-mediated immune responses, humoral immune responses also play a substantial role. Specific IgA response appears to be stronger and more persistent than IgM response. Moreover, IgM and IgG antibodies show similar dynamics in COVID-19 disease.

KEYWORDS: COVID-19; innate immunity; adaptive immunity; T cells; antibodies

INTRODUCTION

Many strategies have been applied in order to gain control over the rapid spread of COVID-19 (Weston *et al.* 2020). This unexpected pandemic, caused by a coronavirus SARS-CoV-2, has affected the population globally and has raised the urge to develop either an anti-COVID-19 vaccine or an anti-COVID-19 therapeutic drug (Prompetchara *et al.* 2020). SARS-CoV-2 is a positive-sense single-stranded RNA (+ssRNA, single linear RNA molecule) virus belonging to the large group of coronaviruses, about 89% identical to bat SARS-like-CoVZXC21 and 82% to human SARS-CoV. It shares major structural and molecular characteristics with other coronaviruses, including the presence of structural proteins S (spike), E (envelope), M (membrane) responsible for the formation and stability of the viral envelope and N (nucleocapsid) interacting with the RNA genome (Chan *et al.* 2020). Interaction of the virus with the host cell (via ACE 2, angiotensin converting enzyme 2) is mediated by the S protein, which has to be processed by another host factor, protease TMPRSS2 (transmembrane protease, serine 2) exposing a fusion peptide, essential step for viral fusion with the host cell. Like other coronaviruses, SARS-CoV-2 also produces non-structural proteins – host immune system and host cell physiology manipulating virulence factors (Chan *et al.* 2020; Zhou P *et al.* 2020). Extensive studies have already revealed the virus origin, the mechanisms of transmission, and the clinical appearance of the infection (Zhu *et al.* 2020). Less is known, however, about the SARS-CoV-2 pathogenesis. It is now clear that there is a close interaction between the virus SARS-CoV-2 and the immune system of an individual resulting in diverse clinical manifestations of the disease (Dong *et al.* 2020). While in some individuals, the COVID-19 disease remains asymptomatic, other individuals present severe complications, such as interstitial pneumonia and a respiratory failure (Dong *et al.* 2020, Rizzo *et al.* 2020). For the development of novel therapeutic protocols, it is necessary to understand the complexity of the virus-immune system interplay. Most of the current knowledge regarding the mechanisms of immune responses in COVID-19 is based on short reports and commentaries. In this review, we discuss the immunological features of COVID-19 with the aim to provide a deeper insight into the disease pathogenesis.

COVID-19 and innate immunity

In spite of the fact that the precise mechanisms of interaction between the innate immune system and SARS-CoV-2 have not been described yet, it is suggestive that the innate immune responses and relevant cell types play a vital role in the clinical symptoms and severity of COVID-19 disease. This assumption is in agreement with the previous studies on the SARS-CoV, the closest relative to SARS-CoV-2, which predominantly infects airway and alveolar epithelial cells, vascular endothelial cells, and macrophages. (Gu *et al.* 2005). It was shown that SARS-CoV triggers various innate recognition and response pathways. To some extent, we can extrapolate the general anti-viral innate mechanisms to SARS-CoV-2, including the fact that "self" vs. "non-self" discrimination is mainly mediated via recognition of the viral nucleic acids as PAMPs by specific pathogen recognition receptors (PRRs) in the cytosol. The hallmark of this concept is in a case of RNA viruses sensing of the double-stranded RNA (dsRNA) as an obligatory intermediate of the viral reproduction cycle. Recognition of the dsRNA is mediated by several receptor systems, particularly important for recognition of the coronavirus RNA is RIG-I like helicase MDA5 synergizing with other host dsRNA PRRs (PKR and OAS) (Zürst *et al.* 2011). Coronaviruses encode multiple proteins that interfere with PRR-mediated viral sensing and subsequent effector viral-controlling mechanisms, most importantly blocking IFN responses or viral RNA recognition via PAMP receptors (Frieman *et al.* 2008). Viral own enzymatic machinery could be involved in this process as coronavirus endoribonuclease (EndoU) targets viral polyuridine sequences to evade activating host sensors (Hackbart *et al.* 2020). Other examples are ribose-2'-O methylation of viral RNA interfering with the recognition by the MDA5 (Zürst *et al.* 2011) or papain-like proteinase with deubiquitination activity attenuating the interferon response (Clementz *et al*. 2010)**.** It was also shown that also structural proteins could be involved in immunomodulation – as M protein inhibits type I Interferon production by impeding the formation of TRAF3, TANK, and TBK1/IKK complex, the same scenario could be expected for SARS-CoV-2 (Siu *et al.* 2009). The SARS-CoV M protein was even shown as unique proteinaceous PAMP promoting type I interferon response via a TLR-related nonclassical TRAF3-independent mechanism (Wang Y *et al.* 2016). Involvement of the Toll-like receptors in sensing SARS-CoV was proven for TLR-3 on a mouse model, as signal-transducing machinery involving adaptor protein TRIF contributed to the protective immune response (Totura *et al*. 2015). It was even shown, that M protein inhibits type I Interferon production by impeding the formation of TRAF3, TANK and TBK1/IKK complex, the same scenario could be expected for SARS-CoV-2 (Siu *et al.* 2009).

Mucosal surfaces, presenting the first line of defense, are protected against the virus via mucosa-associated lymphoid tissues (MALT). Since the SARS-CoV-2 has been described to enter the human body through respiratory tract, oral mucosa and conjunctival epithelium, mucosal IgA presumably protects these physical barriers (Rizzo *et al.* 2020). Okba et al. observed a trend with an increase in the IgA response in severe cases of COVID-19 (Okba *et al.* 2020). Since IgA is considered a major effector molecule to defend the physical barriers against viruses, Padoan et al. aimed to evaluate the role of IgA in COVID-19. It has been revealed, that specific IgA response is detectable in 75% of the patients within the first week and appears to be stronger and more persistent than IgM response (Padoan *et al.* 2020, Rizzo et al. 2020). ACE2 is the main receptor for SARS-Cov-2 and allows the virus entry into the cell. Virus infected epithelial cells produce interferons, which are associated with interferon responsive genes and those allow a robust innate immune response to occur (Jason 2020). Dendritic cells, macrophages, and neutrophils as the first line of defense start the immune reaction and affect its type and intensity. Autopsies on patients who died of COVID-19 revealed a high infiltration of macrophages within the area of bronchopneumonia (Barton *et al.* 2020). Moreover, ACE2 expressing macrophages containing SARS-CoV-2 nucleoprotein antigen were found to highly infiltrate the spleen and the lymph nodes in COVID-19 patients. These macrophages showed a significant production of IL-6 suggesting they may contribute to the excessive inflammation in COVID-19 disease (Park 2020).

The overactivation of the inflammatory immune response can lead to a cytokine storm and subsequent immune exhaustion. The presence of the cytokine storm has been seen in patients with severe clinical manifestation of COVID-19 and was found to correlate with poor therapeutic outcome (Huang *et al.* 2020). Since proinflammatory cytokines play a key role in the disease prognosis, the major involvement of macrophages in the lung damage was discussed (Park 2020). More importantly, Macrophage Activation Syndrome was described as a serious risk factor contributing to lung inflammation. Therefore, it has already been discussed whether strong IL-6 mediated inflammatory response, which is normally responsible for the health regain after the viral infection, could deteriorate the recovery of COVID-19 patients via Macrophage Activation Syndrome (McGonagle *et al.* 2020). In severe cases of COVID-19, the elevation of serum IL-6 has been observed (Zhou F *et al.* 2020). The high production of IL-6 together with the Macrophage Activation Syndrome may explain the high serum levels of CRP, which are normally lacking in viral infections. Similarly, in SARS disease, which represents the closest disease to COVID-19 in humans, high production of IL-6 was also previously described. The intensity of IL-6 production in SARS was even higher than in common viral respiratory diseases (influenza and parainfluenza) (Okabayashi *et al.* 2006). Shakoory et al. suggested a blockade of pro-inflammatory (IL-1, IL-6) cytokines or receptors in patients with overly activated macrophages as a possible therapeutic tool in COVID-19 (Shakoory *et al.* 2016). Since a prolonged inflammation mediated by IFN type II immune response leads to serious damage of tissue, it can be presumed that also in COVID-19 patients, the prolonged inflammation adds to the condition (Opal *et al.* 2005). In contrast, low production of IFN-γ was reported in severe cases of COVID-19 (Chen *et al.* 2020). Additionally, IFN alpha 2b is used as antiviral therapy in severe cases of the disease. (Thevarajan *et al.* 2020) (Yu N *et al.* 2020) (To *et al.* 2020). Nevertheless, the anti-inflammatory effect of type I IFN prevents tissue injury (Smits *et al*. 2010). These findings are consistent with the results of SARS-CoV research and might explain the differences in disease severity according to age groups. As opposed to the cytokine storm, it has been also observed, that several patients do not develop such a rapid response. Moreover, a number of patients remain well and afebrile while carrying the virus. These asymptomatic carriers were already documented in a retrospective study in the minor 2004 SARS outbreak and there is a growing evidence that also in COVID-19, asymptomatic carriers are highly prevalent (Yu X *et al.* 2020). More importantly, asymptomatic COVID-19 carriers were proven to be infectious, while the transmission of SARS only occurs during the symptomatic period (Gao *et al.* 2020). Asymptomatic carriers pose a significant challenge in the public health prevention. It is presumed, that in these asymptomatic patients, the adaptive immune responses preclude disease progression to severe stages. Therefore, it is particularly relevant to implement at these asymptomatic or early stages of disease boosting strategies for the immune responses (anti-sera or pegylated IFNα) (Shi *et al.* 2020). Authors Shi et al. describe two phases of immune responses during the COVID-19 infection. The first phase is represented by an immune defense-based protective phase, while the second phase if characterized by a broad inflammation. Therefore, ehnancing the immunity in the first phase and suppressing the immunity in the second phase may be the crucial approach for COVID-19 therapeutic management.

Even though macrophages might play a crucial role in COVID-19 pathogenesis, other innate immune cells are also involved. Elevated numbers of monocytes were observed in impaired blood-vessels (Yao *et al.* 2020). In severe cases of COVID-19 disease, an increased number of neutrophils was detected (Liu J *et al.* 2020). Furthermore, high proportions of neutrophils and the neutrophil-to-lymphocyte ratio have been associated to bad prognosis of the disease (Liu J *et al.* 2020). SARS-CoV-2 has mainly been detected in the lung tissue isolates. However, in other tissues, signs of severe damage were also reported (Yao *et al.* 2020). These findings suggest that the inflammatory response could be even more destructive than the direct activity of the virus (Yao *et al*. 2020).

Since the maturation and differentiation of the innate immune cells, including neutrophils, macrophages, natural killer cells, and dendritic cells, is modulated by estrogen and testosterone hormones, a question could be raised, whether the sex differences in the clinical manifestation of COVID-19 disease might be associated with the hormonal dependency of the innate immune responses (Jaillon *et al.* 2019). However, the clinical manifestation surely depends on multiple factors, such as genetic background (HLA, gene polymorphisms – such as for ACE2) and the individual variability in environmental/personal risk factors (age, smoking, diet, physical activity, vaccination scheme, contact history with other coronaviruses).

Another general issue causally linked to the higher winter incidence of the respiratory disease (potentially including COVID-19) relevant to innate immunity is vitamin supplementation and availability. Namely, vitamin D could be the key factor with its multiple immunoregulatory functions in the combination with sun exposure (Grant *et al.* 2020).

COVID-19 mortality and severity is not only gender, but also age-biased. It has been shown that SARS-CoV-infected old macaques had a stronger host response to virus infection than young adult macaques. They expressed higher levels of proinflammatory cytokines, whereas expression of IFNs type I was reduced (Smits *et al.* 2010). In contrast to the elevation of macrophages, a significant decrease of NK cells in severe cases of COVID-19 was detected (Zhang *et al.* 2020). A significant increase of NKG2A expression in COVID-19 patients was also observed. Upregulation of NKG2A was associated with the exhaustion of cytotoxic T cells and NK cells at the early stage of SARS-CoV-2 infection, and therefore, was associated to severe disease progression (Zheng *et al.* 2020). So far, the results suggest that in severe cases of COVID-19 myeloid cell lineages, especially macrophages, play the prominent role in the disease progression through their overactivation, whereas NK cell activity is reduced.

COVID-19 and adaptive immunity

Data regarding the adaptive immune responses in COVID-19 are limited. Both cellular and humoral responses were identified and further investigated in COVID-19 (Prompetchara *et al.* 2020). It is presumed that COVID-19 induces a similar Th1 type immune response as other viral infections (Russel *et al.* 2020). The count of $CD8^+$ T cells was reported to be decreased during COVID-19 infection, and, in severe cases, memory $CD4^+$ T cell and T regulatory cell count was significantly reduced (Zhang *et al.* 2020). These findings were accompanied by a decreased number of $CD4^+$ and $CD8^+$ T cells in lymph nodes. Lymph nodes and spleen in COVID-19 patients were described as atrophic, which highlights the role of SARS-CoV2 in potentiating cell degeneration (Zhang *et al.* 2020). In relation to $CD8^+$ T cells, it has been revealed that these cells exhibit functional exhaustion patterns, such as the expression of NKG2A, PD-1, and TIM-3 (Zheng *et al.* 2020, Moon 2020). The expression of NKG2A was, however, decreased in patients who recovered after antiviral therapy (Zheng *et al.* 2020). Therefore, it is reasonable to assume that T cells are able to restore their functional activities after antiviral therapy. Similar observations regarding the expression of NKG2A were also seen in NK cells (Zheng *et al.* 2020). In mild stages and/or in patients presenting with mild symptoms solely, the lymphocyte count was found to be significantly higher as compared to patients with severe disease. This also applied to both T cell ($CD3^+$ cells) and $CD8^+$ T cell ($CD3^+/CD8^+$ cells) populations (Cao *et al.* 2020). In both mild and severe cases of COVID-19, the $CD8^+$ T cell counts were decreased as compared to healthy donors. Moreover, $CD8^+$ T cells presented in COVID-19 patients were found to less degranulate (decreased CD107a externalization) and to produce lower levels of IL-2, IFN γ, and granzyme B as compared to healthy donors (Zheng *et al.* 2020). In peripheral blood T cells isolated from patients in intensive care units (ICUs), the expression of PD-1 was significantly higher as compared to T cells isolated from patients with mild disease or from healthy donors (Moon 2020). Taken together, these findings highlight the strong immunosuppressive abilities of SARS-CoV-2 of the adaptive immune responses.

Since the most common clinical symptom of COVID-19 remains fever, the involvement of pro-inflammatory cytokines is evident. Increased serum levels of IL-6 were observed in more than 50% of the patients (Prompetchara *et al.* 2020). Studies further revealed that as the disease severity progresses, the serum levels of pro-inflammatory cytokines increase as well (Shi *et al.* 2020). This rise of pro-inflammatory cytokines is also associated with the depletion and functional exhaustion of T cells. Specifically, with the upregulation of PD-1 phenotypic pattern (Moon 2020). Although the rice of the cytokines may call for appropriate interventions, such as anti-IL-6 treatment, these interventions need to be considered based on the severity of the disease. Shi el al. has recently shown that different immune responses are associated with mild and severe stages of COVID-19 (Shi *et al.* 2020). These findings led to conclusions that stimulating the immunity in the non-severe (mild) stages of the disease can be beneficial. In contrast, however, once a severe impairment of lung functions had already occurred, further damage is potentiated by the immune system, and, therefore, immunosuppression is required instead (Shi *et al.* 2020).

SARS-CoV-2, similarly to other coronaviruses, restrains antigen presentation by downregulating MHC class I and II molecules, which inhibits the T cell-mediated immune responses (Zheng *et al.* 2020). Nevertheless, humoral immune responses also play a substantial role in COVID-19 infections, even though antibodies may not be sufficient to neutralize the virus (Guo *et al.* 2020). The most concerning evidence regarding the development of antibodies is that each patient has completely different kinetics of humoral responses (Guo *et al.* 2020). Most of the patients develop antibodies after 7 days since the disease onset. During the first seven days of the disease, the detectability of the virus-specific antibodies in COVID-19 patients was less than 40% and, therefore, the use of serology testing is of limited value (Zhao *et al.* 2020). It has been demonstrated, that SARS-CoV2 plasma has a cross-reactivity to SARS-CoV, but does not show cross-reactivity to other coronaviruses (Guo *et al.* 2020). In the early phase of COVID-19 infection, RT-qPCR should be the dominant diagnostic tool (Zhao et al. 2020). It has also been shown that 22% of patients with RT-qPCR-confirmed positivity were IgM negative (Guo *et al*. 2020). Since the day 15 after the disease onset, IgM and IgG antibodies were detected in 94,3% and 79,8% of the patients, respectively (Zhao *et al.* 2020). In some patients, the detection of IgM antibodies was observed at the same time as the detection of IgG (Zhao *et al.* 2020). Also, the IgM and IgG antibodies showed similar dynamics in selected patients (Zhao *et al.* 2020). The duration of IgG antibodies still remains unknown. The only estimation could be done from the immunology memory studies performed on SARS-CoV, where SARS-specific antibodies were maintained for an average of 2 years (Wu *et al.* 2020)). The titer of the virus-specific antibodies was correlated with the disease severity, and it has been shown that a high titer of SARS-CoV2 antibodies serves as an independent risk factor for critical manifestation of COVID-19 (Cao 2020). It has been shown that COVID-19 patients generate SARS-CoV-2-specific neutralizing antibodies. Neutralizing antibodies are produced by B cells after infection with the virus and can block the virus from entering the host cells. Therefore, these antibodies play a critical role in the virus clearance. In a retrospective study by Liu et al., neutralizing antibodies were detectable in SARS patients throughout 2 years follow-up. Moreover, the correlation of neutralizing antibodies titers with age, lymphocyte counts, and blood CRP levels implied there is an active interplay between virus and host immune response (Liu W et al. 2006). Several reports indicated that demonstrated that SARS-CoV-specific monoclonal neutralizing antibodies could cross-neutralize SARS-CoV-2 infection (Wang C *et al.* 2020). Moreover, a convalescent plasma containing neutralizing antibodies has been tested in the passive antibody treatment of COVID-

19 (Rajendran *et al.* 2020). Neutralizing antibodies might, therefore, present a key immune product for vaccine development.

Vaccine development

An important question that raises with the pandemic is whether a vaccination strategy could be a helpful treatment to prevent the disease or at least to suppress its detrimental or even deadly clinical manifestations. Unlike the common cold coronaviruses to which the human population has mostly been exposed, the SARS-CoV-2 is new to the human immune system. As discussed above, neutralizing antibodies may contribute to vaccine development and have already been considered an effective prevention of the virus infection. The level of neutralizing antibodies has been used previously to evaluate the efficacy of vaccines against smallpox, polio, and influenza viruses (Zinkernagel *et al.* 2003). Therefore, neutralizing anti-SARS-CoV-2 antibodies may serve not only as passive antibody therapy but also as a marker of vaccine efficacy. Regarding the cellular immune responses, a recent study has already shown that SARS-CoV-2-unexposed individuals were able to *in vitro* develop a $CD4^+$ T cell response to SARS-CoV-2-derived peptides only in ~50% of cases, and a $CD8^+$ T cell response was noted even only in ~20% of cases (Grifoni *et al.* 2020). In the COVID-19 convalescent patients, the response rate was then much higher: ~100% for $CD4^+$ and ~70% for $CD8^+$ T cells (Grifoni *et al.* 2020). These data showed that a great portion of the SARS-CoV-2-unexposed human population might fail to mobilize the adaptive immune responses after the virus contraction. Whether this failure is then the cause of the overwhelming and detrimental innate immune responses upon the disease onset is not known. However, the data demonstrated that a vaccination strategy could substantially improve the adaptive immune system's response rate. This may not necessarily ensure that vaccination would provide wide and robust protection against the COVID-19. However, the vaccination will certainly help to mobilize the SARS-CoV-2-specific adaptive immune responses, which might at least provide increased protection against the development of the severe and often deadly forms of COVID-19.

Conclusion

The current knowledge about COVID-19 indicates that the immune system plays a crucial role in setting the severity of the disease. SARS-CoV-2 virus efficiently infects the cells in the lower respiratory system, and this induces a fast local immune response, which damages this vital and fragile part of the body. To prevent progression of the severe forms of the disease, the immune system needs to be targeted and modulated alongside the therapeutic interventions that aim at the virus.

Abbreviations:

ACE2 – angiotensin converting enzyme 2, CD – cluster of differentiation, CD4 T cells – helper T cells, CD8 T cells – cytotoxic T cells, COVID-19 - coronavirus disease 2019, CRP – C reactive protein, DAMP – damage associate molecular pattern, HLA – human leukocyte antigen, IFN – interferon, Ig – immunoglobulin, IL – interleukin, MALT – mucosae associated lymphoid tissue, MHC – major histocompatibility complex, NK cells –natural killer cells, NKG2A - CD94/NK group 2 member A, PAMP – pathogen associated molecular pattern, PD-1 – programmed death 1, PRR- pattern recognition receptor, RNA – ribonucleic acid, RT-qPCR – real-time quantitative polymerase chain reaction, SARS-CoV-2 - Severe acute respiratory syndrome coronavirus 2,TANK - TRAF-associated NF-κB activator, TBK1/IKK - TANK-binding kinase 1 and I-κB Kinase, Th1 – T helper 1 cells, TIM-3 - T-cell immunoglobulin and mucin-domain containing-3, TRAF3 - TNF receptor-associated factor

Conflict of Interest:

The authors Jan Paces, Zuzana Strizova, Daniel Smrz and Jan Cerny declare no conflict of interest regarding the publication of this article.

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