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COVID-19: Unanswered questions on immune response and pathogenesis

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The novel coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) revealed an unprecedented threat for humanity. As for other novel pathogens we are virtually disarmed against SARS-CoV-2, while an efficient protection will be possible only after the development of an effective vaccine. In this case, the viral load and the quality of immune response determine different outcomes ranging from damages to the host. Here, we underscore some of the main unsolved problems, mostly focusing on asymptomatic subjects and differences in severity of the disease by age and sex.

Key words: Anti–SARS-CoV-2 immunity, COVID-19 pathogenesis, viral immune evasion

The recent spread of the coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) revealed an unprecedented threat for humanity. As for other novel pathogens we are virtually disarmed against SARS-CoV-2, while an efficient protection will be possible only after the development of an effective vaccine. In these days, while the virus is rapidly spreading through the world, many urgent questions arise on how to combat its deleterious effects in infected patients. This implies to be able to answer many questions on the virus itself, on the pathogenesis of infection, on the host immune response, and to identify therapeutic tools to control virus entry into the cells, its replication and spread, and its lethal effects.

Here, we underscore some of the main unsolved problems, mostly focusing on pathogenetic aspects and host immune response to virus. On this basis, we will also touch important aspects regarding asymptomatic subjects and differences in severity of the disease by age and sex.

Immune responses against viruses are rather heterogeneous. An efficient intervention of innate immunity, with its cellular and soluble components, is fundamental to combat the early phases of a primary infection by cytopathic viruses. In the case of influenza virus, such early phases, characterized by the intervention of type I interferon (IFN-1), natural killer (NK) cells, and other cells of the innate immunity, are followed by the generation of virus-specific CD8⁺ cytolytic T lymphocytes, which may control viremia after 3 to 4 days. The first, low-affinity, IgM-specific antibodies appear after 6 to 7 days, whereas the high-affinity IgG and IgA antibodies require 3 to 4 weeks. This time interval implies that antibodies do not contribute to the recovery from a primary influenza infection, although they protect from secondary infections and can be used for seroprophylaxis. The situation is quite different in noncytopathic virus infections such as hepatitis B virus, in which the immune response itself may cause major damages to the host. In this case, the viral load and the quality of immune response determine different outcomes ranging from complete recovery to fulminant hepatitis. Influenza virus and hepatitis B virus infections are 2 paradigmatic and opposite conditions. However, an array of immune response-virus interactions and consequent pathologies exist. Unraveling such interactions occurring in COVID-19 will be a crucial challenge to better address more effective therapeutic strategies.

First, we do not have information on the innate responses to SARS-CoV-2. For example, the reported lymphopenia may suggest a deficiency in NK cells (which provide an important...
cellular barrier during early viral infections) in patients with severe disease. Both phenotypic and functional studies on NK-cell subsets should be performed in these patients in comparison with paucisymptomatic or asymptomatic ones. It may well be that in the latter individuals, an efficient innate response may control the virus and/or allow sufficient time to mount an efficient T-cell response, resulting in absent or mild symptoms. Detection of high numbers of CD8+ T cells in peripheral blood and evidence of their specificity for viral peptides would provide a clue for an ongoing effective, cell-mediated immune response. Of note, in the case of COVID-19, given the relatively long interval between viral infection and the onset of symptoms, this T-cell–mediated adaptive immune response, crucial in the control of infections by influenza virus, could escape detection.

Importantly, we also lack clear information on the production of specific and neutralizing antibodies (NAbs), crucial for the establishment of a protective immunity. Antibodies specific for the so-called receptor-binding domain (RBD) on S protein are considered the main targets of NAbs, which is a prerequisite for protection against respiratory coronaviruses (rCoVs). Cocktails of antibodies specific for RBD and other regions of the S protein may further improve the effect of NAbs against SARS-CoV-2 and, eventually, its mutant strains. However, it is not yet clear whether human hyperimmune globulins from recovered patients may allow a successful seroprophylaxis. Of note, in patients with SARS, some NAbs targeting non-RBD regions of the S protein caused an antibody-dependent enhancement effect on viral infectivity and disease. A very recent study on 26 patients who recovered from COVID-19 revealed that although most developed antibodies specific for S protein, only in 3 cases they were specific for the RBD domain. Although these data may pose serious questions regarding the establishment of a protective immunity, they may offer an important clue for selecting appropriate epitopes in the development of effective vaccines. Because S protein or mRNA are being used in novel vaccine preparations, the previous finding poses serious problems on the efficacy of response of induced antibodies and imposes to establish the protection degree given by memory T cells. Another hurdle is expected in the use of effective adjuvants, particularly because elderly people are frequently highly susceptible to the virus and poor responders to vaccinations.

Table 1 summarizes the main aspects of immunity toward the 3 rCoVs. Thus, at present, immunologic studies suffer from important limitations related to (1) lack of information on asymptomatic individuals who could mount highly effective cellular and/or humoral immune responses, (2) scarce knowledge of the changes in peripheral blood lymphocyte numbers and composition and function in individuals with progressive disease, and (3) no data on the regulatory mechanisms (cells and cytokines) during infection, impairing the clearance of the virus and favoring its immune evasion.

Further important information on immune mechanisms capable of controlling COVID-19 may come from females and pediatric patients. Indeed, in most instances, children develop a mild form of the disease (if any). Different explanations could be proposed. Thus, children, in view of the high plasticity of their adaptive responses, particularly in their B-cell compartment, could more efficiently clear the virus. To this favorable situation may contribute also innate immunity, challenged/trained by frequent infections and by vaccines/adjuvants administered in early life. In addition, the lower susceptibility of children to SARS-CoV-2 could be due to the lower density of angiotensin-converting enzyme 2 (ACE2) receptors as compared with adults.

The lower frequency of infection and mortality in women could be related to hormonal and genetic differences, to lower incidence of comorbidities affecting the lung (chronic obstructive pulmonary disease, smoke, etc.), or to higher prevalence of autoimmune and allergic disorders, as the result of some decreased regulatory mechanism. In comparison with men, women usually show (1) less viral load levels and less inflammation with higher CD4+ T cells and antibody responses; (2) lower expression of serine protease TMPRSS2 (the S protein priming) in tissues and ACE2 in the lung; (3) overexpressed TLR8, CD40L, and CXCR3 (encoded by X chromosome), all influencing antiviral response; and (4) overexpressed TLR7 (the crucial sensor for RNA viruses such as SARS-CoV-2), which, when triggered by the virus, leads to higher IFN-1 and lower IL-6 production.

Indeed, in the near future, the relationships between the severe COVID-19 infection and the immuno-mediated diseases shall be extensively evaluated. As far as allergy is concerned, some reports of Wuhan hospitalized case series indicate that this group of diseases, in particular asthma, does not represent a risk factor for SARS-CoV-2 morbidity and mortality. The few suitable data, however, do not allow to establish whether these disorders may constitute a protective factor for the infection or the related mechanisms. Among them we could hypothesize that (1) the atopic status itself associated with the impairment of some regulatory mechanism may favor antiviral immunity, (2) the higher proportion of activated eosinophils in allergy may play some protective role, while these cells are usually strongly reduced in COVID-19, and (3) the topical administration of antiallergic therapies may directly protect the patients’ airways from viral spread, taking into account that some coronavirus strains are inhibited in vitro by combinations of nebulized asthma therapeuticals. Finally, very recently it has been shown that epithelial cells of respiratory mucosa from patients with allergy express less ACE2 molecules than healthy donors and that IL-13, a crucial molecule of type 2 response, is negatively related to the ACE2 expression.

Other important missing information regards the mechanisms by which the virus may escape the immune response. Of note, data on rCoVs, including SARS-CoV-2, indicate that these pathogens are particularly prone to evade immune detection and dampen human immune responses. Taking into account that susceptible HLA aplotypes, high viral load, and previously impaired immunity may contribute to the virus escape of immune response, based on the knowledge of other human rCoVs, some other not-mutually exclusive mechanisms of immune evasion can be hypothesized for SARS-CoV-2 (Fig 1). The first mechanism relies on early inhibition of IFN-1 recognition and signaling by infected cells. In rCoVs, IFN-1 is suppressed through different mechanisms directly or indirectly interfering with the signaling of RNA receptors. Present limitations concern whether and how much the reduced IFN-1 production may compromise the viral control, leading to severe consequences to infected host. Data of the timing of IFN-1 response could be informative also for therapy: some intensive care (IC) units in Italy included inhaled IFN-1ß in therapeutic protocols.

Related to the previous mechanism is the possible early functional inhibition/alteration of cells of the innate immunity
TABLE I. Immune responses toward rCoVs

**Cellular immune responses**

SARS-CoV³
- Impaired circulating NK cells and T-cell subsets in mild and severe patients
- Relatively higher frequency of CD8⁺ than CD4⁺ T cells in recovered patients
- Highly activated CD4⁺ and CD8⁺ T cells with predominant type 1 or type 3 profiles in severe patients
- High type 2 cytokines present in sera of patients with severe diseases
- Strong memory T-cell responses correlating with high NAb serum levels
- T cells specific for structural proteins (S, E, M, N epitopes) and memory CD8⁺ T cells detectable over 10 y from infection

MERS-CoV³
- Early onset of CD8⁺ T cells correlating with disease severity
- Predominance of memory CD4⁺ T cells with Th1 or Th17 profiles in survived patients
- Higher T-cell response in survived patients than in fatal cases

SARS-CoV-2
- Time of onset, phenotype, repertoire, functional profile, and amplitude of T-cell response still unknown
- Reduction of circulating NK cells and T-cell subsets in relation to severity of disease⁴
- Few data on the recruitment of NK cells and T-cell subsets and their functions (scRNAseq) in the bronchoalveolar lavage fluid of patients with pneumonia⁵

**Humoral immune responses**

SARS-CoV³
- Seroconversion few days after the disease onset and specific IgG detectable in most patients by 14 d
- Long-lasting specific IgG and NAbs reported 2 y after infection
- NAbs specific for S, N, M epitopes, including the RBD domain
- Delayed or weak antibody responses associated with severe outcome

MERS-CoV³
- Seroconversion within 2-3 wk from disease onset still detectable until 13 mo after infection.
- Delayed or weak antibody responses associated with severe outcome

SARS-CoV-2
- IgM antibodies detectable 7-10 d after disease onset and serocoversion developed in most patients recovered⁶
- Unfrequent antibody specificity for the RBD domain of S protein²

MERS-CoV, Middle East respiratory syndrome coronavirus.

**FIG 1.** Possible mechanisms of immune evasion of SARS-CoV-2. Immune evasion of SARS-CoV-2 may be favored in individuals with compromised ability to mount efficient immune responses such as old people and patients with immunodeficiency or individuals carrying HLA alleles unable to properly present SARS-CoV-2 peptides to T lymphocytes. In addition, a high viral load may overcome the barriers of the immune responses. Notably, viruses escaping control may inhibit IFN-1 and infect cells of both innate and adaptive immunity by exerting a cytopathic effect. In turn, the compromised function of immune cells and the impaired antiviral effect of IFN-1 would further favor immune evasion, resulting in highly detrimental pathological effects. DC, Dendritic cell.
such as macrophages, dendritic cells, and NK cells. Thus, beside a possible cytopathic effect of the virus, viral TLR ligands could directly or indirectly induce an unwanted polarization of these cells toward inefficient type 2 responses. This would have deleterious consequences not only on the antivirus activity of the innate cells themselves (ie, sharply compromised NK-cell cytotoxicity and production of useful cytokines, M2 polarization of macrophages, etc) but also on downstream adaptive responses. These could reflect an impaired NK-cell–mediated dendritic-cell editing, the activity of M2 macrophages, and so forth.19,20 As a consequence neither T_H1- nor Tc1-mediated efficient antivirus responses could be elicited. Concerning the cytopathic activity of the virus, lymphopenia has been described in more than 80% of IC patients and correlates with disease severity. The few data from autopsies indicated that lung infiltrates consist of activated macrophages with minimal lymphocytic component associated with lymphocyte depletion in spleen.21 It has been shown that SARS-CoV and Middle East respiratory syndrome coronavirus directly infect T cells, contributing to lymphopenia and atrophy of lymphoid tissues, thus representing a key component in the viral-induced pathogenesis. It is urgent to confirm and expand these data and to acquire solid information on cytopathic activity of the virus on cell subsets.

Another mechanism concerns the adaptive immune response to the virus: antigen presentation via MHC class I/II may be compromised by infected antigen presenting cells, leading to impaired T-cell response. An unanswered question concerns the rate of viral mutations and its possible superantigenic components, leading to chronic stimulation with exhaustion of T-cell response. In addition, the hyperproduction of cytokines by monocytes/macrophages may favor T-cell suppression or deviation to less protective cell profiles (ie, T_H2).23 The detection of circulating effector and regulatory memory T cells or adaptive cytokines during the early phases of infection when lymphopenia is still mild could be informative for prognosis.4,24 Notably, the identification of conserved immunodominant T-cell epitopes shall have implications for vaccine design.25,26

One or more of the immune evasion mechanisms with other factors such as, for instance, the different viral load or the preexisting impaired immune response can likely contribute to the immunopathogenesis of COVID-19 (Fig 2). Of note, when the infected organism is unable to mount an adequate immune response to the virus, as a compensatory mechanism, a persistent monocyte/macrophage-induced hyperinflammation predominates. Among other sensors, viral RNAs are essentially recognized by TLRs (as TLR7/8, TLR3), activating 3 intracellular pathways leading to the IFN-1 production (through MyD88 and IRF7), expression of costimulatory molecules for T-cell activation (through TRAF6 and IRF3), and production of proinflammatory molecules (through p38MAPK and AP-1). It has been hypothesized that the inhibition by rCoVs of the first 2 pathways at different levels may favor the hyperactivation of p38MAPK in infected monocytes/macrophages, leading to hyperproduction of cytokines, chemokines, and acute-phase proteins.27 Indeed most IC patients with COVID-19 show high levels of cytokines (IL-1β, IL-6, TNF-α) and chemokines (CCL2, CCL3, CCL5, CXCL8, CXCL10), indicating overexpression of a proinflammatory condition (the so-called macrophage activation syndrome) in...
disease progression and explaining the massive homing of immune cells into tissues. Indeed a proportion of severe patients develops a hypersensitivity-like pneumonia and a subsequent acute respiratory distress syndrome sustained by a cytokine release syndrome. Others can develop a secondary hemophagocytic lymphohistiocytosis, a hyperinflammatory condition characterized by a fulminant and fatal hypercytokinemia with multiorgan failure, just reported in some cases of COVID-19. Finally, some patients can develop a disseminated intravascular coagulation due to the cytokine-driven epithelial and endothelial cell damage/apoptosis with increased vascular leakage, hyperexpression of tissue factors, and chronic triggering of coagulation. Acute respiratory distress syndrome, cytokine release syndrome, secondary hemophagocytic lymphohistiocytosis, and disseminated intravascular coagulation constitute the final pathological pictures of COVID-19, each being able to set off the multiorgan failure and death (Fig 2). Importantly, comorbidities and concomitant therapies (ACE inhibitors?) can influence/enhance the above cascade of events.

Therefore, during infection it is crucial to monitor the cytokines responsible for hyperinflammation and to block their activity by using approved therapies addressed to reduce the rising mortality (ie, tocilizumab [anti–IL-6R-], anakinra [IL-1RA-], TNF-α blockers, JAK inhibitors). Notably, clinical trials with tocilizumab and anakinra have been recently approved in different countries. In addition, the autopic reliefs indicate the presence of widespread microthrombi with widening of the downstream vessels: for this reason, the use of low-molecular-weight heparin has been approved in severe COVID-19 by Drug Italian Agency.

In conclusion, we urgently need to acquire basic information on how the immune system recognizes SARS-CoV-2 and how it may contribute to the severity of the disease process. Understanding the immunopathogenesis of COVID-19 may provide important clues for effective treatments of this disease.

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