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IMMUNITY FROM SARS-COV- 2

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Understanding the immune response to SAR-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus) is essential for understanding the pathogenesis of disease and the utility of therapies such as hyperimmune globulin and human plasma convalescent, and for the development of antiviral vaccines and monoclonal antibodies. The efficient attenuation of SAR-CoV-2 transmission is estimated to start once the collective immunization at 70% of the population. There is no pre-existing immunity to SARS-CoV-2 in the population, except for cross-reactivity (common viral antigens) with other coronaviruses. It is not yet known whether pre-existing immunity to normal human seasonal corraviruses could provide some degree of cross-protection. Clearly, an effective and safe vaccine for COVID-19 would be an ideal way to achieve immunity of the population. Understanding the kinetics, durability and degree of protection provided by vaccine-induced antibodies will be crucial. The immunological corelations of the SAR-CoV-2 protection are unknown and the roles of specific antibodies and T-cells in the elimination of infection have not yet been definitively identified in humans.

Key words: SAR-CoV-2, antibodies, T-cells, COVID-19.

INTRODUCTION

Over the past 18 years, three new coronaviruses have crossed the barrier of the species, infecting humans and causing human-to-human transmission. In addition, four seasonal human coronaviruses (229E, NL63, OC43 and HKU1) have been identified as causing up to one third of the higher respiratory infections acquired by the community Although most human coronaviruses are beta-coronaviruses, two of the seasonal viruses (*i.e.* 229E and NL63) are alpha-coraviruses, which shows that both viral subgroups are important human pathogens[1]. In December 2019, a new coronavirus causing severe acute respiratory syndrome (SARS) appeared in Wuhan, China and was called SARS-CoV-2 and has since caused a pandemic affecting virtually all countries.

Coronaviruses form a family within the *Nidovirales* order and are a single-stranded positive-sense large RNA virus (27–32 kb), gene coding for 16 non-structural proteins and 4 major structural proteins: membrane (M) protein, the envelope (E) protein, the spike (S) glycoprotein and nucleocapside protein N. The ARN genome and the N protein form a helical symmetric nucleocapside surrounded by the external shell. Protein S is composed of two functional subunits: S1 and S2. The S1 Subunit consists of the N-terminal domain (NTD) and the receptor binding domain (RBD). The S2 subunit contains fusion peptide (FP), regions: Heptad repeat 1 (HR1), central helix (CH), connector domain (CD), heptad repeat 2 (HR2), transmembrane domain (TM) and cytoplasmic domain (CT) and is intended to merge the viral membranes with host cells[1].

Nucleocapsidic protein N forms complexes with viral RNA, and is involved in the transcription and replication of viral RNA, the assembly of the encapsulated genome in virions, and interferes with cell cycle processes of host cells. Moreover, in many coronaviruses, including SARS-CoV-2, protein N has high immunogenic activity and is expressed abundantly during infection[1].

In SARS-CoV-2 infection the organism shall determine a complex immune response against the virus, including the production of specific antibodies against viral antigens, with spike (S) and nucleocapsidic (N) protein being the main

immunogenic[1]. The virus enters the host cell by linking the S-protein to the receptor of the Angiotensin 2 (ACE2) conversion enzyme, which is present on the surface of many cells, including alveolar cell type 2 in the lungs and the epithelial cells of the oral mucosa, *i.e.* the ACE2 connects the subunit S1 of the virus, by receptor binding domain (RBD).

For reasons that are not fully clarified, immunity to seasonal human coronaviruses tends to be short-lived, between 80 days and several years. Reinfection was also demonstrated in patients with SARS-CoV-2. It remains unclear whether such reinfection is due to low protection from the same strain or different strains of the same virus or both[2]. For patients infected with SARS-CoV-1 or MERS (the coronavirus responsible for Middle East respiratory Syndrome), the detection of humoral markers of immunity was possible for 2–3 years, but these markers were absent when patients were retested 5–6 years later[2]. Understanding the mechanisms for installing short-term immunity after a viral infection is important as these processes could have considerable implications for the protection and duration of immunity induced by vaccines. As the number of patients infected with SARS-CoV-2 continues to increase, it becomes even more important to identify, assess and understand the immune response to the SARS-CoV-2 infection.

There is little data on post-infection immunity with SAR-CoV-2 and biological and genetic factors responsable for the broad spectrum of severity of the disease remain unclear. Data suggest that the action of neutralizing antibodies and the responses of CD4+ and CD8+ T cells could be associated with the severity of COVID-19, age being a risk factor [3].

HUMAN IMMUNITY TO SARS-COV-2

Humorale immune responses to SAR-CoV-2 are mediated by antibodies that are specific to viral surface glycoprotein, mainly glycoprotein Spike and nucleocapside protein. The 180 kDa spike glycoprotein contains two subunits (ie, N-terminal S1 and C-terminal S2) and is considered an important antigenic determinant capable of inducing a protective immune response[1]. Subunit S1 contains a receptor binding domain (RBD), which mediates the viral binding to functional ACE2 receptors on sensitive cells and is the primary target for SARS-CoV-2 neutralizing antibodies.

SARS-CoV-2 specific functional neutralizing antibodies which are produced as a result of infection, vaccination or both are considered important for viral neutralization and viral clearance and are quantified by using in vitro neutralizing antibody test. For these reasons, the antibody titer could be a good biomarker for the effectiveness of antibody protection and the immune response after exposure to SARS-CoV-2. The presence of SARS-CoV-2 IgG, IgM and IGA antibodies in patients with COVID-19 has also been reported, and these responses correlate well with the titer of nucleapside antibodies[4]. The majority of COVID-19 patients or convalescent patients have virus-specific IgM, IgA and IgG responses on days after infection, suggesting that the antibodies mediate immunity to SARS-CoV-2 [5].

The general kinetics of the SARS-CoV-2 antibody response is analogueous to that for SARS-CoV-1, and is characterized by robust seroconversion (IgM and IgG) 7–14 days after the onset of symptoms and the antibody concentration persists from weeks to months after infection[6]. A study assessing kinetics of anti-glycoprotein Spike antibodies in patients with COVID-19 found that IgA antibodies are produced early (in the first week) and reached a maximum concentration of 20–22 days, while IgM antibodies have reached a strong titer at 10–12 days and subsequently dropped to 18 days after the onset of symptoms[7]. A seroprevalence study that examined IgG response to the spike glycoprotein after the onset of symptoms in 40 patients with COVID-19, reported that the IgG titer increased in the first 3 weeks and started decreasing from the 8th week[8].

In pacient with the mild form of COVID-19, a rapid decrease in the titer of RBD-specific IgG antibodies over a period of 2–4 months has been observed in several studies, suggesting that the humoral immunity induced by SARS-CoV-2 may not be long-term in persons with slight symptoms.

Similar results were reported in responses to SARS-CoV-2 nucleocapsid protein specific antibodies[9].

Patients with COVID-19 and low IgG titer (*i.e.*, the peak titre 1-2 fold the cutoff value for a positive result) had a higher rate of viral clearance compared to patients with high IgG titer COVID-19 (strong antibody response), again suggesting that a strong antibody response is associated with more severe symptoms, and low antibody responses may be associated with higher virus clearance rates[10].

A comprehensive study on adaptive immunity to SARS-CoV-2, which also examined the association with the severity of the disease, showed that the titer of neutralizing antibodies was not correlated with the severity of COVID-19, indicating that cell immune responses are also important for the elimination of infection with SARS-CoV-2 [3].

A report on the immunological evaluation of patients with acute SARS-CoV-2 infections, symptomatic and asymptomatic, found that the IgG titer was much higher in symptomatic persons than in asymptomatic individuals in the weeks 3–4 after exposure to SARS-CoV-2. In convalescence phase (*i.e.* 8 weeks after leaving hospital), the IgG titer in symptomatic persons remained significantly higher than in asymptomatic persons. IgG titer dropped during the convalescence phase to 30 (97%) of the 31 symptomatic individuals and 28 (93%) of the 30 asymptomatic individuals, while four (13%) symptomatic individuals and 12 (40%) asymptomatic individuals became seronegative IgG within 2–3 months after infection[11]. There are other factors (e.g. smoker status, age, ethnicity, race, gender, body mass index) which could influence serological and immune responses during infection with SARS-CoV-2. In a group of 20 patients with COVID-19, S1-specific IgG responses were significantly higher in older women (>40 years old) than younger women (40 years old) and men, this means older women could develop antibody responses more effectively than other groups [9].

CELL IMMUNITY TO SARS-CoV-2

Initial reports on cell immunity to SARS-CoV-2 consisted of case reports with a small number of patients and indicated that the proportion of CD38+ cells and HLA-DR+ cells returned to the basal level around day 20.Serious symptoms were also linked to a higher decrease in the number of peripheral CD4+ and CD8+T cells compared to mild symptoms, suggesting a link between the severity of the disease and the size of the cell immune response. However, more extensive studies are needed to further support a correlation[12,13].

Recognition of SARS-CoV-2 antigens by pre-existing T cells and cross-reactivity of T cells created during previous human coronaviruses infection could also contribute to the frequent presence of reactive T cells at SARS-CoV-2 in patients with COVID-19. The cell response consists mainly of T-Helper-1 (Th1) cells, characterized by strong IFNγ secretions directed to the structural spike glycoprotein, membrane protein and nucleocapside protein (in this order), although non-structural proteins may also be targets. CD8+ T cells produced as a specific response to SARS-CoV-2 produce IFNγ and tumor necrosis factor (TNF)α. Gamma interferon (IFNγ) favors the accumulation of macrophages at the site of the cell immune response and their ability to destroy intracellular micro-organisms[14,15].

LABORATORY TESTS FOR THE DETERMINATION OF NEUTRALIZING ANTIBODIES

Many anti-Covid vaccines are under development, many of which are based on an immune response for RBD. Serological tests can play an important role in understanding viral epidemiology in the general population and in identifying persons that are susceptible to viral infection. Electrochemiluminescent (ECLIA) testing uses a recombinant protein representing the RBD of the S antigen in a double antigenic sandwich, which facilitates the quantitative determination of high affinity antibodies against SARS -CoV-2. Antibody quantification helps to determine specific antibody titer and dynamic monitoring of patient immune response[16,17].

CONCLUSIONS

There is little data on post-infection immunity with SAR-CoV-2 and biological and genetic factors responsable for the broad spectrum of severity of the disease remain unclear

Many anti-Covid vaccines are under development, many of which are based on an immune response for RBD.

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