Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to Editors

The importance of cell-mediated immunity in COVID-19 – An opinion

Introduction

In December 2019, first outbreak of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was reported in Wuhan, China [1–3]. The illness caused by this virus was named coronavirus disease 2019 (COVID-19) which rapidly spread across all continents distressing millions of lives [4]. The pandemic has affected over 8 million cases worldwide with more than 400,000 deaths so far. The treatment of patients critically ill with COVID-19 has become a major challenge for physicians and there is an urgent need of reliable biomarkers that can aid in managing patients timely and efficiently. Testing of COVID-19 is largely dependent upon reverse-transcription polymerase chain reaction (RT-PCR), based on its ability to amplify and detect presence of SARS-CoV-2 in upper respiratory tract specimens. In addition to the gold standard RT-PCR, serological analyses (most commonly enzyme-linked immunosorbent assay- ELISA) to detect presence of anti-SARS-CoV-2 antibodies has also become a widespread diagnostic means especially useful in identifying the asymptomatic infected and/or recovered population [5]. While physicians have been using seroconversion data as correlates of protection, it is important to acknowledge that antibody titers do not always correspond with disease severity. While some patients display a linear relationship between rising antibody titers and improving clinical symptoms, others display a disconnect between serum antibody levels and the magnitude of organ damage [6–8]. Indeed, previous SARS-CoV-1 studies also suggest that patients with severe illness display faster peak antibody responses than other groups with mild-moderate disease [9–11]. An important question that stems from these SARS-CoV-2 antibody data analyses is whether high loads of anti-viral antibodies could be disadvantageous in controlling the disease. Additionally, should researchers be looking for another element of the immune system, besides antibodies, that can better predict disease severity and patient prognosis.

The role of antibodies in SARS-CoV-2 infection

During the course of viral infection, host plasma cells produce immunoglobulins (Igs) that recognize various antigenic determinants. Antibodies are essentially produced by either B-cell receptor (BCR) driven intracellular delivery of viral antigens or by activation of B cells via viral antigen-specific helper T cells. Among the substantial pool of anti-viral Igs, most efficient ones are the neutralizing antibodies (NAbs), which directly bind virus particle to limit its entry, fusion and/or egress. Conclusively, CD4 + T cell-dependent humoral immune response produces long-lived plasma cells followed by persistently existing quiescent memory B cells and helper T cells. Analogous to an enveloped virus-provoked-immune response, SARS-CoV-2 also triggers the B cell to produce virus-specific Igs. Seroconversion times after SARS-CoV-2 exposure are approximately 4–6 days for IgA and IgM peaks and above 10 days for peaks of virus-specific IgG [12,13]. Furthermore, specific viral surface protein analysis has revealed that Igs that recognize receptor-binding domain (RBD) or heptad repeat (HR) domains of the SARS-CoV surface spike (S) protein have neutralizing effects [7,8,14]. Studies show RBD of the SARS CoV 'S' protein binds to the Angiotensin-Converting Enzyme 2 (ACE-2) receptor whereas HR1 and HR2 domains aid in fusion of the viral and host cell envelopes [14]. Thus, NAbs identifying these viral epitopes will prevent viral attachment, entry and intracellular replication and promote viral clearance, complement activation and natural killer cell activation. However, in rare instances, antibodies can enhance viral disease, a phenomenon called antibody-dependent enhancement (ADE) [15,16]. Various factors such as antibody affinity, isotype and total concentration in serum can determine whether the presence of those Igs will protect the host or promote the pathology. Non-neutralizing antibodies have been suggested previously to promote ADE in SARS virus infection [17,18]. It is critical to compare this phenomenon with various COVID-19 Ig studies presented thus far. Analyses conducted on anti-nucleoprotein (NP) and anti-RBD IgM and IgG responses to SARS CoV-2 infection demonstrate that the antibody responses vary from individual to individual, with some patients having clinically severe disease despite early peak levels of Igs [8]. Another cohort study by Wu et al. examined NAb antibody titers in 175 patients recovered from SARS-CoV-2 infection [7]. This study also established that there was no correlation between Nab titers and disease severity. Moreover, approximately 30% of those recovered patients had very low limit of antibody titers and 10 of those patients exhibited NAb titers below detectable limits. Another retrospective analysis in Chinese patients, by Zhao and co-workers, established that higher titers of anti-SARS-CoV-2 antibody were associated with worse clinical outcomes [13]. Parallel to current studies, Lee and group retrospectively analyzed SARS-CoV-1 IgG responses and concluded that patients who seroconverted earlier in the course of disease progressed to a more severe illness [9]. Another study on SARS-CoV-1 affected patients indicates that deceased patients infected with SARS-CoV-1 developed faster peak Nab responses when compared with patients who recovered [10]. Altogether, these studies indicate that detection of anti-SARS-CoV-2 Ig is merely a diagnostic marker of seroconversion after infection. It is yet, a far-fetched suggestion to consider that SARS-CoV-2 seroconverted individuals, even those with detectable Nabs, will have improved clinical outcomes. Moreover, these studies also necessitate testing for Nabs: Non-Nabs ratio in convalescent plasma to accurately determine its benefit in COVID-19 treatment.

Hypothesis

Given that humoral arm of adaptive immune response is not linked with disease severity, it is substantial to analyze the cellular arm of adaptive immune response. Based on this perception, we can hypothesize that SARS-CoV-2 pathology is correlated with absolute numbers and activity of CD8 + cytotoxic T-cells and/or Th1-biased

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CD4 + helper T cells. Additionally, we can presume with supporting evidence that clearance of COVID-19 depends upon activation, differentiation, survival and long-lived memory of CD8 + and/or Th1 type CD4 + T cells.

Immune defenses above and beyond antibodies

Current literature on SARS-CoV-2 infection indicates that we still lack an immune system biomarker that can consistently predict disease severity and progression. We suggest that extensive analysis of cell-mediated immunity in SARS-CoV-2 infection which includes a differential count of lymphocytes and their molecular markers will provide us with significant data to further our understanding of COVID-19. Existing SARS-CoV-2 antibody analyses indicate that clinical severity of COVID-19 is consistently correlated with decreased total lymphocyte counts [8,13,19–22]. Studies conducted by Chen et al. precisely show that absolute counts of T lymphocyte population were significantly decreased despite presence of normal or high total numbers of B lymphocytes [22]. Diao and coworkers retrospectively investigated the total T cell counts and serum cytokine concentrations in patients with COVID-19 [23]. This study determined that both Non-ICU and ICU patients had significantly low T cell counts, and more importantly the critically-ill group displayed decreases in CD8 + T cells. Furthermore, T cells from this group also expressed a higher concentration of cell-surface marker PD-1 which implies ineffective and exhausted T cells [23]. Another report by Ganji et al, indicates that total expression of CD8 molecule on cytotoxic T cells was significantly increased in SARS-CoV-2 infected individuals, whereas CD4 molecule expression and CD4:CD8 T cell ratio remained unchanged [24]. Moreover, study presented by Dong and group assessed RBD and NP induced B and T cells responses in a COVID-19 recovered patient cohort. The results showed that along with strong antibody responses against RBD and NP, numbers of IFN-γ-secreting T cells was significantly higher in recovered individuals than healthy controls, suggesting that they had developed SARS-CoV-2-specific T cell responses [25]. Moreover, a comprehensive analysis of the immune system in COVID-19 patients was presented most recently by Grifoni and group [26]. This study also revealed that SARS-CoV-2-specific CD8 + T cells expressing IFN-γ and granzyme B were detected in majority of COVID-19 patients. Another recent report focusing critically-ill group of COVID-19 specifies T cell profiles in these patients [27]. This study indicates that SARS-CoV-2-specific CD4 + and CD8 + T cells appear within first two weeks of symptoms, their frequency increase during the course of infection. Upon ex vivo stimulation of peripheral blood mononuclear cells (PBMCs), Th1 subset of CD4 + T cell population along with Th1-cytokines IFN-γ, TNF-α and IL-2 dominated over Th2 or Th17 phenotype. In addition to SARS-CoV-2 data, previous studies of SARS-CoV-1 analysis provide further evidence to support our hypothesis. A cohort study examining SARS-CoV-1 immune responses showed that NAb and CD8 + T cell responses were the major mechanisms of SARS-CoV-1 virus clearance [28]. In a report presented by Channappanavar et. al., 8–10 month old mice were immunized with a T-cell dominant 'S' protein epitope (either S525 or S436) and later challenged with a lethal dose of SARS-CoV-1 virus. Consistent with the number of CD8 + T cells, nearly 60–80% of the mice were protected from lethal infection [29]. Furthermore, a follow-up study on SARS-CoV-1 measured memory B cell and T cell responses in recovered individuals. This study showed that there was an absence of long-term B cell memory and virus-specific-IgG, however, SARS-CoV antigen specific IFN-γ-secreting T cells were found 6 years post-infection [30]. This data substantiates the role of cytotoxic T cells in providing long-term protection against SARS-CoV and influences research workers to identify T cell specific epitopes of SARS-CoV-2 virus.

Discussion

The current knowledge of COVID-19 indicates that Nabs play a substantial role in clearing the virus, however, a population of individuals with undetectable Nabs also recover successfully. This data point as well as the fact that antibody titers poorly correlate with clinical severity of the disease, prompt us to improve our concept of immune response during and post SARS-CoV-2 infection. While much data has been presented regarding the B cell and Ig responses in SARS-CoV-2 infection, we still await detailed analysis of short-term and long-term immunity provided by T cells or NK cells. Additional data from immunological assays such as flow cytometry on patient serum samples to precisely determine the absolute and differential counts of circulating lymphocytes will further our understanding of SARS-CoV-2 infection. A thorough analysis of expression of T cell surface markers will help determine naïve, effector (activated or exhausted) and memory T cell subsets. We will further benefit from T cell cytokine expression studies such as intracellular cytokine staining (ICS) and enzyme-linked immunospot (ELISpot) assay using peripheral blood mononuclear cells (PBMCs) from known cases of mild, moderate and clinically severe COVID-19. With this detail, researchers can provide a T cell-grounded classification of disease severity for physicians. If T cell data consistently correlates with disease severity, clinicians can propose a management plan for newly identified cases based on the results from a comprehensive immune status panel [31]. The given immune studies for SARS-CoV-1 and SARS-CoV-2 indicate that T cell-specific viral epitopes are distinct and are more widely distributed throughout the viral proteome as compared to B cell-specific epitopes, which are more concentrated on the SARS virus 'S' protein [28,32]. Considering that T cells may provide a longer lasting immune response against SARS-CoV-2 infection, it is essential to determine the entire repertoire of B and T cell-specific viral epitopes to develop an effective vaccine. In conclusion, additional data on T lymphocytic responses in COVID-19 will expand our knowledge of SARS-CoV-2 infection, probabilities of re-infection and the disparities in COVID-19 host responses. In addition, it will help design appropriate treatment plan and modification of vaccine strategies, if necessitated.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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