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REVIEW ARTICLE

Long-term immunologic effects of SARS-CoV-2 infection: leveraging translational research methodology to address emerging questions

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The current era of COVID-19 is characterized by emerging variants of concern, waning vaccine- and natural infection-induced immunity, debate over the timing and necessity of vaccine boosting, and the emergence of post-acute sequelae of SARS-CoV-2 infection. As a result, there is an ongoing need for research to promote understanding of the immunology of both natural infection and prevention, especially as SARS-CoV-2 immunology is a rapidly changing field, with new questions arising as the pandemic continues to grow in complexity. The next phase of COVID-19 immunology research will need focus on clearer characterization of the immune processes defining acute illness, development of a better understanding of the immunologic processes driving protracted symptoms and prolonged recovery (ie, post-acute sequelae of SARS-CoV-2 infection), and a growing focus on the impact of therapeutic and prophylactic interventions on the long-term consequences of SARS-CoV-2 infection. In this review, we address what is known about the long-term immune consequences of SARS-CoV-2 infection and propose how experience studying the translational immunology of other infections might inform the approach to some of the key questions that remain. (Translational Research 2022; 241:1–12)

Abbreviations: AIM = activation induced marker; COVID-19 = coronavirus disease 2019; ELISpot = Enzyme-linked immunospot; ICS = intracellular cytokine staining; IL = interleukin; MAIT cell = mucosa-association invariant T cell; PASC = post-acute sequelae of SARS-CoV-2 infection; RBD = receptor-binding domain; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; SOT = Solid organ transplant

OVERVIEW

Many questions remain regarding the immunologic consequences of SARS-CoV-2 infection. While initial studies were crucial in demonstrating that most individuals develop long-term humoral and cell-mediated immunity to infection with the virus, the current era of COVID-19 is characterized by emerging variants of concern, waning vaccine- and natural infection-induced immunity, debate over the timing and necessity of vaccine boosting, and the emergence of post-acute sequelae of SARS-CoV-2 infection (PASC). As millions of individuals worldwide continue to become infected, there is an ongoing need for research to promote understanding of the immunology of both natural infection and prevention. In this review, we address what is known about the long-term immune consequences of SARS-CoV-2 infection and propose how experience studying the translational immunology of other

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infections might inform the approach to some of the key questions that remain.

LONG-TERM PROTECTIVE IMMUNITY FOLLOWING INFECTION

Most individuals with SARS-CoV-2 infection develop robust and persistent immunologic responses following natural infection, and as of the time of this review, many studies have characterized the humoral[1-23] and cell-mediated[19, 24-33] immune responses during convalescence for periods of up to 1 year. While the magnitude of the immune response to natural infection is at least in part determined by the severity of the illness,[3 ,6,7,15,28,30] the predictors of the duration of natural immunity are not fully understood and may be determined by a variety of clinical and measurement factors.[15,18] Despite this complexity, there is general consensus that, in most cases, natural immunity persists for up to at least 8 months. Despite the observation that antibody levels may wane over time, several studies have now demonstrated persistence of virus-specific lymphocytes over 12 months following natural infection by various intracellular cytokine staining (ICS), activation induced marker (AIM), and EliSpot assays. These assays quantify T cell cytokine expression (ICS/EliSpot) or surface markers of T cell activation (AIM) following antigenic stimulation with various virus-specific peptide pools. For example, the percentage of virus-specific CD8 and CD4 T cells as measured by ICS or AIM range from approximately 0.01%−10% during this extended time period across multiple studies,[25,26,28,29,31,32] with the median or mean percentage typically <1%. Spot forming cells/units in ELISpot assays tend to range from 10 to >1,000 in response to SARS-CoV-2 peptides, including HLA-restricted pools.[27,34] These responses wane slowly over time in all assays depending on initial disease severity and various clinical factors but can typically be detected across a range of virus gene regions (eg, Spike, Nucleocapsid, Membrane).

These immunologic findings have been borne out by the clinical observation that re-infection with similar viral variants was relatively uncommon in the first year of the pandemic, with some exceptions.[35] During the first year of the pandemic, re-infection seemed exceedingly rare and fewer than 50 cases were reported in the literature,[35] although the true burden of re-infection is difficult to estimate given the scale of the pandemic, the high proportion of asymptomatic infections, and the variability in access to testing. While there was initially hope that those with prior SARS-CoV-2 infection would aid efforts toward herd immunity and could be at lower risk for re-infection, more recent studies have demonstrated that natural immunity within a population itself is likely insufficient to fully protect against re-infection, particularly with novel variants of concern.[36] The study of long-term natural immunity has been complicated by the relatively widespread rollout of highly efficacious vaccines with inconsistent uptake across demographic and geographic locales in addition to the recent authorization or approval of booster vaccine doses across the United States and Europe.

BREADTH AND DEPTH OF T CELL IMMUNE RESPONSES AND CROSS-REACTIVITY TO OTHER CORONAVIRUSES

Data regarding the breadth of SARS-CoV-2-specific T cell immune responses following natural infection and the potential for cross-reactivity with other human beta-coronaviruses are rapidly evolving and were reviewed by Grifoni and colleagues.[37] Thus far, over 1400 unique CD8 and CD4 T cell epitopes have been identified,[37,38] although only a handful of antigens comprise >85% of these. Interestingly, immunodominant regions of the spike protein for CD4 T cells are relatively limited, whereas distribution for CD8 cells are more homogeneous.[37] Nonetheless, a prior study estimates that an individual may recognize 17 different CD8 and 19 different CD4 immunologically important epitopes.[38] In addition, we and others have shown that SARS-CoV-2 specific CD4 T cell responses, and to a much lesser extent CD8 T cell responses, are significantly correlated with antibody responses including total levels and neutralization capacity.[30,39,40] CD4 and CD8 T cells also appear to play unique roles in clinical disease, or respond differently to natural infection, and it is important to impart that these cells play different roles in immune responses to infection and should not be thought of as a unified T cellular response. For example, we and others have demonstrated that the magnitude of virus-specific CD4 T cells appears to correlate with initial disease severity and with levels and neutralization capacity of antibody responses.[25,30,41,42] These associations were not consistently observed with CD8 T cell responses in our study, which appear to be influenced by other clinical factors. For example, we previously reported that pre-existing lung disease is independently associated with higher long-term SARS-CoV-2-specific CD8, but not CD4, T cell responses. Regardless of the differences between CD4 and CD8 T cell responses, there is now data emerging that virus-specific T cell reactivity is not significantly disrupted

by viral variants, such as Delta.[43,44] Little data are currently available on the emerging Omicron variant.

There is also data emerging that some individuals may have cross-reactive T cell responses to other human beta-coronaviruses, with detectable responses in those without a history of known infection.[25,28,41,45,46] Some of these responses may be a result of occult, asymptomatic prior SARS-CoV-2 infection that led to aborted or rapidly wainig antibody levels,[29,47] but data exist suggesting that pre-existing memory responses from endemic, less-pathogenic coronaviruses or other pathogens occur in some individuals.[34,48] Interestingly, pre-existing cross-reactive T cell responses may be better detected by assays that measure the capacity for T cells to proliferate in response to SARS-CoV-2 Spike protein stimulation *ex vivo* rather than by intracellular or cell surface markers of response.[49] Regardless, it is poorly understood to what extent or for how long pre-existing cross-reactive immunity may protect from acute infection or modulate disease severity and the development and persistence of post-acute sequelae. Further study is urgently needed.

VACCINE INDUCED ANTIBODY AND T CELL RESPONSES

Initial vaccine trials predmoniaty enrolled healthy adults, and those currently approved or pending approval for use in the United States and Europe (Pfizer/BioNTech BNT162b1, AstraZeneca ChadOx1, Moderna mRNA-1273, Janssen Ad26.COV2, Novavax NVX-CoV2373) lead to robust antibody binding and neutralization titers.[50] Antibody responses generally mirror protection from asymptomatic through severe disease, hospitalization and death. However, efficacy has been shown to wane over time leading various regulatory agencies in Europe and the United States to approve or authorize boosters or supplementtal doses for adults,[51-55] with or without underlying immunomodulatory conditions or belonging to risk groups. Despite waning antibody titers and increased cases of mild infection, vaccines continue to protect against severe disease and hospitalization for up to 6 months.[51-55] As of now, vaccines remain active against the predominant circulating strains of SARS-CoV-2, and variants that may be more resistant to vaccination, such as Mu, appear to have a replication disadvantage compared with the widely circulating Delta variant. Whether this will remain the case with Omicron is unknown. Whereas levels of nasopharyngeal shedding have been reported to be similar in persons who acquired infection after full vaccination compared with those who were previously unvaccinated, the duration of viral shedding and symptoms are significantly shorter, and infection may be more compartmentalized to non-shedding tissues.[56] Further research is warranted to more precisely determine the impact of vaccination on infectivity of breakthrough infection. Regardless, vaccine use has had a dramatic positive effect on reducing morbidity, mortality and community spread of SARS-CoV-2.

Data on T cell responses from vaccine trials are more sparse, and systematic study of adaptive cellular responses varied across initial studies (as reviewed elsewhere[50]). A majority of approved or authorized vaccines, however, have demonstrated development of CD4, CD8 or total T cell responses as measured by spot forming colonies per 10^6 cells in EliSpot assays (40 to >2600 spot forming colonies). Data on the decay of T cell responses following vaccination over time are currently lacking, and it is not known what role vaccine-elicited virus-specific T cell responses play in preventing primary infection or modulating the course of acute and post-acute disease.

To date, the immunologic response prior to SARS-CoV-2 vaccination has been characterized for over 12 months.[57,58] The recent surge of the Delta variant of SARS-CoV-2 globally has revealed that vaccine-induced immunity might be insufficient to prevent infection and more severe disease in many cases. Furthermore, the duration for which vaccine-induced immunity can protect against severe disease and hospitalization remains unclear, although boosting is likely to significantly extend the duration of protection.

IMMUNITY IN IMMUNOCOMPROMISED INDIVIDUALS

It is now well established that antibody and T cell responses can be severely impaired following vaccination,[59] and to a lesser extent, natural infection,[60-62] in immunocompromised individuals, including solid organ transplant (SOT) recipients, cancer patients, and others receiving immunomodulatory medications for various conditions. For example, there is growing evidence that SOT recipients do not develop detectable antibody levels or measurable neutralizing capacity following two-dose vaccination,[63-68] and current clinical experience demonstrates higher rates of post-vaccine infection and hospitalizations in this population. An additional third or even fourth dose appears to increase antibody responses, however.[69,70] Patients with cancer, especially those with hematological malignancies on cytoreductive or anti-B cell therapies

and certain rheumatologic diseases also exhibit reduced antibody responses to vaccination,[71-73] albeit to a lesser degree than those who have received SOT but may experience high rates of vaccine breakthrough.[74] Various medications that have anti-proliferative mechanisms of action (such as mycophenolic acid derivatives) may be associated with more impaired antibody, B and T cell responses. As above, anti-B cell agents and systemic corticosteroids impact antibody responses and memory B cell responses and combinations of immunomodulatory agents are likely to have more profound and lasting negative impacts on these immune responses.[64,65,67,75-77]

T cell responses are also impaired in the setting of immunosuppressive medications and diseases, but data are now emerging that these responses are somewhat discordant with antibody responses following vaccination. For example, nearly half of kidney transplant recipients in one study that did not develop antibody responses following vaccination had detectable SARS-CoV-2-specific T cell responses.[64] Whether or not these T cell responses are protective, as discussed above, is not known and requires further study. However, recent data show that persons that receive anti-B cell therapy (eg, anti-CD20 for multiple sclerosis) have a paradoxical increase in SARS-CoV-2-specific CD8 T cell responses, despite significant impairment of humoral responses.[75,78-82] The increase in CD8 T cell responses may reflect an immune compensatory mechanism,[78,83,84] but it is still not clear what role virus-specific CD8 T cells have in protection from infection or modulation of disease severity. It is also interesting to note that despite the potential for increased CD8 T cell responses, individuals with impaired humoral responses have increased risk of more severe infection.[78,85]

Immunity in other immunocompromised individuals, such as those living with HIV infection, is more variable. Recent work has suggested a lower magnitude of humoral and cell-mediated immune responses[86-88] or shorter duration of the antibody response in comparison to the general population,[89] although both observations require further study. Recent studies suggest that the immune response following vaccination is equal amongst PLWH and the general population[90] and in comparison to those with other immunocompromising conditions,[91] but further work is needed to confirm these findings given the global concern for sustained immunity to SARS-CoV-2 and the known poorer responses to immunization for other infections among PLWH.[92-97] This includes suboptimal responses to vaccination to prevent against yellow fever,[93,94] Hepatitis B,[96], influenza,[95] polio, diphtheria, and tetanus.[97] It is likely that inadequate CD4 T cell immune reconstitution, chronic inflammation, and T cell exhaustion underlie these observations,[93] and careful studies will be needed to understand how HIV and COVID-19 vaccination durability overlap.

HUMAN INFLAMMATORY RESPONSES IN COVID-19

COVID-19 can lead to profound inflammatory responses in acute infection and to increased levels of various cytokines, such as TNF-α, IL-6, and IP-10, which are associated with more severe disease and organ damage.[98-103] Especially among those hospitalized with COVID-19, inflammation during the acute and early post-acute phase of infection has been associated with poor outcomes.[104-110] In addition, many individuals present with profound lymphopenia, including a marked decrease in circulating NK, CD8 and CD4 T cells, including helper and regulatory T cells.[98-103] Lower numbers of circulating monocytes, eosinophils and basophils have also been observed. In contrast, leukocyte counts tend to be higher in patients with severe clinical manifestations.[111,112] Despite lymphopenia in more severe SARS-CoV-2 infection, increased frequency of T cells responding to various antigens such as, Spike, Nucleocapsid, membrane, and accessory (functional) protein (eg, ORF 1ab) peptide sequences develop within the weeks following infection.[29] Although lymphocyte counts return and virus-specific adaptive immunity develop early during clinical recovery, increased markers of T cell exhaustion and reduced functional diversity of T cell subsets have been reported in the early convalescent period.[98,99,113,114]

Emerging data suggest that inflammation related to acute SARS-CoV-2 infection can persist for weeks or even months.[115,116] One study found that convalescent plasma donors with prior COVID-19 demonstrated elevations in certain markers of inflammation compared to historical controls, even though they presumably felt well enough to donate plasma.[115] These markers include interferon (IFN)-gamma, certain interleukin (IL) proteins (eg, IL-12p70, IL-13, IL-1β, IL-2, IL-4, IL-5, IL-33), and monocyte chemoattractant protein (MCP)-1 and suggest ongoing immune activation. Another study of a large cohort of individuals hospitalized with asymptomatic, mild, and severe disease showed that individuals who recovered from COVID-19 had elevated levels of proinflammatory and angiogenic markers at 6 months in comparison to healthy controls.[116] There is an ongoing need for work exploring the clinical implications of persistent inflammation following SARS-CoV-2; while such elevations are clearly significant in chronic infections like HIV,[117,118] this is less well understood for acute infections like SARS-CoV-2 which is not thought to persist over the long-term.

IMMUNOLOGIC AND INFLAMMATORY MANIFESTATIONS OF POST-ACUTE SEQUELAE OF SARS-COV-2 INFECTION (PASC)

Recently, there has been recognition that a significant proportion of individuals recovering from SARS-CoV-2 infection experience new or persistent symptoms that did not pre-date their infection.[119-122] Investigation into PASC is only just beginning, and the pathophysiology of the condition is thus far entirely unknown. While well-designed epidemiologic studies are beginning to identify certain risk factors for PASC, including female sex, older age, severity of initial infection, number of symptoms during acute illness, and sociodemographic factors, the condition remains poorly understood.[119-122]

One major question is whether PASC is an immunologic phenomenon, either from long-term sequelae following an immunologic insult that occurs early in the course of the infection (ie, a "hit and run" mechanism) or related to an ongoing immunologic or other perturbation, potentially in the setting of ongoing viral persistence in tissue. So far, the clues have been limited. A handful of studies have identified higher levels of binding or neutralizing antibodies in those with PASC,[122,123] suggesting that persistent symptoms could be a manifestation of more severe illness (which is known to be associated with higher antibody levels and correlated with higher risk of developing PASC) or possibly persistent immune stimulation.[14] Other studies have found that the humoral response appears *lower* among those with persistent symptoms.[124-126] For example, ongoing viral shedding in the gut is associated with lower RBD-specific antibodies,[124] suggesting suboptimal immune responses may result in persistent viral antigen. Furthermore, a handful of studies have correlated PASC with lower SARS-CoV-2 specific antibody responses,[126] and have shown that those with lower titres during early recovery might be more likely to have persistent symptoms. Cellular immune studies are limited and have thus far not revealed obvious differences, although our recent work has suggested lower[126] or differential decay in the magnitude of SARS-CoV-2 specific CD8 T cell responses among those with PASC.[30] While intriguing, the precise implications of this finding is not known, and could either represent a more exhausted pool of viral-specific cells that develop over time or other immune dysregulation or detrimental systemic inflammation leading to decay in the frequency of these CD8 T cells. Understanding the relationship between immune responses during early recovery and the persistence of PASC symptoms could guide diagnostic or therapeutic decision making for millions of individuals recovering from COVID-19.

Studies that have evaluated persistent inflammation have suggested potential elevations in biomarkers,[116,127] although no clear immunologic pathways have yet to be consistently implicated. We recently demonstrated that during early recovery (ie, one to two months after initial infection), those who went on to develop PASC generally had higher levels of biomarkers including significant elevations in circulating TNF-alpha and IP-10, and a trend towards higher IL-6 levels. During late recovery (4 months following infection), levels of TNF-alpha and IP-10 levels decayed and converged with levels in participants without PASC, whereas IL-6 elevations became more pronounced. These differences tended to be more pronounced among those with a greater number of PASC symptoms suggesting that PASC is associated with increased immune activation over time, which may underlie some symptoms which persist for more than 3 months following SARS-CoV-2 infection. IL-6 elevations may result from immune cell activation and signaling, degradation of gut mucosal integrity and translocation of bacterial and other infectious agents, B cell activation, among many other processes.[117,128,129] Further characterization of such biological pathways and the processes that might drive them could lead to the identification of therapeutic targets for those experiencing PASC. In a prior study, we did not observe differences in markers of aberrant blood clotting, such as D-Dimer in those with and without persistent symptoms, despite disorders of hemostasis contributing to some individuals with severe COVID-19.[30] However, further study is certainly warranted given sample size limitations and the lack of a standard working definition of PASC.

Aberrant autoimmune responses are present during acute COVID-19 and have been proposed as a potential underlying etiology of PASC,[130-132] and recent study showed that over 40% of patients in a longitudinal study have positive antinuclear antibody (ANA) titers >1:160 12 months following infection.[133] A majority of the cohort reported PASC symptoms and the number of symptoms reported were higher in those with a positive ANA. In our long-term COVID-19 cohort, however, we were unable to detect positive ANAs in any of 49 participants approximately 4 months after initial infection and detected positive ANAs in just 3 of 69 participants 8 months after acute infection, which is similar to the percentage of people in the general population without known autoimmune disease that have detectable ANAs. Our cohort included individuals with and without PASC, including those with >20 symptoms 8 months after initial infection and perturbation in activities of daily life.[134] As a result, further studies of potential autoimmune

mechanisms behind PASC are needed in order to understand these disparate findings.

Finally, the alterations of both inflammation and immune responses in the setting of convalescent COVID-19 may also influence future risk of various conditions such as cardiovascular, pulmonary and neurological diseases. Unfortunately, time will be needed to understand the longer impact of SARS-CoV-2 infection more fully beyond persistent symptoms.

TISSUE PERSISTENCE OF SARS-COV-2 INFECTION: A POTENTIAL MECHANISM FOR PASC?

It is well established that immunocompromised individuals are capable of shedding SARS-CoV-2 RNA from oral/nasopharyngeal tissues months after acute infection, and novel variants may have arisen in such individuals under the setting of partial immune pressure.[135,136] However, RNA shedding usually resolves within a month in immunocompetent patients,[137] and we recently observed no persistent RNA shedding in convalescent COVID-19 patients who exhibit PASC approximately 4 months after initial infection.[30] Despite this, there has been limited but intriguing data suggesting that SARS-CoV-2 proteins can be identified in gut tissue months after initial infection.[14] If SARS-CoV-2 remains transcriptionally and/or translationally active in various tissue reservoirs following clearance from nasopharyngeal tissues, this could represent a potential mechanism underlying the development and maintenance of PASC.[138] There are also data emerging that COVID-19 may lead to intestinal damage and microbial translocation.[139] In chronic infections, such as Human Immunodeficiency Virus (HIV), persistence of virus in gut-associated mucosal tissue leads to gut barrier dysfunction and bacterial/fungal translocation that may lead to elevated markers of immune activation (including IL-6) and inflammation, even in those on suppressive antiretroviral therapy.[128] Furthermore, elevations in IL-6 and other inflammatory markers in the setting of chronic HIV infection are associated with worse clinical outcomes,[140-142] and the longer-term clinical impact of persistent IL-6 elevations identified in our PASC cohort requires further investigation.

Although the mechanisms of PASC are not known, the current thinking is that PASC is a multifactorial process that manifests in diverse clinical and demographic phenotypes. In addition, there is yet to be a standard working definition of PASC and objective phenotypes have yet to be determined. As a result, studying the pathophysiological basis of PASC is going to be challenging and require large numbers of study participants with well curated control groups.

LEVERAGING THE STUDY OF OTHER CHRONIC INFECTIONS TO UNDERSTAND POST-ACUTE SEQUELAE OF SARS-COV-2 INFECTION

The infectious disease research community has developed tools over the last two decades that can be leveraged to support research into duration of immunity and immunologic consequences following SARS-CoV-2 infection. Decades of research in chronic viral infections, such as HIV as mentioned above, shows that tissue persistence and ongoing inflammation and immune activation can lead to increased morbidity across multiple organ systems. In addition, other chronic viral infections, such as CMV, may lead to long-term inflammation in those with various immune suppressing conditions such as HIV and organ transplantation and increases risk of cardiovascular disease through chronic immune dysregulation,[143-147] and tools have been developed to study tissue-based disease that can be applied to the long-term pathogenesis of COVID-19. Although SARS-CoV-2 is predominately thought of as a respiratory illness, viral receptors, suchy as ACE2, can be found throughout various tissues in the body, including endovascular tissue and many organ systems.[148-150] Given data hinting at potential viral persistence in gut tissues, many of the potential drivers of PASC could require tissue investigation for meaningful in-depth mechanistic studies.

First, there is an urgent need to understand the long-term immunological and inflammatory impact of SARS-CoV-2 infection in mucosal, gastrointestinal and respiratory tissues. Whereas procedures such as bronchoalveolar lavage can be relatively easy in patients requiring mechanical ventilation for diagnostics or therapeutics, invasive or semi-invasive sampling in the setting of convalescent disease presents greater challenges. Although challenging, the HIV research community has developed a wide range of translational research tools to study viral persistence and immune and inflammatory responses in various mucosal, lymphoid and other tissues which can be applied to the study of COVID-19 and PASC. For example, gut tissue biopsies and lymph node sampling are routinely performed in the clinical and translational HIV research settings, and the study of these tissues has revealed much

information on how HIV persists in the setting of suppressive antiretroviral therapy over time and how immune responses (or lack thereof) interact with infected cells.[151-161] In addition, *in situ* study of viral infection within an anatomic histological context has proved critical in elucidating the burden of infection and impact on immune and inflammatory responses.[153,162-165] In addition to the gastrointestinal studies as discussed above, studies of COVID-19 are now emerging examining the role of T and B cell memory responses in various tissues (eg, lung-associated lymph nodes in adults or tonsillar tissue in children) and mucosa-association invariant T cells in lower airways. It is likely that analysis of human nasopharyngeal, respiratory, lymph node, gut and vascular endothelium will be necessary to fully understand the long-term immune and inflammatory implications of COVID-19. Given the challenges of studying tissues that are not routinely accessible to clinical sampling, such as the brain, heart, liver, spleen, and deeper lymph node chains, to name just a few, non-invasive technologies to determine the burden of SARS-CoV-2 infection and short- and long-term immune and inflammatory sequelae are urgently needed. Table 1 summarizes many unanswered translational science questions related to long-term immunologic consequences of COVID-19.

Table I. Translational science questions related to long-term immunologic consequences of COVID-19

Humoral and Cellular Immunology
What is the role of T cells in preventing or mitigating the severity of acute infection or re-infection in those with prior SARS-CoV-2 infection or vaccination?
At what point following infection or vaccination is protection from hospitalization and severe illness lost? At what point is protection from re-infection lost?
How does post-infection immunity compare with post-vaccine immunity? How does this immunity compare across emerging variants of concern?
What is the functional half-life of SARS-CoV-2-specific T cells and amnestic potential following infection or vaccination?
How do novel SARS-CoV-2 therapeutics, including antivirals and immunomodulatory agents, affect long-term immunity following natural infection?
Are the compensatory T cell responses observed in immunocompromised patients with imparied humoral immunity following vaccination protective?
Mucosal Immunity
What are the key factors in determining the presence and duration of protective mucosal immunity?
How does immune memory differ between what has been observed in peripheral blood with various tissues (eg, mucosal and organized lymphoid tissues, lower respiratory tract, etc.)
What is the role of secretory and circulating IgA antibodies?
Post-Acute Sequelae of SARS-CoV-2 Infection
Are there immune mechanisms active during acute infection that predict the development of post-acute sequelae of SARS-CoV-2 infection (PASC)?
Are there immune mechanisms that are initiated during the recovery phase (ie, after acute infection has resolved) that are associated with PASC?
If immune mechanisms are found to underlie PASC, can we distinguish persistent immune perturbations from the sequelae of so-called "hit-and-run" mechanisms?
Does SARS-CoV-2 antigen persist beyond the period of mucosal viral shedding, either in the form of replication-competent or non-replication-competent virus? If so, at what body sites?
Do inadequate or excessive immune responses (including autoimmune responses) contribute to PASC?
If immune mechanisms drive PASC, are there interventions which can prevent or treat PASC symptoms?
Will PASC lead to increased risk of cardiovascular or neurologic diseases over time?
Quantifying Tissue SARS-CoV-2 Burden and Sequelae
What tissue-based measurements will be informative in determining whether SARS-CoV-2 genetic material or protein persist in tissues? What measurements will be acceptable in those who have entered the convalescent phase?
Are there non-invasive methods of measuring whole-body immune responses or inflammation in the setting of SARS-CoV-2 infection?

CONCLUSION

SARS-CoV-2 immunology is a rapidly changing field, with new questions arising as the pandemic continues to grow in complexity. The next phase of COVID-19 immunology research will focus on clearer characterization of the immune processes defining acute illness, development of a better understanding of the immunologic processes driving protracted symptoms and prolonged recovery (ie, PASC), and a growing focus on the impact of therapeutic and prophylactic interventions on the long-term consequences of SARS-CoV-2 infection.

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