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# Biological Significance of Anti–SARS-CoV-2 Antibodies

## Lessons Learned From Progressive Multifocal Leukoencephalopathy

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## Abstract

### Objective

To discuss the pathogenic and diagnostic relevance of cellular and humoral immune responses against severe acute respiratory syndrome novel coronavirus (SARS-COV-2) and pertinent observations made in progressive multifocal leukoencephalopathy (PML).

### Methods

Review of pertinent literature.

### Results

There is at least 1 precedent for an antibody response against a viral pathogen that fails to provide host protection in the absence of immune-competent $CD4^+$ T cells. PML is an infection of the CNS caused by JC virus (JCV), which commonly occurs during treatment with the therapeutic monoclonal antibody natalizumab. In this context, the humoral immune response fails to prevent JCV reactivation, and elevated anti-JCV serum indices are associated with a higher PML incidence. The more relevant immune-competent cells in host defense against JCV appear to be T cells. T cell–mediated responses are also detectable in convalescing patients with SARS-COV-2 irrespective of the humoral immune response.

### Conclusion

Based on pathogenic lessons learned from PML under natalizumab therapy, we suggest the incorporation of functional assays that determine neutralizing properties of SARS-CoV-2–specific antibodies. In addition, we outline the potential role of T-cell detection assays in determining herd immunity in a given population or in studying therapeutic responses to vaccines.

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## Glossary

**GMFR** = geometric mean fold rise; **GMT** = geometric mean titer; **Ig** = immunoglobulin; **IRIS** = immune reconstitution inflammatory syndrome; **JCV** = JC virus; **PML** = progressive multifocal leukoencephalopathy.

The emergence of the novel coronavirus severe acute respiratory syndrome novel coronavirus (SARS-COV-2) and the evolving coronavirus disease 2019 (COVID-19) pandemic have brought about an unprecedented surge of social, economic, and health burdens. There is growing recognition that achieving naturally acquired immunity on a population level will be associated with an unacceptable burden of mortality and disease-related comorbidities.[1] Consequently, the development of curative treatments or protective vaccines will be required for disease control.

Starting with smallpox inoculations more than 300 years ago, vaccines have been used to generate a protective immune response against a pathogen.[2] Vaccines are administered for primary or secondary disease prevention and contain immunogenic proteins, peptides, polysaccharides, or DNA or RNA that encode a dominant antigenic determinant. In the majority of human vaccines, serum antibodies against the inoculate or the pathogen have long served as a meaningful biomarker of immunogenicity and efficacy.

Currently, numerous vaccine candidates are being evaluated for their ability to generate SARS-CoV-2–specific immune responses. Recently reported preliminary results on 1 vaccine candidate showed a positive and dose-dependent serum immunoglobulin (Ig) G response of binding serum antibodies to the S-2P antigen of SARS-CoV-2 encoded by an RNA vaccine as measured by ELISA.[3] Studies have also demonstrated the vaccine-induced neutralizing activity of anti–SARS-CoV-2-IgG by using a pseudo-typed lentivirus reporter single-round-of-infection neutralization assay and live wild-type SARS-CoV-2 plaque reduction neutralization testing.[3]

In theory, naturally acquired immunity should accomplish the same goals as vaccination. Experiments in rhesus macaques (*Macaca mulatta*) provided evidence that in higher nonhuman primates, infection with SARS-CoV-2 results in detectable antibody responses and provides protective immunity to experimental animals.[4,5] However, these 2 observations may not be causally linked. For instance, it was shown by other investigators that naturally acquired humoral immunity in survivors may not be sustained beyond weeks or months.[6,7] Unfortunately, most studies that investigated immunologic biomarkers associated with SARS-CoV-2 exposure were inconsistent in their use of methodology and data acquisition. Assays that were used to measure serum IgM, IgG, or IgA responses include ELISA, lateral flow immune assays, and chemiluminescence immune assays. Data that were generated in these studies do not allow for an accurate association of humoral immune responses to SARS-CoV-2 and clinical disease activity.

Even more concerning, some studies appear to suggest an association between disease severity and higher anti–SARS-CoV-2 titers in COVID-19.[8–10] Plausible interpretations of this observation are (1) a lack of a neutralizing effect by anti–SARS-CoV-2 antibodies or (2) an antibody-dependent enhancement. Epidemiologic data from some of the hardest hit communities during the first wave of the COVID-19 pandemic appear to support this interpretation: With their corresponding outbreaks almost over, seroprevalence studies suggest a dissociation between the rate of disease propagation and seroconversion in these communities.[11,12] Taken together, these observations call into question a universally protective anti–SARS-CoV-2 antibody response.

There is at least 1 precedent for an antibody response against a viral pathogen that fails to provide host protection. Progressive multifocal leukoencephalopathy (PML) is an infection of the CNS caused by the human polyoma virus JC virus (JCV). PML is almost exclusively observed in individuals with severe and prolonged immunosuppression. One setting that allows PML to occur is treatment with the humanized recombinant monoclonal antibody natalizumab, which binds to α4-integrin and prevents its interaction with its ligands vascular cell adhesion protein 1 in the CNS and mucosal addressin cell adhesion molecule 1 in the gastrointestinal tract.[13] Natalizumab reduces the ability of leukocytes to migrate into the brain and spinal cord, creating a relatively immune-deficient microenvironment that is likely permissive for JCV activation to occur.

A prerequisite for PML is an infection with JCV, which occurs very commonly in most populations and which is typically followed by a period of viral latency. Upon the primary infection with JCV, the virus is recognized, and a cellular[14,15] and humoral[16,17] adaptive immune response is generated. Anti-JCV antibodies are detectable in 50%–85% of all adults.[18–20] Given that almost all patients under natalizumab who develop PML are anti-JCV IgG positive, it is currently thought that the humoral immune response is not able to prevent reactivation of JCV and the development of PML.[21] In fact, although causality has not been demonstrated, higher anti-JCV serum indices are associated with a higher incidence of PML under natalizumab.[22] We will argue below that the more relevant immune-competent cells in host defense against JCV appear to be T cells.[14,15]

A critical role for T cells reactive to JCV was also demonstrated in patients with MS who developed PML and subsequently immune reconstitution inflammatory syndrome (IRIS) after natalizumab cessation. IRIS was characterized

immunologically mostly in individuals infected with the HIV. Following the introduction of antiretroviral therapies to clinical practice, an increase in the number of circulating $CD4^+$ T cells was associated with an increased incidence in organ specific inflammation that was not specific to the CNS.[23] Some patients also developed PML as a manifestation of IRIS.[24] Aly et al.[25] performed a histopathologic evaluation of biopsy material from patients with MS who developed PML under natalizumab and IRIS after treatment cessation and showed a prominent T-cell infiltrate driven mostly by $CD4^+$ T cells. In addition, B lymphocytes, plasma cells, and monocytes were also detected in affected tissue. Within the CSF, there were high levels of anti-JCV antibodies. Brain-infiltrating $CD4^+$ T cells were highly reactive to peptide determinants from several JCV proteins, particularly the major capsid protein VP1. Although these findings strongly suggest that JCV-specific $CD4^+$ T cells play an important role in IRIS, the functional contribution of B-cell subsets or anti-JCV antibodies was not clarified in this study or by other investigators. Of interest, the same investigators recently investigated the effects of natalizumab on intrathecal antibodies to viral pathogens and demonstrated that anti-JCV IgG was detectable in 20% of patients with MS before natalizumab initiation.[26] Once natalizumab was administered, the frequency of patients with intrathecal anti-JCV IgG declined. Total CSF IgG and IgM levels also diminished significantly. Because natalizumab substantially reduces the number of intrathecal T cells,[27,28] and the presence of intrathecal anti-JCV IgG, one may argue that both are relevant in host defense against JCV. However, although a reduction in the number of T cells is an event that can be ascertained almost immediately after natalizumab administration, a decline in CSF IgM and IgG levels appears to be a later phenomenon, and at least in 1 study, this was not demonstrated after 14 months of therapy.[29] Many patients with MS on natalizumab therapy have been diagnosed with PML in the first year of treatment, which would favor our argument that the cellular antiviral response is essential for the prevention of PML.

In summary, the humoral acquired immune response against JCV is a reliable diagnostic and prognostic biomarker, but it fails to correlate with host defense in the absence of Ag-specific cellular immune response. Although this phenomenon is incompletely understood, the generation of anti-JCV IgG may present a forme fruste of an adaptive immune response.

A similar role for SARS-CoV-2 binding serum antibodies is conceivable in infected patients, in whom the infection can lead to lymphopenia in patients with a severe disease cause [30,31] or in recipients of anti–SARS-CoV-2 vaccines in whom the vaccine fails to mount a robust T-cell response. Ongoing clinical trials assess humoral immune responses, including the longitudinal geometric mean titer (GMT) of SARS-CoV-2–specific neutralizing antibody, the geometric mean fold rise (GMFR) of SARS-CoV-2–specific neutralizing antibody, quantified levels or GMT of S protein–specific binding antibody, and the GMFR of S protein–specific binding antibody (for instance: clinicaltrials.gov/ct2/show/NCT04470427). Although using assays that assess the neutralizing capabilities of anti–SARS-CoV-2-IgG is paramount for quality control, other adaptive cellular immune responses should also be interrogated. With exception to adaptive immune responses against complex sugars, antibody isotype switching from IgM to IgG does not occur without the involvement of $CD4^+$ T helper cell reactive to the same antigen.[32,33]

Of interest, studies that investigated adaptive T-cell responses against anti–SARS-CoV-2 have yielded some perhaps unexpected results. These investigators detected SARS-CoV-2–reactive $CD4^+$ T helper cells and $CD8^+$ cytotoxic T cells in patients with a known exposure to SARS-CoV-2, but also in individuals in whom blood samples were obtained years before the onset of COVID-19,[34] or in up to 50% of study participants without a known viral exposure.[35–38] T-cell receptor promiscuity has been described for $CD4^+$[39] and $CD8^+$ T cells,[40] and the aforementioned observations likely reflect memory T-cell reactivity to common cold coronavirus.

As stated above, for B-cell subsets to generate an antigen-specific antibody response, $CD4^+$ T cell help is required for most types of antigen. Thus, it appears counterintuitive to believe that an amplified humoral immune response would be associated with impaired $CD4^+$ T-cell function. However, the composition of $CD4^+$ T cells in pertinent tissues may affect quantitative antibody responses. For instance, interleukin-4 is a major driver of T-cell cross-activation of B cells that lead to antibody expression and maturation.[41] Also, the expression of CD40L by $CD4^+$ T cells is critical for the same purpose,[41] and one could envision that low expression of this costimulatory molecule may affect B-cell cross-activation.

In conclusion, serum-based detection assays for anti–SARS-CoV-2 binding antibodies may not prove sufficient in ascertaining herd immunity in a given population or in studying therapeutic responses to vaccines. Functional assays will be required to determine neutralizing properties of SARS-CoV-2–specific antibodies. In addition, it may be meaningful to assess cellular adaptive immune responses against SARS-CoV-2 and specifically against pertinent spike peptides.[34,35,37,38] The detection of antigen-reactive T cells by customized HLA tetramers is considerably more complex than antibody detection assays with regard to assay verification and implementation. Furthermore, vaccine recipients would have to be HLA genotyped. Other methods, including activation induced marker assays, have been used to detect antigen-specific T cells in blood and lymphoid tissues,[42–44] including T cells reactive to SARS-CoV-2 epitopes.[45] A pharmacologically and biologically plausible diagnostic approach will enable the medical community to overcome the challenges of the COVID-19 pandemic and will inform on diagnostic strategies for future pandemics.

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