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INSIGHTS

Down but far from out: The durability of SARS-CoV-2 immunity after asymptomatic infection

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The dynamics of immune responses in asymptomatic SARS-CoV-2–infected subjects remain to be fully characterized. The work presented in this issue of *JEM* by Le Bert et al. (2021. *J. Exp. Med.* https://doi.org/10.1084/jem.20202617) sheds some light on these issues and ultimately provides some degree of confidence in the magnitude and persistence of immunity over time after asymptomatic infection with SARS-CoV-2.

Since the beginning of the SARS-CoV-2 pandemic, questions have circulated around the generation and maintenance of protective immunity to the virus and its relationship to the severity of infection. Older data from the original severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS; Sariol and Perlman, 2020), as well as early analysis of immunity after COVID-19 (Long et al., 2020), raised concerns as to the durability of protective immunity resulting from SARS-CoV-2 infection. Relevant to this concern was the representation of asymptomatic infections in the total case load, around which estimates ranged as high as 80% (Ing et al., 2020). Again, early data suggested that the magnitude of immune response in an individual correlated with their severity of disease, elevating the worry that high rates of asymptomatic infections might further compromise the durability of protective immunity (Cervia et al., 2021; Long et al., 2020).

The Emerging Infectious Disease collaborative program between Duke University and the National University of Singapore (DUKE-NUS) teamed up with other research institutes in Singapore to follow the immune responses of residents from a migrant worker dormitory during the initial months of the SARS-CoV-2 outbreak. Le Bert et al. (2021) were able to identify and track the immune responses of those that initially reported as seropositive as well as those that seroconverted during the course of the study, providing reasonable estimates for the duration of time elapsed after infection. Because of the regular screening for symptoms at the dormitory (see below), they were able to reasonably satisfy the concern that the data obtained were not influenced by disease severity. Serum was obtained to evaluate basic serology and neutralizing antibody titers at multiple time points. Peripheral blood mononuclear cells (for T cell analysis) were obtained from a smaller subset of individuals from those seropositive at recruitment, as well as those seroconverting mid-study. These were compared with antibody and T cell responses from samples obtained from symptomatic subjects who had been hospitalized with mild to severe symptoms. Collectively, the dataset allowed the authors to track the relationship between various T and B cell parameters to symptomology and time from initial infection.

To the question of the stability of anti–SARS-CoV-2 serology, the authors found different answers depending on what aspect of the serology was measured. As in previous reports, N-specific antibodies declined over time, though initial titers were generally robust and correlated well with a surrogate assay for determining neutralization capacity. Importantly, not only did neutralizing titers tend to outpace those of N-specific antibodies, they also did not decline as precipitously; titers of anti-N were lost in 25% of individuals found positive at recruitment, whereas only 9% of those seropositive for neutralizing antibodies at baseline became negative over the same time frame.

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Regardless of antibody persistence, the authors found robust T cell frequencies against all viral proteins analyzed (N, M, and S). Interestingly, the magnitude of T cell reactivity and its hierarchy (M-specific T cell responses dominated) were similar between symptomatic and asymptomatic individuals, an indication that the range of T cell responses was neither augmented nor limited by pathology. In regards to the

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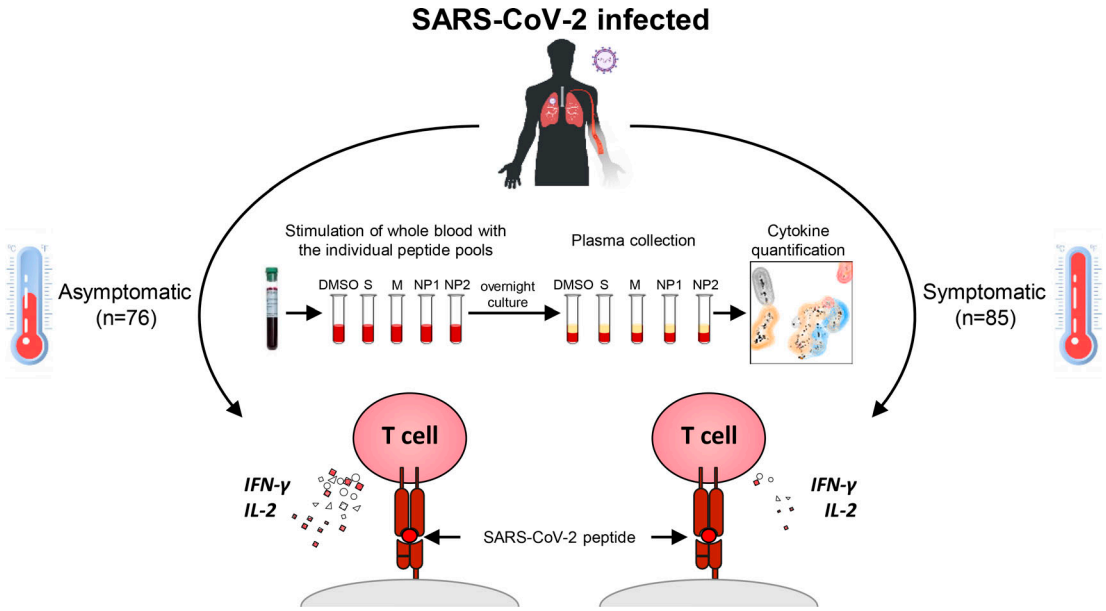

SARS-CoV-2–specific T cells were assessed in a cohort of asymptomatic and symptomatic COVID-19 patients. Similar frequencies of viral-specific T cells were reported between asymptomatic and symptomatic individuals. SARS-CoV-2–specific T cells from asymptomatic patients presented increased IFN-γ and IL-2 production, while increased inflammatory cytokines were secreted by SARS-CoV-2–specific T cell in symptomatic individuals. Figure is reprinted with permission (Le Bert et al., 2021).

durability of the response, some patients lost responses to specific antigens after viral clearance. As others have reported, there was evidence that this decline was greater in asymptomatic than symptomatic individuals, and as a function of declining antibody responses to N. However, the majority of asymptomatic individuals did maintain readily detectable T cell responses against multiple antigens, and their T cells actually made more IL-2 and IFN-γ than those from symptomatic individuals.

The obvious utility of these data is in understanding the influence of disease severity on the quantity and quality of anti–SARS-CoV-2–specific immunity generated. This is actually not a trivial accomplishment, mostly because validating any infection as truly asymptomatic (and not just "presymptomatic") is far from straightforward. The present study by Le Bert et al. (2021) accomplished this by enrolling migrant workers quarantined in a dormitory in Singapore, allowing them to establish the general timing of seroconversion and related symptomology; each participant was monitored twice daily for temperature and pulseOx readings and evaluated by medical posts within the dormitory. Further, each worker "received a monetary compensation provided by the Singapore government," hopefully avoiding any "economic incentive to report an asymptomatic state for fear to economical loss." With this as the background for the study, the data strongly support the conclusion that asymptomatic infections may indeed generate protective immunity, especially when one takes into account the durability of both neutralizing antibody titers as well as T cell frequencies over the course of the study.

Their results also support the general longevity of anti–SARS-CoV-2–specific immunity after infection. Since the beginning of the pandemic, the durability of protective immunity after COVID has been questioned and scrutinized. These concerns were bolstered by early emerging pandemic-related data that antibody responses were declining precipitously after recovery from COVID (Cervia et al., 2021; Long et al., 2020). Though later, more comprehensive analysis of samples from >30,000 patients largely dispelled the early notion of catastrophic loss of protective immunity (Wajnberg et al., 2020), concerns remain, not only for immunity after COVID but also after vaccination as well. Most early results relied on the analysis of serum antibody titers, leaving undetermined the certain role for SARS-CoV-2–specific T cell responses in maintaining host defense. The data here provide that information along with how it is influenced by disease severity. The authors did find reduced T cell responses in asymptomatic individuals at later time points after infection, but no differences early after infection. As stated by Le Bert et al. (2021), these data "further support the accumulating evidence that the quantity of SARS-CoV-2–specific T cells is not proportional to disease severity during the early phase of infection." Secondarily, though statistically reduced over time relative to their symptomatic counterparts, the T cell responses in asymptomatic individuals were by no means negligible and showed enhanced cytokine production, as noted above. Though the question of protective immunity cannot be definitively answered in these data, they are sufficient to provide some confidence in reasonably lasting cellular immunity, even in post-asymptomatic infected individuals.

As noted above, the longevity of SARS-CoV-2–specific antibody responses has been under great scrutiny, and the results presented here indicate that the answer to that question depends on what one is looking for. Immunity often wanes after infection as a general rule, and the response to coronavirus is clearly no different. The authors' data agree with those of others in that anti-N IgG declines over time, even more so in asymptomatic individuals (Cervia et al.,

2021; Long et al., 2020). This seems to indicate some relationship between infection symptomology/pathology and durability of antibody levels. However, these previous reports also noted differences in the rate at which the antibody response to N or the spike protein change over time. In the present report, Le Bert et al. (2021) took the approach of monitoring virus neutralization as a surrogate for spike-specific antibodies. Counter to the results for N-specific antibody levels, neutralization titers remained stable over time regardless of infection symptomatology. This is not only good news for the prospects of durable protective immunity, it is also consistent with the hypothesis that there may be ongoing affinity maturation in response to residual antigen after viral clearance, leading to improved quality of the antibody over time. The greater decline in N-specific responses for asymptomatic individuals might then indicate that the severity of disease contributes more to the amounts of residual nucleocapsid antigen than to spike.

Lastly, the data presented here imply that perhaps estimates of the frequency of asymptomatic infections depend on what part of the planet you are from. Le Bert et al. (2021) state that out of all of their seropositive individuals, 93% reported as asymptomatic. All subjects most likely to present as a symptomatic infection (older than 65, having hypertension or diabetes) were moved into other facilities, a fact that almost certainly influenced their high frequency of asymptomatic infection to some degree. Even if those individuals had been included, however, it seems unlikely it would have produced an asymptomatic rate more commensurate with the current consensus, somewhere around 20% (Rasmussen and Popescu, 2021). All subjects in this study were men from either India or Bangladesh, both locations in which the COVID-related fatalities, while terrible, have not been as high as in the US, especially when correcting for population size and density. Similar observations have recently been made for sub-Saharan and western Africa (Lawal, 2021), which have seen far fewer per capita fatalities as compared with Europe and the US. This has prompted speculation that elevated preexisting/cross-reactive immunity may exist in different parts of the world. If true, then this might logically be expected to produce a higher frequency of asymptomatic infections from these regions. While yet to be determined, the availability of pre–COVID-19 samples from multiple parts of the world would make this hypothesis highly testable and amenable to study.

Collectively, these data raise our expectations for the quality and durability of immunity after asymptomatic infection. This study was performed early on in the pandemic, so there are no data on, nor any real expectation of, cross-protective antibodies against the newer variant strains. However, as the new variant strains have fewer mutations outside of the spike protein, the persistence of T cells specific for multiple SARS-CoV-2 antigens in both symptomatic and asymptomatic individuals might support the expectation of at least some degree of T cell cross-reactivity, and therefore cross protection. As we move into the next, and hopefully last, phase of the present pandemic, contrasting the durability of both humoral and cellular immunity to SARS-CoV-2 after infection, vaccination, or both will provide important insights for managing future emerging infections.

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