# SARS-CoV-2 immunity and reinfection

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**Commentary**

In the accompanying article, Selhorst et al. describe a case of symptomatic reinfection in a young immunocompetent healthcare worker 185 days after primary symptomatic SARS-CoV-2 infection [1]. The timeline appears consistent with other reports of reinfection in the literature and lay press [2-7]. These cases illustrate several important issues regarding SARS-CoV-2 immunity, and the authors have done a nice job to make this particular case instructive.

Establishing reinfection or recrudescence of SARS-CoV-2 is not a simple feat. A few case reports have demonstrated phylogenetic confirmation of reinfection [5-7]. It is well established that viral RNA can be detected in the nasopharynx many months after initial infection, particularly in immunocompromised individuals. Demonstrating reinfection necessitates phylogenetic analyses to confirm that a virus detected during subsequent illness is a unique variant. This is made even more difficult by the relatively slow evolutionary rate of SARS-CoV-2, driven by the proofreading ability of SARS-CoV-2 viral polymerase complex [8]. The ability to limit evolution means that unique viral lineages may differ only by several nucleotides. In this case, whole genome sequencing identified 18 nucleotide differences between the initial and the subsequent infection, strongly supporting reinfection. However, as the epidemic progresses, many new lineages are coming into existence. We are already seeing some lineages that have modified potential epitopes, and we may soon see “escape” variants with adaptation to human or vaccine derived immune responses [9-11].

To determine if reinfection has occurred, it is essential to assess if the individual first developed a SARS-CoV-2-specific immune response. These types of analyses include (1) enzyme linked immunosorbent assays (ELISAs) to quantify antibody titers to SARS-CoV-2 proteins, (2) neutralizing assays to evaluate the potency of SARS-CoV-2 antibodies to neutralize virus, and (3) assays to quantify SARS-CoV-2 $CD4^+$ and $CD8^+$ T cell responses [12-14]. Selhorst et al. utilized a pseudovirus neutralizing assay to determine if their patient’s antibodies could neutralize SARS-CoV-2 spike protein [14, 15]. The authors first tested a neutralizing response at Day 94 post symptom onset. Unfortunately, there was no blood sample taken at 3 to 4 weeks post primary infection to quantify a peak neutralizing titer. The standard neutralizing assay is a live virus assay which evaluates the ability of any SARS-CoV-2 antibody to neutralize the live virus [15, 16]. Given the robust correlation between anti-Spike IgG and anti-Receptor Binding Domain (RBD) IgG with neutralizing antibody titers, the surrogate pseudovirus neutralizing assay is a suitable alternative to evaluate for

the development of neutralizing antibodies [14, 17]. As the pseudovirus neutralizing assay evaluates antibodies that only interact with the viral RBD within the Spike protein, this assay neglects any neutralizing antibodies generated against other SARS-CoV-2 proteins.

Long term protective immunity to viral infection, in part, requires continued presence of virus-specific B cells to generate neutralizing antibodies. While infection with some RNA viruses provides long lasting immunity (e.g, measles, polio), protection is much more limited for other viruses (e.g. influenza). The immune response to common cold coronaviruses (OC43, HKU1, NL63, and 229E) wanes by four months to one year post infection, permitting reinfection [18, 19]. Determining the duration of the immune response is critical for understanding protection [20, 21]. In SARS-CoV, neutralizing antibody titers persisted in some individuals for more than 200 days post symptom onset [22, 23]. For SARS-CoV-2 infection, more severe the illness has been associated with a greater the neutralizing antibody titer [24]. In the accompanying paper, the authors noted that the patient had an initial mild illness for which she was managed as an outpatient. At Day 94 post infection, she had a detectable neutralizing antibody titer. Upon reinfection, the patient developed a more than 6-fold increase in neutralizing antibody titer at Day 7 post reinfection. As a surrogate for neutralization, the authors showed elevated RBD IgG titers at Day 94 post primary infection, which doubled at Day 7 post reinfection. Interestingly, the nucleocapsid IgG remained comparable both pre-reinfection and post-reinfection. Though antibody titers eventually wane with any disease, SARS-CoV-2-specific memory B cells persist [20, 25] to quickly generate an antibody response leading to milder disease [26] , as in the case of this 39 year old healthcare worker. We are only beginning to understand the expanse of SARS-CoV-2 immunity.

How a circulating neutralizing antibody titer translates to mucosal protection for SARS-CoV-2 remains unknown [27]. Whereas circulating IgA exists predominantly as a monomer, secretory IgA exists as a dimer and patrols the mucosal epithelium to prevent inflammation and/or injury by microbes [28]. For SARS-CoV-2, serum anti-Spike and anti-RBD IgA correlate with saliva anti-Spike and anti-RBD IgA [29]. With regards to SARS-CoV-2 severity, viral levels are similar between patients with mild and severe/critical illness presentations, suggesting that the host mucosal immunity may dictate disease severity [30]. Patients with mild SARS-CoV-2 disease have detectable mucosal anti-SARS-CoV-2 IgA in tears, nasal fluid, and saliva with anti-SARS-CoV-2 secretory IgA inversely correlating with age [31]. Of interest, experimentally derived anti-SARS-CoV-2 dimeric IgA was 15x more potent at neutralizing SARS-CoV-2 than monomeric anti-SARS-CoV-2 IgA [32]. Overall, this suggests the importance of mucosal IgA in preventing reinfection and perhaps mediating disease severity.

Finally, the major questions on everyone's mind are (1) how long will immunity with the SARS-COV-2 vaccine last and (2) will vaccine induced immunity be inferior to immunity from primary infection. Current FDA approved SARS-CoV-2 vaccines include pegylated lipid nanoparticles containing mRNA encoding the Spike protein developed by Pfizer and Moderna [33, 34]. Thus far, the duration of the immune response following mRNA vaccination has lasted up to 3 months post vaccination, as measured by neutralizing antibody titers to the Spike or RBD proteins [35]. Pegylated lipid nanoparticles encase the mRNA to facilitate uptake by the innate immune system while preventing rapid degradation of the mRNA encoding vaccine antigen [36]. Of note, the induction of mucosal Spike IgA following vaccination has yet to be established. Future SARS-CoV-2 vaccines may utilize other techniques to enhance antigen presentation and the duration of the immune response, including different adjuvants, increased valency for protein-based vaccines, and antigen persistence for viral vector-based vaccines. The optimal approach still remains an open question.

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