Review of: "A Single Dose of COVID-19 mRNA Vaccine Induces Airway Immunity in COVID-19 Convalescent Patients"

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Martinuzzi et al. provide an explanation, based on due consideration of the operation of the mucosal immune system, for the observation that vaccination of subjects who were previously infected with SARS-CoV-2 develop stronger immunity to COVID than uninfected subjects, with enhanced mucosal IgA antibody responses in nasal fluids.

Ironically it is still under-appreciated that, although SARS-CoV-2 is primarily an infection of the upper respiratory tract (URT), it induces mucosal immune responses in the form of secretory IgA (SIgA) antibodies. This has been repeatedly demonstrated in several reports that show IgA antibodies to the virus in secretions collected from infected individuals, including saliva, tears, nasal fluids, tracheo-bronchial fluids, and even breast milk of lactating women (1-7). Instead, attention has overwhelmingly been focused on the circulating antibody responses represented mainly by IgG. In addition, the vaccines developed so far are all administered by parenteral injection, with the objectives of inducing circulating, virus-neutralizing IgG antibodies (“seroconversion”) as well as anti-viral cytotoxic T cells, and to alleviate serious disease and prevent death. The reasons why these efforts have been successful are that the severe acute respiratory syndrome known as COVID-19 develops when the virus descends into the lower respiratory tract (LRT) and establishes infection of the lungs with dysregulated inflammatory responses that are a major factor in the pathology. The dominant immunoglobulin isotype found in the terminal airways and alveoli is IgG, derived from the circulation largely by passive transudation. Thus virus-neutralizing plasma IgG antibodies can have a major beneficial impact on the pulmonary viral infection, facilitate recovery, and forestall death – all highly desirable outcomes especially in the early phases of the pandemic. A downside is that IgG antibodies can also be inflammatory by activating complement and by recruiting and activating neutrophil phagocytes, both of which contribute to COVID pathology.

Since the introduction of COVID vaccines in 2021, it has become clear that “breakthrough” infections can occur, even in well-immunized individuals. However, it has also been observed that immunization is more effective in preventing infection in subjects who were previously infected with SARS-CoV-2 (8-10). Reasons advanced for this observation have included enhanced induction of cellular immunity represented by cytotoxic T cells, and even the ”training” of innate immune mechanisms. What has been unaccountably neglected is consideration of the role of mucosal priming in enhancing protective

immune responses (see Science eLetter published July 17, 2021: https://www.science.org/doi/10.1126/science.abj2258). This important preprint by Martinuzzi et al. (11) provides a mechanistic explanation for these findings.

This study enrolled 20 subjects who were convalescent from COVID-19 (“infected group”) and 23 well-matched healthy individuals who had not been previously infected (“naïve group”), all under informed consent. Both groups were immunized with the Pfizer BNT162b2 mRNA vaccine, the infected group with one dose, and the naïve group with two doses at the prescribed 3-week interval. Antibody responses were monitored in plasma collected on days 0 (first immunization), 7, and 21, and in nasal fluids collected on days 0 and 21, and also on days 28 and 42 for the infected group. Infected subjects, but not the naïve subjects, displayed IgA and IgG antibodies to SARS-CoV-2 spike protein in both plasma and nasal fluids prior to immunization. A single dose of the vaccine was sufficient to elevate spike-specific IgA and IgG antibodies in nasal fluids in most of the infected subjects, and virus-neutralizing activity was detected. Some of the naïve subjects also developed nasal IgA antibodies, after two doses of the vaccine. Antibodies to the receptor-binding domain of the spike protein paralleled the responses to the whole spike protein. Plasma IgG and IgA antibody responses were similar in both groups.

Because the mucosal immune system operates by the dissemination of precursors of IgA-secreting plasma cells from mucosal inductive sites to mucosal effector sites through the circulation, the authors postulated that virus-specific IgA antibody-secreting cells (ASC) should be detectable in the blood approximately one week after immunization, as previously demonstrated (12). Accordingly they found elevated numbers of virus-specific IgA ASC in the blood of infected subjects 7 days after the single immunization dose. Importantly, these IgA ASC expressed integrin beta7, which forms part of the homing receptor, alpha4beta7 that recognizes the addressin, MAdCAM-1, which is selectively expressed on high endothelial venules in mucosal tissues (13). Naïve subjects did not develop beta7+ IgA ASC even after the second immunization. The infected subjects also displayed higher numbers of circulating IgG ASC than the naïve subjects in response to vaccination.

Thus this study demonstrated that subjects who had previously been infected with SARS-CoV-2 were mucosally primed to respond promptly to a single dose of the Pfizer vaccine, with the production of mainly IgA (and also IgG) anti-SARS-CoV-2 antibodies in their nasal fluids, most likely from ASC displaying mucosal homing characteristics. Naïve subjects given the scheduled two doses of Pfizer vaccine did not develop these mucosal SIgA antibody responses to the same degree, and did not show circulating virus-specific IgA ASC with mucosal homing characteristics.

The significance of these findings lies in two important aspects: (i) that initial infection by SARS-CoV-2 might be effectively prevented by means of SIgA antibodies in nasal (and oral) fluids, where the virus is first acquired; (ii) that transmission of the infection is through virus-containing droplets and aerosols generated from the nose and mouth, their respective secretions being the vehicles of transmission. If these fluids contain anti-viral antibodies, especially SIgA, which has been demonstrated to be highly effective in viral neutralization (14), then the infectivity of these emissions will be substantially

reduced. Now that vaccines have been successfully introduced to prevent serious disease and death, which is what they were primarily designed to accomplish, control of the pandemic will increasingly depend on preventing transmission of the virus. The report by Martinuzzi et al. (11) shows that this can be advantageously achieved by vaccinating previously infected subjects who have been primed to mount effective recall mucosal antibody responses. For subjects not previously infected, this implies that initial mucosal immunization, for example by the intra-nasal route, might be more effective at inducing the desirable SIgA anti-viral antibodies in the nasal fluids and saliva, where the virus is first encountered. Subsequent parenteral vaccination might then be used to recall the mucosal antibody responses, or possibly booster mucosal immunizations might be given.

Several efforts are in progress to develop intra-nasally administered COVID vaccines, which can be expected to induce superior protective mucosal immunity to systemically injected vaccines, even in naïve subjects. However, none have yet advanced into Phase III clinical trials. Conventional systemic immunization is usually poorly effective at inducing SIgA antibodies in mucosal secretions, most of which contain very little IgG anyway. By far the predominant type of immunoglobulin in most human secretions is SIgA, mainly a dimeric form of IgA associated with the secretory component, by means of which it is selectively transported across mucosal epithelia into the secretions, having been locally synthesized by plasma cells resident in the lamina propria and secretory glands. Thus the mucosal immune system is largely separate from the circulatory immune system, and has its own distinct populations of cells that are induced in specialized immune inductive sites such as the intestinal Peyer’s patches and Waldeyer’s ring of tonsils and adenoids (15).

We have argued that mucosal immunity to SARS-CoV-2 has been unduly neglected, but is essential for understanding the course of the infection, which in many cases appears to remain relatively mild and limited to the URT (16). It is clear that mucosal IgA antibodies are induced by SARS-CoV-2 infection, although their quantitative impact has not be adequately addressed.

Several additional aspects of this paper by Martuzzi et al. (11) deserve comment:

It is noteworthy that nasal fluid antibodies were normalized against total corresponding IgA and IgG concentrations. This is critically important for the quantitative evaluation of mucosal antibody responses. Unlike plasma which is maintained by homeostatic mechanisms to keep immunoglobulin concentrations within normal limits over time and between individuals, the immunoglobulin concentrations in secretions can vary considerably between and within individuals, even over short time periods, due to a variety of stimuli that affect not only local immunoglobulin production and transport, but also the secretion flow rate which inversely affects their concentrations. Unless these effects are compensated, erroneous and misleading results will be obtained.

Nasal fluids were collected by the insertion of absorptive swabs. It is possible that this procedure may have increased the contribution of plasma-derived IgG (and IgA) to the fluid collected, because it has been demonstrated that even seemingly mild absorptive swabs can sufficiently irritate delicate epithelia to increase the exudation of tissue fluids, even in the absence of overt bleeding. One way to assess this is to

assay serum albumin, which is not normally present in secretions. Alternatively, fluids should be collected by washing procedures which are less prone to disrupt the epithelia. While washing introduces an unknown dilution factor, this can be compensated by assaying total immunoglobulin concentrations, as mentioned above.

It would be interesting to assay the antibody responses also in saliva, which is easily collected, although appropriate measures should be taken to minimize contamination with blood-derived materials, and to correct for substantial variation in total immunoglobulin content, as mentioned above. It would be expected that SIgA antibody responses to parenteral immunization of previously infected subjects should also be found in saliva, similar to those in nasal fluid. Saliva is of potentially huge importance as a vehicle for SARS-CoV-2 transmission, by the emission of droplets and aerosols even during normal speech, but more so during shouting and singing.

It would be interesting to know if plasma IgA antibodies induced by vaccination of previously infected subjects were polymeric or monomeric. Besides being of relevance to the source of such antibodies within mucosal as opposed to systemic compartments, polymeric IgA antibodies are much more effective at viral neutralization, including against SARS-CoV-2, than are monomeric IgA antibodies (14,17,18). Similar considerations might apply also to IgA subclasses, since IgA2 is enriched in secretions where it may contribute up to 50% (or more in some secretions) of total IgA, whereas plasma IgA is predominantly IgA1. Although few functional differences have been demonstrated between IgA subclasses, some differences have recently been ascribed to their different glycosylation patterns (19).

The authors correctly mention the limitations of the study, which include a relatively small number of study subjects. Nevertheless, these findings align well with what is already known about the operation of the human mucosal immune system. It is also true that relatively little is known about the immunoglobulin composition and functions of nasal fluids which have been inadequately studied. However, their potentially huge importance for comprehending SARS-CoV-2 infection and transmission should encourage further study. Finally, the kinetics of the responses induced by immunization of previously infected subjects deserve further study, from the perspectives both of determining the persistence of immune protection against COVID, and of better comprehending memory within the mucosal immune system.

Overall, however, this paper contributes novel and important insight into a neglected area of immunity to SARS-CoV-2 infection. Further studies are awaited with great interest.

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