A summary of the latest evidence about SARS CoV2 Immunology:

3rd June 2020 Wendy Barclay

Antibody response and implications for testing, vaccines and antibody therapies.

It is now apparent from many studies around the world that most, if not all, people infected with SARS CoV2, even if only a mild infection, make an antibody response to the major antigens that include the spike protein S and the nucleoprotein N. Previous reports that a high proportion of those with mild disease fail to show a response were based on earlier versions of the antibody test, that have now been superseded.

Many different assay are reported that measure these antibodies including ELISA assays in laboratories and point of care type assays such as lateral flow tests. (Suthar et al. BioRix; Amanat et al Nature Medicine 2020; Wajnberg et al MedRix 2020).

It is still not clear how long naturally induced antibodies will persist. There is no evidence in the public domain on longitudinal studies documenting antibody titres in recovered people over time.

There is still no evidence that the immune response protects people from reinfection, largely because not many people have been re-exposed yet. Limited descriptions of people testing positive for a second time are more likely to be from persistent shedding of viral RNA rather than new infections although sequencing is required to confirm this.

However, recent evidence from animal models (hamster or mouse) showed that antibodies against spike protein administered passively protected against subsequent challenge with SARS CoV2 virus (Rogers et al. biorix; Wu et al. Science 2020). This was the case when the antibodies were neutralizing, (ie in *in vitro* assays, they protect cells from infection by the virus by blocking the interaction between the spike receptor binding domain and the ACE2 receptor). However, other antibodies that bound to spike protein but did not neutralize in in vitro tests, did not protect against infection.

Most people make a polyclonal response after virus infection that targets many different proteins and epitopes within them (Brouwer et al. biorix). Thus we expect that most people will make neutralizing antibodies that are protective against reinfection. However the specific protective (neutralizing) antibodies are not measured in all the different antibody tests that have been developed so far. For example some tests measure antibodies against N protein only.

Some of the latest serological tests do measure antibodies that target the spike receptor binding domain specifically, or even measure the antibody's ability to disrupt the interaction between spike and ACE2 receptor (Amanat et al. Nature Medicine 2020; Quinlan et al BioRix. 2020). A positive, high titre result in these tests would give some reassurance that an individual was 'immune' and unlikely to be susceptible to reinfection.

However we still don't know what titre of neutralizing antibody is required for immunity (the animal studies give us some clues), and we don't know how long the antibodies persist, and whether they will be effectively boosted by a memory response on re-exposure. We also need to know if the antibody in the blood (or in oral fluids as new tests are developed) is relevant to protection against lung disease (which appears probable) and against infection of the nose and pharynx (less likely).

The success of the passively transferred antibodies paves the way for antibody therapy either with convalescent plasma or with cloned antibodies, although we need to be cautious about using single antibodies that might drive virus escape (see for example ter Meulen PNAS 2006).

Neutralizing antibody or even other antibodies might be correlates of immunity that can be measured to indicate vaccine efficacy. Early results from non human primate vaccine and challenge studies performed on the chAdOx Oxford vaccine showed low levels of antibody and some protection against disease but not against infection in the upper airway (van Doremalen et al. MedRix 2020). However, the studies were performed very early after vaccination and with a high dose of challenge virus delivered both to the upper and lower airway. This design of the study was inherently likely to give suboptimal protective signal.

Early results from the Imperial vaccines study in mice demonstrate very high levels of neutralizing antibody (McKay et al BioRix. 2020).

The implications of these recent findings on SARS CoV2 antibodies are:

1. **Tests that measure antibody responses can indicate how many people have been infected.**
2. **Tests that measure neutralizing antibody responses might indicate the level of 'immunity' to reinfection or disease in the population.**
3. **Vaccines that induce good neutralizing antibody titre in serum are likely to confer protection.**
4. **Therapies that transfer neutralizing antibodies are likely to show efficacy if sufficient levels of antibody are given.**

<u>T Cell response:</u>

To generate an antibody response, it is essential that the specific B cells are stimulated by cytokines secreted from neighbouring specifically stimulated T helper (CD4+) cells. The T cells respond to antigen presenting cells that present fragments of virus (T cell epitopes) in conjunction with MHC class II. T cell epitopes have recently been described for SARS CoV2 (Grifoni et al Cell 2020) and are located in spike, nucleoprotein and M proteins as well as in other non-structural viral proteins.

The presence of CD4+ T cell epitopes in spike protein suggests that vaccines that consist only of spike will elicit an immune response but it will differ from that after natural infection.

A substantial number (>40%) of people unexposed to SARS CoV2 were found to have CD4+ T cell responses that were likely elicited by infection with previous seasonal coronavirus infections (Grifoni et al, 2020). Pre-existing CD4 reactivity was shown to moderate influenza outcome during the pH1N1 2009 pandemic (Sridhar e al 2013) and the detection of cross reactive CD4 cells that respond to SARS CoV2 may explain some of the variability in COVID.

CD8+ T cells responses were also detected in people recovering from SARS CoV2 infections. Some reacted to spike proteins, but several other proteins were more dominant. Nonetheless spike only vaccines can likely elicit CD8 responses as well as antibody responses.

In another study CD8 + T cells were present in lungs of people with mild but not with severe disease suggesting they may modify disease outcome (Bost et al Cell 2020). Vaccines that direct expression

of spike protein from RNA or recombinant virus may elicit CD8+ T cells responses. Purified spike protein vaccines are unlikely to elicit CD8 responses unless formulated with an adjuvant that promotes such responses. The protective value of CD8 responses is currently undetermined.

Implications of these recent findings about T cell immunity are:

1. **People with pre-existing T cell immunity generated after infection with seasonal coronaviruses may experience less disease, which might explain some of the variation in outcome.**
2. **T cell epitopes have been identified in the spike protein and vaccines might be formulated to induce such responses. However, a T cell vaccine could also include many other viral proteins.**

Harmful 'Immune' responses

<u>Antibody dependent enhancement of disease:</u>

There has been some concern around antibody dependent enhancement of disease (Iwasaki and Yang 2020). There is still no robust evidence of this for SARS CoV2. In one paper using passive transfer of antibodies to hamsters there was a small signal of increased weight loss over control for the groups receiving low dose antibody (Rogers et al. BioRix 2020). Numbers were too small to conclude this was ADE and no mechanistic was performed.

<u>Proinflammatory response:</u>

Severe COVID is associated with very high levels of inflammatory chemokines such as IL-6 (Herold et al. MedRix 2020). This can then lead to downstream pathology such as blood clots. Use of the anti-IL6 antibody as a treatment (Tocilizumab) is based on this concept.

Transcriptomic studies suggest SARS CoV2 drives a high proinflammatory response whilst very effectively suppressing the antiviral interferon response (Blanco-Mello et al. Cell 2020). This may imply that SARS CoV2 infections might pave the way for coinfection by other viruses. Indeed some coinfections with influenza and SARS CoV2 have been reported (Promed mail).

Implications of these findings:

1. **ADE might be important but there are no robust data as yet.**
2. **Severe COVID is associated with harmful chemokine responses and this justifies immunomodulatory therapies in severe COVID.**
3. **Because all viruses manipulate the immune system to some extent but by different mechanisms, SARS CoV2 may predispose to worse outcomes during consecutive or concurrent infection by other respiratory viruses. These concerns justify efforts to ensure good uptake of influenza vaccines this coming winter.**

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