

Update on Immunity to SARS CoV2

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Antibody responses.

Key questions are:

1. What type and what levels of antibody response confer protection from infection or disease?
2. Can people get reinfected with SARS CoV2 and if so, do they have disease and can they transfer virus onto others?
3. Is the virus evolving in any way that might impact the efficacy of current vaccines that are designed on early strains?
4. If antibodies are protective, how long will protection last?

Viral antigens N and S

The SARS CoV2 virus has a large positive sense RNA genome, that is associated in the virus particle with many copies of a virally encoded nucleocapsid protein, N. The virus particle has an external envelope with lipids derived from the host cell on which many copies of a trimeric spike protein, S, are displayed.

S is the virus attachment protein through which virus binds to target cells, by interacting with the primary receptor, ACE2. The domain of S that interacts with ACE2 is known as the receptor binding domain RBD. Since the viral antigens are foreign proteins, people infected by SARS CoV2 generate an immune response and make antibodies that bind N and S, and other viral proteins.

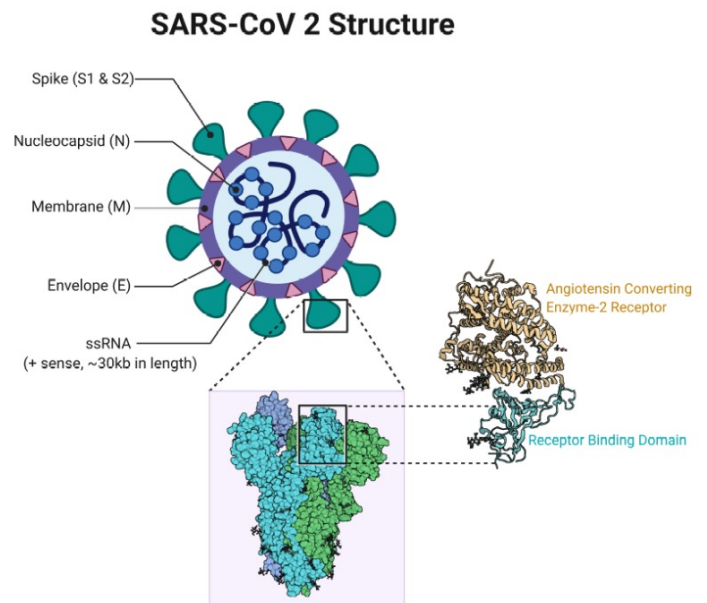


Figure 1. SARS- CoV 2 Structure. Contributed by Rohan Bir Singh, MD; Made with Biorender.com

The presence of antibodies specific for SARS CoV2 viral proteins therefore indicate that a person has been infected by the virus. Tests that measure antibodies to N or S can be used in seroprevalence studies. Antibodies that bind the RBD of S and block the ability of virus to attach to target cells are most likely to neutralize virus infectivity.

Serological responses to SARS-CoV-2.

Early studies suggest SARS-CoV-2 behaves similarly to SARS-CoV and MERS-CoV. In a study of 173 people, the seroconversion rate for total antibody to the spike receptor binding domain (RBD) was 93.1% (161/173), and for IgM to the Spike RBD was 82.7% (143/173) and IgG to the nucleoprotein 64.7% (112/173). The median time to seroconversion for total antibodies was 11 days, IgM 12 days and IgG 14 days, although some of these differences could be due to ELISA assay format. For samples collected between 15-39 days from disease onset, seroconversion for total antibodies was detected in 100%, IgM in 94.3% and IgG in 79.8% of patients to the RBD and nucleoprotein respectively.¹⁰

Several studies have shown a trend of higher antibody levels with severe compared to mild disease, but this may depend on the assay used to quantify antibody responses. This effect was not seen by Wajnberg *et al*, in whom over 99% of the patients with self-reported or laboratory documented infection developed IgG (FDA approved 2 step ELISA) in a community cohort with mild disease. Their findings suggest IgG developed over 7-50 days from symptom onset with a median of 24 days, suggesting the optimal testing for is 3 -4 weeks post symptom onset and at least 2 weeks after symptom resolution.

Further work UK work from the National COVID-19 Scientific Advisory Panel which included mild, severe and asymptomatic patients (Figure 2) detected IgM or IgG in 34/40 individuals with a confirmed history of COVID infection (sensitivity 85%, 95%CI 70-94%), vs. 0/50 pre-pandemic controls (specificity 100% [95%CI 93-100%]) and demonstrated high sensitivity for IgG from day 10 following symptom onset.¹¹

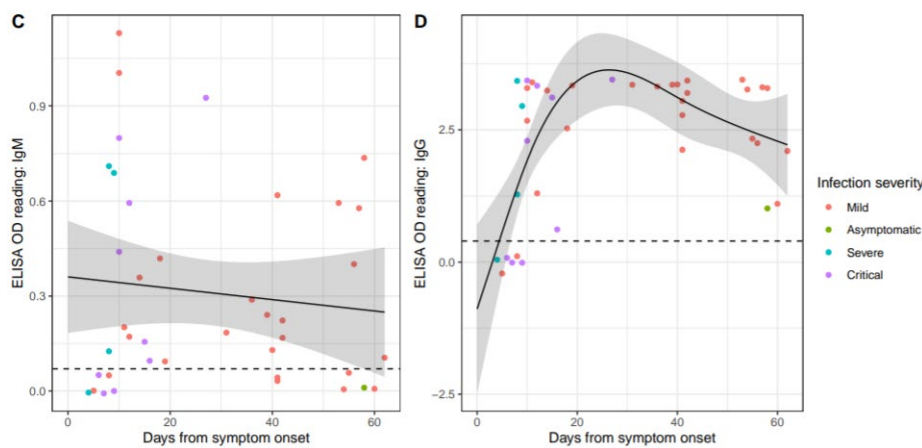


Figure 2

In a comprehensive summary on the humoral immune responses it was noted that antibody responses are detected in most individuals between 10-14 days after infection.¹²

A recent large study from Iceland reported that 25 days after diagnosis by qPCR, 1107 of the 1215 COVID-19 patients (91.1%) were seropositive [[NEJM Sept 01 2020](#)]. Over 90% of qPCR-positive persons tested positive with two pan-Ig SARS-CoV-2 antibody assays and remained seropositive 120 days after diagnosis, with no decrease of antibody levels (Figure 3). SARS-CoV-2 antibody levels were higher in older people and in those who were hospitalized.

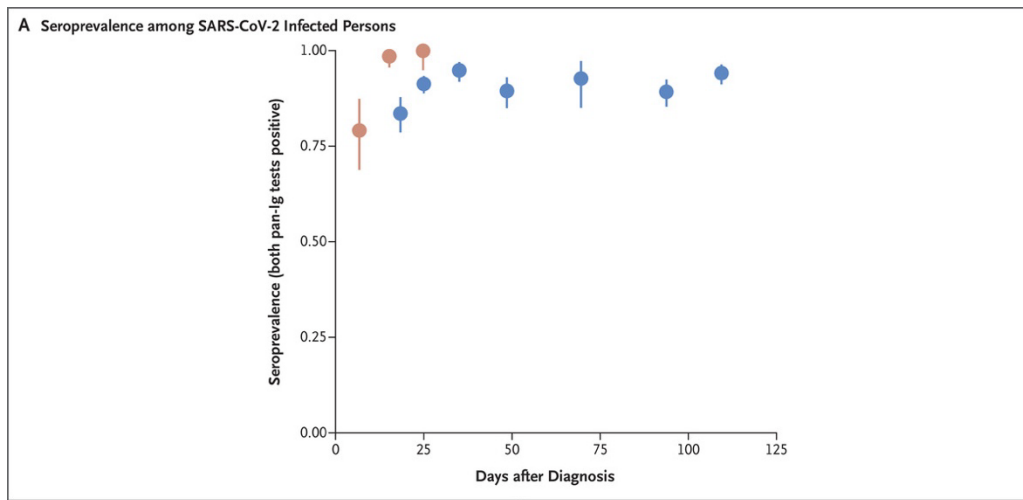


Figure 3. Antibody Prevalence and Titers among qPCR-Positive Cases as a Function of Time since Diagnosis by qPCR. DF Gudbjartsson et al. N Engl J Med 2020. DOI: 10.1056/NEJMoa2026116

Neutralising antibodies are associated with reduced infectivity

In a study of virus shedding and antibody in 129 hospitalized COVID-19 patients (89 intensive care, 40 medium care), van Kampen et al <https://doi.org/10.1101/2020.06.08.20125310> measured viral RNA, subgenomic viral RNA, serum neutralizing antibody. Infectious virus detected in 23/129 cases (18%) with a median shedding duration 8 days after symptom onset (IQR 5 – 11). Infectious virus found in <5% after 15.2 days post onset of symptoms. Samples with $>10^7$ RNA copies/mL SARS-CoV-2 most likely infectious and serum neutralising antibody of $>1:20$ predicted non-infectiousness

They conclude that patients with severe/critical COVID-19 shed infectious virus for longer, but that infectious virus is undetectable once serum neutralizing antibodies are present.

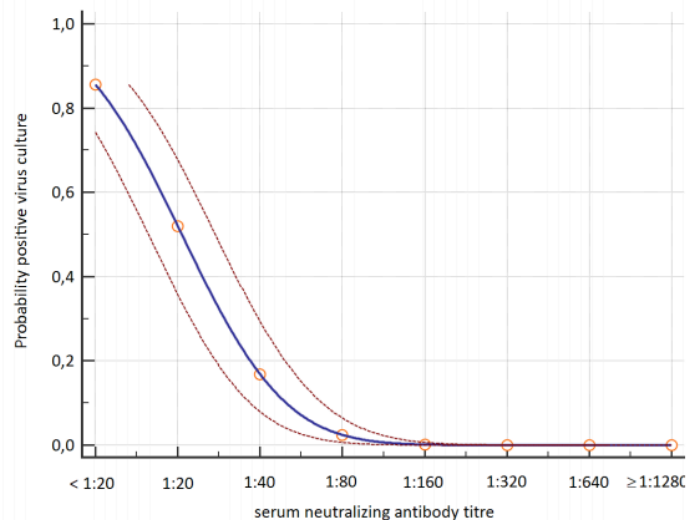


Figure 3. Probit analyses of the detection of infectious virus in respiratory samples with cell culture by serum neutralizing antibody titer. Blue line represents the probit curve and the dotted red lines represent the 95% confidence interval. Circles are marker points. Serum neutralizing antibody titers are expressed as plaque-reduction neutralization titers

Neutralising antibodies mediate protection in animal studies

In animal models, the presence of neutralizing antibodies correlates with protection. Animals that had been previously infected and mount a polyclonal antibody response (i.e. diverse antibodies against different sites/proteins) that includes neutralizing antibody did not become infected when re-challenged after 28 days (Imai et al PNAS.2020).

Animals to which single (monoclonal) neutralizing antibody had been passively transferred are also protected from infection at high doses of transferred antibody, and from disease at lower doses. This indicates that neutralizing antibodies are necessary and sufficient for protection, at least in those animal models. This concept underpins the use of therapeutic antibodies or convalescent sera for treatment of patients with COVID.

What is not currently known is what level of antibody is required to confer protection in humans against a natural dose of SARS CoV2, such as would be faced during a transmission event.

Do neutralising antibodies mediate protection in humans?

Some recent preprint releases and publications shed some light on these issues:

1. *The Seattle fishing boat incident:*

<https://www.medrxiv.org/content/10.1101/2020.08.13.20173161v1.full.pdf>

An outbreak on a fishing vessel that departed from Seattle with 122 crew who agreed to testing

Crew were assessed for virus by RT-PCR and antibody by Abbott Architect assay that detects antibodies to SARS CoV2 N protein before departure. 6 crew members had N antibody above the cut off value of 1.4.

Three of these were amongst the total of 104 who became infected during the outbreak (85% infection rate). Their N antibody scores were the lower than the crew members who resisted infection. When tested with antibody assays that measure neutralizing antibody against the Spike protein RBD, these 3 infected individuals lacked antibody to S.

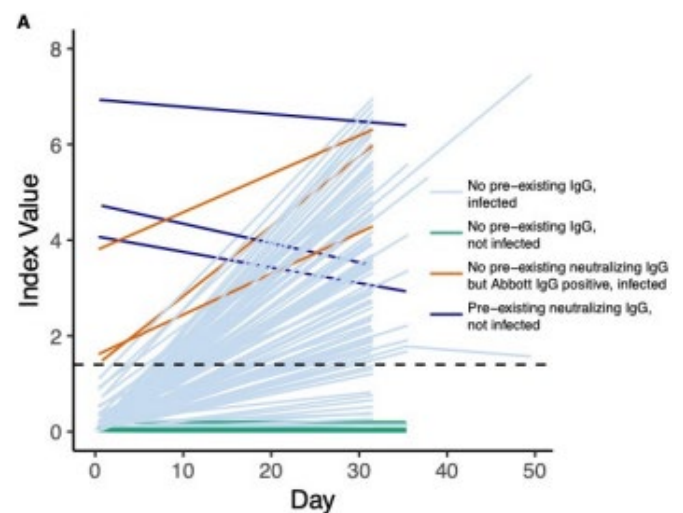


Figure 4: Antibody levels before and after the fishing trip in relation to susceptibility to infection

It is possible that the 3 individuals with low level antibodies were infected at the time of ship departure, but were negative on PCR (as are some COVID cases). They had made a rapid N antibody response but not yet made an S antibody response.

However, their Ct scores 18-21 days later when the ship returned were indicative of high viral loads, ranging from 17 to 23, suggesting they were shedding high levels of virus at 3 weeks after infection, a kinetic that is not seen in other studies. Alternatively, the initial N antibody tests might have been false positives or that they had been previously infected with SARS CoV2 but with low levels of seroconversion or waning antibody that allowed reinfection. There was no documentation of clinical

status or of contact patterns on the ship and it is not known if the 3 potentially reinfected individuals were symptomatic.

On the other hand, the 3 other people with high levels of N antibody prior to departure also had neutralizing RBD antibodies and remaining uninfected despite high levels of exposure on board the boat, (as did 15 other crew members). This was a highly significant finding (Fisher exact test $p=0,002$) suggesting that these robust titres of neutralizing antibodies conferred sterilizing immunity and protected from infection. This study suggests that a neutralizing antibody titre of 1:160 was sufficient to confer protection from infection during natural exposure, but is based on small numbers of cases.

2. The Hong Kong reinfection event and potential for antigenic drift

The first confirmed reinfection was reported. <https://academic.oup.com/cid/advance-article/doi/10.1093/cid/ciaa1275/5897019>

A 33-year-old otherwise healthy individual who had been infected 142 days previously with mild symptoms was found to be virus positive following travel abroad and re-entry screening upon return to Hong Kong. The virus sequence on first and second infections were different and matched contemporaneous isolates. One explanation for the reinfection could be that the antigenicity of the second virus was different than the first and this represent immune escape following SARS CoV2 evolution. There were 3 amino acid changes in the S protein of the second virus relative to the first. Antigenicity was not assessed but a recent paper indicates similar strains are not antigenically distinct. Dearlove, B., et al. (2020). "A SARS-CoV-2 vaccine candidate would likely match all currently circulating variants." Proceedings of the National Academy of Sciences: 202008281. <http://www.pnas.org/content/early/2020/08/28/2008281117.abstract>

An alternative explanation is that the individual was not robustly immune following the first infection: The antibody status (IgG to N protein using Abbott assay) at the start of the second infection was negative suggesting that any antibody response made to the first infection had waned over the intervening 4 ½ months. The individual seroconverted by the Abbott assay 5 days into the second infection. The second infection was asymptomatic. The Ct value for NP swab was 26, maintained for 3 days before viral clearance a level compatible with isolation of infectious virus suggesting this individual could be infectious to others on reinfection.

This is in line also with the Ct values for the 3 potentially reinfected people in the ship episode, whose Ct values ranged from 17 to 23.

The conclusion is that people who have experienced mild or asymptomatic SARS CoV2 may have waning immunity over 4-5 months, allowing them to be reinfected and shed virus at levels compatible with onwards transmission.

A second possibility is that virus is evolving in humans and this may affect efficacy of natural and vaccine induced immune responses. This was not assessed. A number of studies have shown using experimental evolution that such antigenic drift by SARS CoV2 S protein is possible, but currently the consequence of the escape mutations on virus fitness pathogenicity and transmissibility is not assessed.

Weisblum, Y., et al. (2020). "Escape from neutralizing antibodies by SARS-CoV-2 spike protein variants." [bioRxiv: https://www.biorxiv.org/content/10.1101/2020.07.21.214759v1](https://www.biorxiv.org/content/10.1101/2020.07.21.214759v1)

Baum, A., et al. (2020). "Antibody cocktail to SARS-CoV-2 spike protein prevents rapid mutational escape seen with individual antibodies." [Science: eabd0831](https://www.sciencemag.org/lookup/doi/10.1126/science.abb0831).

<https://science.sciencemag.org/content/sci/early/2020/06/15/science.abd0831.full.pdf>

Lou, Y., et al. (2020). "Cross-neutralization antibodies against SARS-CoV-2 and RBD mutations from convalescent patient antibody libraries." *bioRxiv*: 2020.2006.2006.137513.

<http://biorxiv.org/content/early/2020/06/06/2020.06.06.137513.abstract>

Seroprevalence and antibody waning.

Current seroprevalence studies have relied on either antibody tests using N antigen or LFT tests which also largely use N.

These indicate a seroprevalence following the first wave of the pandemic of around 6% in general population in UK and higher in London (13%) BAME individuals (17%) and age group 18-24 (7.9%), measured using self test LFT (REACT 2).

<https://www.medrxiv.org/content/10.1101/2020.08.12.20173690v2>

ONS/PHE reported seroprevalence is similar. Whether antibodies induced after the first wave infection are beginning to wane is not clear.

Some reports find antibodies wane faster from people who were infected asymptotically (Long et al. *Nat Med* 2020).

<https://www.nature.com/articles/s41591-020-0965-6.pdf>

A follow up study of REACT 2 suggest a fall in seroprevalence in those who did not report symptoms (unpublished).

This is in line with a recent report from Ibarondo et al *NEJM* 2020, in which a half-life of 36 days was measured in RBD IgG in sera from 34 people mostly with mild illness.

<https://www.nejm.org/doi/full/10.1056/nejmc2025179>

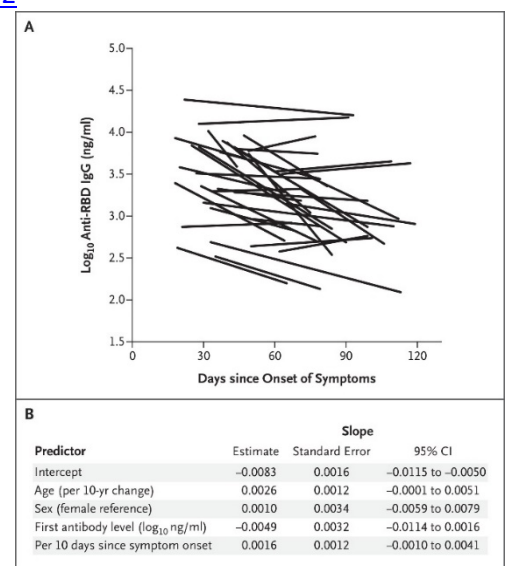


Figure 5: RBD antibody vs. time

A recent pre-print <https://doi.org/10.1101/2020.07.18.20155374> measured kinetics of antibody to receptor-binding domain (RBD) of the spike (S) protein of SARS-CoV-2 in 259 symptomatic patients up to 75 days, and tested 1548 samples prior to the pandemic. They found IgG, IgA, or IgM antibody responses to RBD in recently infected individuals, with 100% specificity and a sensitivity of 97%, 91%, and 81% respectively.

IgA and IgM antibodies against RBD were short-lived with most individuals estimated to become seronegative again by about 50 days after symptom onset. IgG antibodies lasted longer and persisted through 75 days, showing a slower decline in the latter stages. IgG antibodies to SARS-CoV-2 RBD were highly correlated with neutralizing antibodies. The method used to curve-fit is critical in the interpretation of these studies.

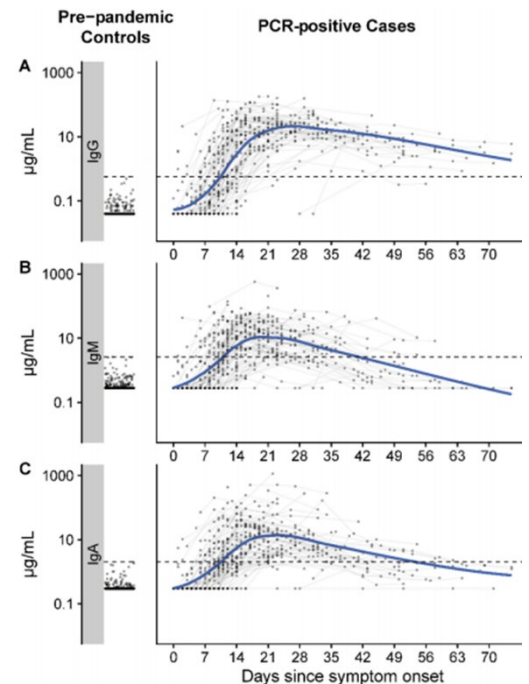


Figure 6: Kinetics of IgG, IgM and IgA

Another longitudinal study from a London hospital also found waning antibody over 3 months since infection, that was quite variable between patients and more obvious in patients with mild disease. <https://www.medrxiv.org/content/10.1101/2020.07.09.20148429v1.full.pdf>

However, a recent large study from Iceland reported that over 90% of qPCR-positive persons tested positive with two pan-Ig SARS-CoV-2 antibody assays and remained seropositive 120 days after diagnosis, with no decrease of antibody levels (figure 3). It is not currently clear why the Icelandic study did not detect antibody waning.

Conclusions:

1. Antibody responses are seen as early as day 10-14 in most individuals, but this depends on the assay used to measure antibody (moderate confidence).
2. Serum antibody levels peak at about one month and then settle to a lower level (high confidence).
3. There is a general correlation between antibody to the receptor binding domain and virus neutralisation (high confidence).
4. People with high levels of neutralising antibody may be resistant to infection and are likely to be less susceptible to severe lower respiratory tract or systemic disease (moderate confidence).
5. The rate of decay of antibody beyond 3 months remains uncertain.
6. If a poor antibody response is made, or when antibody wanes, individuals can be reinfected even with antigenically similar strains as for their first infection. Such individual shed viral loads compatible with onwards transmission (moderate confidence).

B. T cells

There has been considerable recent interest in antiviral T cells and what they do, for example:

<https://reason.com/2020/07/01/covid-19-herd-immunity-is-much-closer-than-antibody-tests-suggest-say-2-new-studies/>

Key questions include:

1. Do T cells protect against SARS-CoV-2 infection?
 2. How does T cells compare with antibody in protection against coronaviruses?
 3. Can vaccines induce T cell immunity?
 4. Might T cells be involved in immune enhancement of disease?
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Background

T cells come from the bone marrow, migrating to the thymus to undergo a process called affinity selection. This happens in two stages: 1. 'positive selection', in which cells which bind to MHC (major histocompatibility) proteins associated with self-peptides divide; 2. Deletion of cells that react too strongly to self ('negative selection'). Only about 2% of all the cells that arrive in the thymus emerge as mature T cells.

These mature cells migrate out to the peripheral organs, especially to mucosal surfaces. Here they play a key role in tackling infections but mainly act by coordinating other immune responses (boosting antibody and enabling immunological memory), in addition to killing cells that contain virus. Unless a peptide sequence from a specific pathogen has already been encountered, specific T cells that see a particular peptide are very rare indeed. They only expand if they encounter the MHC-peptide combination that they are destined to recognise.

The main relevant types of T cell are helper cells (CD4 cells), killer T cells (CD8) and regulatory T cells (Treg). T cells can be found (and measured) in the blood, but these cells are themselves not capable of defending against viruses. T cells have to be in clusters of cells to be functional. There are assays that are used to help diagnose tuberculosis based on T cell activity, but most tests for detecting past infection are based on measuring specific antibody, not T cells.

Do T cells protect against SARS-CoV-2 infection?

Not if they are in the blood. However, the number of cells in the blood may reflect cells that are in mucosal surfaces under some conditions, so circulating cells may correlate with protection.

Studies on COVID-19 cases from Oxford and ISARIC4C found T cells in the blood recognising 39 peptides containing CD4+ and/or CD8+ epitopes with 6 immunodominant epitope clusters. The virus-specific T cells made IFN-g TNF-a and IL-2; CD8 T cells expressed CD107a (cytotoxic marker). The proportion of CD8 vs. CD4 correlated with severity and memory T cell responses were greater in severe cases. These T cell responses correlated with spike, RBD and NP-specific antibody. <https://www.biorxiv.org/content/10.1101/2020.06.05.134551v1>

In a large Scandinavian study, SARS-CoV-2-specific T cells were detectable in some antibody-negative exposed family members and convalescent individuals with a history of asymptomatic and mild COVID-19, suggesting that the presence of primed T cells may be a very sensitive way to demonstrate viral exposure <https://doi.org/10.1016/j.cell.2020.08.017>

Another recent study from the Karolinska Institute focused on mucosa-associated invariant T (MAIT) cells which function as innate-like sensors and mediators of antiviral responses. They showed that cells of this type were very low in number in the circulation of patients with COVID-19, but that these cells were present and strongly activated in the airways. MAIT cell levels normalized in the convalescent phase, indicating that they are recruited to the infected site during infection and subsequently released into the circulation as disease resolves <https://doi.org/10.1101/2020.08.27.20182550>.

How do T cells compare with antibody?

T cells can clear virus from infected sites if the virus is present in living cells. T cells have no effect on free virus in body fluids or in mucus, whereas antibody has effects in liquid phase.

Ferretti et al. used an unbiased viral genome-wide screen to map the epitopes recognized by memory CD8⁺ T cells from convalescent patients with COVID-19 with prevalent HLA types. SARS-CoV-2-specific memory CD8⁺ T cells recurrently targeted a limited set of immunodominant epitopes, which were unique to SARS-CoV-2 and not from highly variable regions of surface protein S. In fact, only 10% of these epitopes corresponded to the S protein, stressing the relevance of developing vaccines that promote T cell responses against other viral targets, such as ORF1ab and N protein. <https://doi.org/10.1101/2020.07.24.20161653>

Can vaccines induce T cell immunity?

Live virus infections typically induce strong T cell responses, while purified antigen does not. This can be affected by the use of adjuvants, but inactivated or subunit vaccines tend to induce poor T cell responses. Live vaccines (such as the adenovirus-based vaccines) will be expected to induce both T and B cell immune responses and more durable memory. For example, the ChAdOx SARS-CoV-2 vaccine ([https://doi.org/10.1016/S0140-6736\(20\)31604-4](https://doi.org/10.1016/S0140-6736(20)31604-4)) stimulates T cells that make interferon gamma on restimulation as do other vaccines that express the antigen from inside a host cell such as the saRNA vaccines. <https://www.nature.com/articles/s41467-020-17409-9>

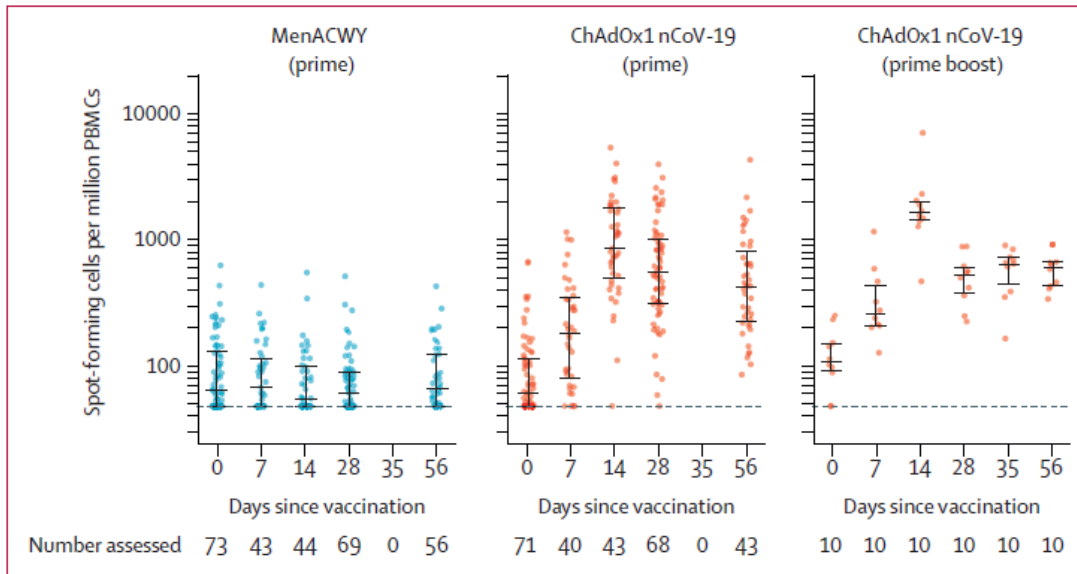


Figure 6: Interferon- γ ELISpot response to peptides spanning the SARS-CoV-2 spike vaccine insert
 Error bars show median (IQR). The lower limit of detection, indicated with the dotted line, is 48 spot-forming cells per million PBMCs. PBMC=peripheral blood mononuclear cell. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2. ELISpot=enzyme linked immunospot. MenACWY=meningococcal group A, C, W-135, and Y conjugate vaccine.

Might T cells be involved in immune enhancement of disease?

There is good evidence from studies of other viral infections (e.g. RSV) that immune enhancement is seen when there is a strong T cell response in the absence of neutralising antibody. It is possible that immune enhancement will not be seen immediately, but might develop after the protective responses wane.

Conclusions:

1. T cell responses are seen after infection with SARS-CoV-2.
2. There may be some cross-recognition of other coronaviruses by such T cells.
3. Mucosal resident memory T cells might help antiviral defence in early stages of infection.
4. T cell memory is important for sustained immunity against re-infection.
5. T cells assist in the elimination of virus from infected cells in later stages of infection.
6. It is possible that some T cells could be pathogenic and contribute to disease.