



Review Article

Antibody Formation in COVID-19 and Immunisation

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ABSTRACT

Antibody testing is vital in the study of the dangerousness, spread, identification of high-risk individuals and vaccine production against SARS-CoV-2. Viruses leave trails in the infected body and the immunologists turn out to be the detectives examining these viral footprints. There are two major types of tracks or memory responses that can be utilized by the body against reinfection: B cells which produce antibodies and T cells organising responses through cytotoxic cells and restricting viral replication. T cells' memory of SARS-CoV-2 appears to last longer than antibodies and immunisation can activate T cells as well if it fails to induce long term antibody production. The immune history of SARS-CoV-2 is a huge jigsaw puzzle; several pieces are still missing. This viral research has met with a shortage of valuable foundational knowledge because of the sudden appearance of the virus and its spread. COVID-19 has posed an existential problem for the whole of human society.

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Introduction

The immune system is made up of a network of cells, tissues and organs that work together to protect the body. Leukocytes form one of the important types of cells involved in immunity. The cells of the adaptive immune system are special types of leukocytes, the lymphocytes. B cells and T cells are the major types of lymphocytes and are derived from haematopoietic stem cells in the bone marrow. B cells develop in the bone marrow and are responsible for producing antibodies. There are thousands of different B cells in our bodies, each of which produces a unique antibody. T cells develop in the thymus and help coordinate a rapid and tailored immune response to specific infectious organisms. With regard to COVID-19, even if there is only short-term antibody production in recovered patients and only a short-term vaccination is possible, this would still be beneficial in stopping the social and medical pandemic. Existing evidence suggests that the levels of SARS-CoV-2 antibodies resulting from the viral infection may vary in terms of disease severity, and such a finding is in keeping with previous coronavirus pandemics.

Antibodies

Antibodies are proteins produced by the immune system to target a virus, bacterium or other pathogens. They destroy the pathogen by binding to it and rendering it harmless or by flagging it for destruction by the immune cells. They typically linger in the bloodstream after an infection in case the virus returns. If it does return, the immune response is much faster; patients who have recovered have resistance to reinfection. Indeed, for most viruses, the first time a person is infected, their body will take time to develop the requisite antibodies, so the body should be better equipped to fight off the infection a second time. Unfortunately, it may not be as simple with COVID-19. However, one recent paper looked at reinfection in rhesus macaques, which, after being infected with a standard dose of the virus, did not catch the infection a second time.

There are two major types of memory responses that can be utilised by the body against reinfection. They are the B cells, which produce antibodies, and the T cells, which organise responses through the cytotoxic cells and restrict viral replication. T cells' memory of SARS-CoV-2 appears to last longer than that of antibodies, so immunisation

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can also activate T cells if it fails to induce long-term antibody production. If B cells serve the function of manufacturing antibodies, T cells bind to and kill infected cells. Both of these cells can exist in the body for a long time and respond together to an invading toxic pathogen. A robust immune response usually involves T cells and B cells cross-checking with one another. Fading antibody levels may indicate that B cell immunity declines after a few weeks, but that does not necessarily mean that T cell levels drop at comparable rates. A recent study from patients who recovered from SARS has been found to still possess SARS-reactive T cells more than 15 years after the SARS outbreak in 2003. There are suggestions that some patients with no detectable antibodies still maintains T cell immunity to SARS-CoV-2. But we do not know yet whether T cells are functional without their counterpart antibodies.

Dosage of the Virus

The dose of virus exposure is a crucial factor. The dose that a person receives may vary depending on whether the person breathed in airborne particles or touched a contaminated surface and rubbed their eyes. If someone is only exposed to a small dose of the virus in the first instance, nobody can predict how they may react if the second dose is much greater (Link). It is unclear whether having a stronger dose-response will leave someone any better off. In general, two kinds of antibodies are produced by a virus. The first one shows up 5-6 days after exposure to the virus and vanishes after approximately 20 days; this first antibody indirectly shows the presence of infection and is slowly substituted by another antibody as the person heals. The second antibody indicates that a person has contracted the virus, and when only the second antibody is detected, it means that the person is probably free of the pathogen. Therefore, the potential serological tests for COVID-19 can only give a positive result after nearly 20 days. Antibodies can develop in infected people and those who may be asymptomatic. It is not yet clear whether COVID-19 imparts short or long-term immunity. Virologists examining patients recovering from a COVID-19 infection are noting high levels of neutralising antibodies in the blood of some severely ill patients.

The hope is that recovered patients have developed enough COVID-19-specific antibodies to fight off a secondary infection. However, in one study on convalescent patients in China, 30% of those studied had very little or no detectable antibodies in their blood plasma (Link). One hypothesis is that people with positive molecular testing and mild symptoms who are found to have low levels of neutralising antibodies or even none at all may run the risk of reinfection [1]. In contrast, those who become severely ill attain robust immunity [2].

Reinfection

The WHO has published guidance on adjusting public health and social measures for the next phase of the COVID-19 response [3]. There is currently no evidence that people who have recovered from COVID-19 and have antibodies are protected from a second infection. The WHO is continuing to review the evidence on antibody responses to SARS-CoV-2 infection [4-8]. Most of these studies show that people who have recovered from infection have antibodies to the virus. However, some of these people have exceptionally low levels of neutralising antibodies in their blood, suggesting that cellular immunity may also be critical for

recovery [9]. None of these studies have evaluated whether the presence of antibodies to SARS-CoV-2 confers immunity to subsequent infection by this virus in humans. However, there are currently unconfirmed reports that Public Health England has approved a blood test, developed by the Swiss pharmaceutical company Roche, to determine whether people have already been infected with COVID-19. While antibody tests are currently being carried out in surplus numbers in the NHS, it is too early to make any conclusive interpretations in this regard.

One recent study that analysed the immune response of more than 90 patients and healthcare workers via blood tests revealed that 60% had developed a strong antibody response during their infections, but only 17% retained the same potency three months later. On the flip side, in some cases, the antibody levels were undetectable. Most other coronaviruses that cause the common cold do not assure long-term immunity, so it is arguable that COVID-19 is headed down the same path. Reinfection is a possibility once the antibodies have declined, but if reinfection occurs, subsequent cases would likely be less severe because the retention of immune memory may make reinfection less severe. In such a scenario, like the flu vaccination, an annual coronavirus booster dose may assure sustained levels of protective antibodies. Herd immunity may become a distant phenomenon if that is at all achievable.

In South Korea, recovered patients have tested positive again, causing confusion and pessimism about the chances of immunity. One interpretation is that these patients might have relapsed rather than having been reinfected. Another possibility is that there might have been false-positive or false-negative tests along the line. Viruses like HIV and the chickenpox virus can break into the nucleus of human cells and stay latent for years before reactivating, but SARS-CoV-2 stays outside the host cell's nucleus and does not break into it. It is therefore, unlikely, that it can be reactivated. The severe acute respiratory syndrome (SARS) outbreak ended after about 8,000 cases and instances of reinfection ceased to be reported; T cells could be a sign of ongoing immunity [10]. A later vaccine study in mice found that memory T cells protected the animals from the worst effects of SARS when attempts were made to infect them again [11].

Declining Antibodies

A recent Chinese study detected the presence of antibodies in recovered patients, but they tended to decline after a few months. The research, which studied 37 symptomatic patients and 37 asymptomatic patients, found that of those who tested positive for the presence of IgG antibodies, over 90% showed sharp declines within 2-3 months [12]. In the study, the median percentage decrease was more than 70% for both the symptomatic and asymptomatic patients. For neutralising serum antibodies, the median percentage decrease among the symptomatic individuals was 11.7%, while for the asymptomatic individuals, it was 8.3%. The cellular memories of the infection can swiftly reproduce antibodies in the event of reinfection. A Swedish study indicated the presence of T cell immunity among recovered patients, and 30% of the healthy blood donors studied were found to have developed T cell immunity.

Immunological Tests

More research on current antibody tests is needed to determine whether they are reliable and accurate. False-positive tests, which inform someone that they have had COVID-19 when they have not, or false negatives, which tell someone they have not had COVID-19 when in fact they have, could potentially put lives at risk and can be counterproductive in arresting this pandemic. In line with other coronaviruses, most virologists have proposed that immunity against COVID-19 will only last a year or two. If this is the case, it is likely to become endemic with seasonal peaks of infection and may become less deadly. In the worst-case scenario, the virus could mutate to become more lethal. Thus, there are conflicting results about the immune response to COVID-19.

Laboratory tests that detect antibodies to SARS-CoV-2 in people, including rapid immunodiagnostic tests, need further validation to determine their accuracy and reliability. These tests also need to be able to accurately distinguish between past infections from SARS-CoV-2 and those caused by the known set of six human coronaviruses. Four of these viruses cause the common cold and circulate widely. The remaining two are the viruses that cause Middle East Respiratory Syndrome (MERS) and SARS. People infected by any one of these viruses may produce antibodies that cross-react with the antibodies produced in response to infection with SARS-CoV-2. Many countries are now testing for SARS-CoV-2 antibodies at a population level or in specific groups, such as health workers, close contacts of known cases or within households [13].

Nanobodies

Nanomedicine is a fast-developing field, and nanobodies are a novel and unique class of antigen-binding fragments derived from naturally occurring heavy-chain-only antibodies present in the serum of camelids (family Camelidae). Antibodies derived from llamas have been shown to neutralise SARS-CoV-2 in laboratory tests, and one finding has shown that the immune system of Fifi (one of the 'Franklin llamas') has produced different antibodies from those already identified in COVID-19 sufferers, which will enable cocktails of nanobodies to be tested against the coronavirus (Link) [14]. It has been found that the nanobodies can bind tightly to the spike protein of SARS-CoV-2, thereby blocking it from entering human cells and halting any infection. It is hoped that nanobodies could be used in future treatments and that they have the potential to be used in a similar way to convalescent serum, effectively stopping the progression of the virus in patients who are ill. These findings could help develop new therapeutics against COVID-19.

Vaccination

Research groups across the globe are competing to discover a vaccine for COVID-19, which is hoped to mark the endpoint of the coronavirus pandemic. Vaccination involves procedures in which the adaptive immune system is manipulated in an antigen-specific fashion in order to mimic infection by a pathogen and to activate protective immunity against it. Since 10 January, 2020, when the SARS-CoV-2 genetic sequence was posted online, multiple international organisations have been working to rapidly develop a COVID-19 vaccine; the development

of this vaccine builds on and benefits from work on the SARS and MERS vaccines [15].

It has been reported that an mRNA vaccine (mRNA-1273) has been shipped to the National Institute of Allergy and Infectious Diseases in the US for phase I clinical trials [16]. The vaccine comprises a short segment of genetic code copied from the virus. The trial started in humans on 16 March, 2020. The vaccine is being fast-tracked and has bypassed the phase I stage. Clinical trials in humans have also started on an experimental adenoviral vector vaccine in China [17]. AstraZeneca claims to have developed a treatment by 'combining two antibodies' in one injection to reduce the chance of resistance developing to one antibody. This treatment is intended to help vulnerable individuals who may have a poor response to vaccination.

Barriers

There are still barriers before global immunisation can be achieved. In the first place, clinical trials are subject to practical problems because of coronavirus mitigation efforts that include self-isolation and avoiding healthcare centres where symptomatic patients congregate for medical care and where randomized trials are typically conducted. Such mitigation efforts may interfere with all aspects of a successful clinical trial, including efficient accrual and randomization, intervention adherence and delivery and outcome collection [18]. Nevertheless, the vaccines must pass clinical trials, and they will have to be mass-produced. There are at least eight potential vaccines in development to battle this virus, and it is too early to ascertain which are the safest and most effective.

Immunologists will have to check extremely carefully for signs of dangerous side effects. During the search for a SARS vaccine in 2004, scientists found that one candidate caused hepatitis in ferrets. Another serious concern is 'antibody-induced enhancement', where the antibodies produced by a vaccine actually make future infections worse. This effect caused serious lung damage in animals given experimental vaccines for both SARS and MERS. How the pandemic will pan out is difficult to predict. It could become less virulent as the years pass.

Past Experiences

Immunity to smallpox after vaccination can be long-lasting [19]. The success of the smallpox vaccine has apparently established overconfidence in vaccination technology. Smallpox infection leads to viremia and higher levels of antibody production. COVID-19 does not lead to viremia, and fatality is far lower in comparison to smallpox, which resulted in deaths in one in three people. Infection with varicella-zoster, which causes chickenpox and shingles, imparts long-term resistance. Vaccination against *Clostridium tetani* does not guarantee long-term immunity, and those who receive vaccination are expected to obtain booster injections. The presence of any form of antibodies in the body does not guarantee that they will impart immunity against future infection; only neutralising antibodies provide immunity against reinfection without time specificity. Neutralising antibodies are the proteins that reduce and prevent infection by attaching to the part of a virus that connects to the host cells and freeing the host cells from the pathogen. Non-neutralising antibodies still recognise parts of the

invader, but they do not bind effectively and therefore do not prevent it from invading cells. These may be the private nightmares of microbiologists.

On the other side of the spectrum, HIV sufferers may have large amounts of non-neutralising antibodies in their blood that do not provide any immunity at all. Unlike HIV, however, negative testing of patients recovering from COVID-19 rules out the possibility of the continued presence of the virus in the body. Only testing suspected cases and testing for antibodies can enlighten us about the specificities of COVID-19. Obviously, it is still too early to draw any conclusions. Unlike HIV, coronavirus only has a low shielding with glycans. The observed lesser glycan density indicates that there are fewer barriers for the immune system to neutralise the virus with antibodies. This is positive information for vaccine development.

Short-Term Immunity

Diagnostic tests are used to confirm the presence and amount of the virus, whereas antibody tests help determine whether or not someone has previously been infected. Such a test is also applicable to asymptomatic infections. Immunity functions on a spectrum or a continuum. Only the presence of neutralising antibodies guarantees immunity. COVID-19 was only publicly recognised seven months ago. Therefore, long-term data concerning the antibody status of COVID-19 patients who have recovered for at least 14 days is lacking. After more time has passed, it may turn out that immunity will not be long-lasting. Even though there has not yet been a high incidence of reinfection of SARS-CoV-2, it is disturbing to consider that seasonal viruses only offer short-term immunity and there is a high incidence of reinfection each year. According to antibody tests, host immunity against SARS-CoV peaks at around four months, and the immunity fades away after two to three years. COVID-19 shares a good amount of genetic material with SARS-CoV and its immunity status could follow the same line.

There are indications that SARS-CoV-2 infection initiates the production of neutralising antibodies, and animal (link) studies suggest such antibodies prevent reinfection, at least for a couple of weeks [20]. The crucial IgG antibodies were detected in the blood of British COVID-19 patients, but they appeared to fade appreciably after just two months. More serological tests of recovered patients are warranted to confirm that COVID-19 confers neutralising antibodies, so we can be optimistic about the development of a vaccine. Some antibodies seem to recognise and react to the spike proteins on multiple coronaviruses (link), including SARS-CoV and MERS-CoV, and this information from previous coronavirus outbreaks is useful and insightful into the behaviour of COVID-19.

Vaccine-Induced Immunity

A well-developed vaccine is to sidestep the limitations of natural infection and optimize the vaccine in a way that ensures a robust, long-lasting immune response. Coronaviruses often do not induce high-level immunity. In many cases, flu vaccines may not prevent reinfection, but they do lessen the symptoms in reinfected people who have received the vaccine. Likewise, COVID-19 vaccines, if they do not block the transmission of the infection for a long period, could still reduce the

severity of symptoms. Accordingly, they could be preventive and therapeutic. It is therefore not fully correct to think that a vaccine that offers immunity for less than a year does not have much clinical value. The immunity puzzles of COVID-19 are entangled in several extraordinarily complex uncertainties, and life on this planet appears to be on hold until the medical sciences outpace the coronavirus and discover a safe and effective vaccine or effective treatment strategies in the event that vaccine production is delayed. Only the presence of neutralising antibodies would ensure that SARS-CoV-2 would give in to immunisation. Vaccine-induced immunity is not similar to infection-induced immunity, and immunisation by vaccination is achievable, even when infection does not confer immunity. Since the outbreak of COVID-19, we have truly gained only little immunological information about this novel virus [21].

Vaccination is the greatest achievement of immunology, and a quicker COVID-19 vaccination will go down as a great achievement and triumph in the history of the medical sciences. To this end, the UK – a country with a rich history of pioneering vaccine technology starting with Edward Jenner – has set up a task force in a bid to find a vaccine against this viral abuser. A preventive vaccine is a target, but if that is not achievable, a therapeutic or passive vaccine will become the goal. Many of the vaccine development claims will turn out to be aspirational rather than factual, and the newly developed vaccines will have to pass through rigorous scientific experimentation. Even a short-term vaccine will help to break the transmission cycle and stop the pandemic in its tracks. When we consider that three decades on, attempts to produce a vaccine against HIV have still not been fruitful, expectations need to be tempered [22, 23].

Vaccine Trials

It is encouraging to note that there is global scientific and political enthusiasm to discover a vaccine to combat this viral epidemic, even though there is a time factor before anything can actually be put in place. World health authorities have cautioned against the ultra-optimistic views of developing a vaccine as a quick fix and escaping the bitter reality of living with this threat for the foreseeable future, and they urge that adjustments, including lifestyle changes, be made. It would also be prudent to focus on novel treatment strategies until we develop a vaccine or in the event that vaccine development is delayed.

Immunologists across the globe are now working towards a vaccine faster than ever. As many as 86 teams are presently working to develop a COVID-19 vaccine, including those undergoing clinical trials. According to the WHO, there are 13 vaccines undergoing clinical trials at present. It is claimed that the Oxford vaccine trials have been successful to date, and it is in phase III trials. The vaccine is reported to have produced the antibodies and T cells that are required to fight off the virus.

The Oxford vaccine, called ChAdOx1 nCoV-19, is made from a virus ChAdOx1, which is a weakened version of a common cold virus that causes infections in chimpanzees, which has been genetically changed so that it is possible to grow in humans. By vaccinating with ChAdOx1 nCoV-19, the body is taught to recognise the viral intruder and build an immune response to the spike protein that will aid in blocking the entry

of SARS-CoV-2 into human cells [24]. It is directed at the spike protein of the coronavirus which facilitates receptor binding and entry to host cells. The study enrolled 1077 healthy adults (median age 35 years) who were randomized to receive either the test vaccine (543) or a meningococcal conjugate vaccine which acted as a control. A sub-group of 10 patients from the COVID-19 vaccine group received a booster dose on day 28. In the test vaccine group, spike-specific antibodies peaked by day 28 and remained elevated to day 56. Neutralising antibodies were initially detected in only 32 patients after a single dose of the test vaccine but in the sub-group of 10 patients, neutralising antibodies were detected in all 10 patients by day 42. Systemic adverse effects reported in the test vaccine group included fatigue (70%), headache (68%), muscle ache (60%), malaise (61%) and chills (56%). Phase II and III trials that include a wider group of patients are making progress.

The vaccine, mRNA-1273, encodes the SARS-CoV-2 spike glycoprotein which mediates host cell attachment and is needed for the virus to enter cells [25]. Phase I study of this vaccine trial included 45 healthy volunteers (aged 18 to 55 years) who were split into three groups, with each group receiving either a 25, 100 or 250mcg dose of the vaccine, which was administered on days 1 and 29. The results which cover a period of 43 days after the second vaccination showed that after the first dose, antibody responses were greatest for the two higher strengths and increased further after the second vaccination. In addition, although neutralising antibodies were detected after the first vaccination in less than half of the participants, all had detectable antibodies after the second dose. The vaccine was well tolerated with mild bearable side effects. A Phase III trial is expected to start shortly.

While 129 human trials are in the pre-clinical evaluation phase, on Monday 29 June, India received approval to conduct human trials for a potential coronavirus vaccine. Covaxin – developed by the Indian vaccine and biotherapeutics manufacturer Bharat Biotech – is the first COVID-19 vaccine to obtain approval from the Drug Controller General of India. The drug regulatory body has now permitted the pharma company to conduct phase I and II clinical trials.

Placing emphasis on the development of a vaccine is a good move; let us hope that this will not turn out to be a forever-distant horizon. The hasty development of a vaccine is risky, but the development of novel, efficient drug treatments, including specific antiviral agents, would reduce panic among the general public, just as the discovery of antibiotics alleviated anxieties related to various bacterial infections. Even if vaccine developments are delayed, we can predict with certainty that in the years to come, monotherapy or combination drug therapy against this novel virus will emerge, and such advances will at least lessen the fear of this infection, if not eradicate the virus. The medical ending of the pandemic will come about once an effective vaccine is discovered, like the success of the smallpox vaccine.

Conclusion

It seems that we will all have to brace up and face COVID-19 at some point in the future and survive the assault of this unseen enemy. Tests, novel treatments, and vaccines are the three tools needed to combat COVID-19. The antibody studies reinforce the message that nobody can assume that someone who has had COVID-19 cannot get reinfected just

because they initially became antibody positive. It also means a negative antibody test now cannot exclude the possibility of having had a COVID-19 infection a few months previously either in symptomatic or asymptomatic form. It suggests that vaccines will need to be better at inducing high levels of longer-lasting antibodies than the natural infection or that doses may need to be repeated to maintain immunity. A safe and effective vaccine with long-term immunological properties would drastically change the situation for good. Thus far, the research findings of the pandemic are inconsistent, and the unseen SARS-CoV-2 has become the metaphorical elephant in the room, meaning that there are many dimensions of this pandemic that require further clarification. COVID-19 appears to be a test of self-immunity.

In a way, this pandemic has put us in the position of the Spartans – survival of the fittest – but according to international moral and ethical teachings, the fittest has the responsibility to look after the less fit. Collective efforts from all medical specialities are warranted in defeating the medical pandemic; the social pandemic and the medical pandemic ought to be distinguished. Medical professionals, irrespective of their speciality are at the forefront of this challenging threat. There is an Indian saying, ‘Once you catch the tiger, either the tiger will kill you or you have to kill the tiger’, meaning that this pandemic can either destroy human society economically and health-wise or we have to act collectively and solve it. The politicians have to deal with the economic issues and the social pandemic; the medical professionals will have to solve the medical pandemic.

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Conflicts of Interest

None.

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