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Assessing natural anti-Covid immunity: serology, cellular immunity

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Abstract

It is important to evaluate natural immunity against Covid-19: it is stronger, longer lasting and of better quality than vaccine immunity

Only humoral (antibody) or cellular adaptive immunity can be assessed; innate immunity is not measurable. Serology is the only routine test, it is the measurement of the antibody level.

The measurement of the antibody level leads to an underestimation of the seroprevalence which is already high and above 50% of the population in most countries.

The reasons for this underestimation are:

The tests are designed against the strain isolated in 2019 in China and the calibration is against convalescent blood collected before June 2020.

There is considerable heterogeneity in the commercial tests available.

A large percentage of the infected population may have negative serology within months of infection

Cellular immunity testing could eliminate these false negatives but it is not routinely applicable and is expensive.

Introduction

I have recently shown that natural immunity to Covid-19 (following infection) is stronger, longer lasting, and of higher quality than vaccine immunity (Banoun, 2021a)

The reasons for this were recently outlined by Sonigo et al.

Before the introduction of the 3rd dose, French authorities advised a single dose of vaccine for those already naturally immune. (HAS, 12 February 2021). For the time being, the HAS does not take a position on the need for a 2nd dose in people who have already been infected with Covid (HAS, July 16, 2021). It is therefore important to evaluate the methods for measuring natural immunity acquired after infection.

But healthy people can also clear a virus through their nonspecific innate immunity: this immunity is not known to be assessed, and in these people no trace of their encounter with the virus may be readily detectable.

In Covid, natural infection begins in the mucous membranes of the nasopharynx, which are an immune

sanctuary (Sonigo et al., 2021). Innate immunity at this level may be sufficient to eliminate viruses without significant intervention of adaptive immunity and thus without significant production of specific antibodies. Moreover, the infection can be fought by the cross-immunity already acquired against common cold coronaviruses (Banoun, 2020).

Specific (adaptive) immunity can be assessed by measuring circulating anti-SARS-CoV-2 antibodies and by measuring immune cell memory.

Reminder on Innate and Acquired Immunity

Innate immunity

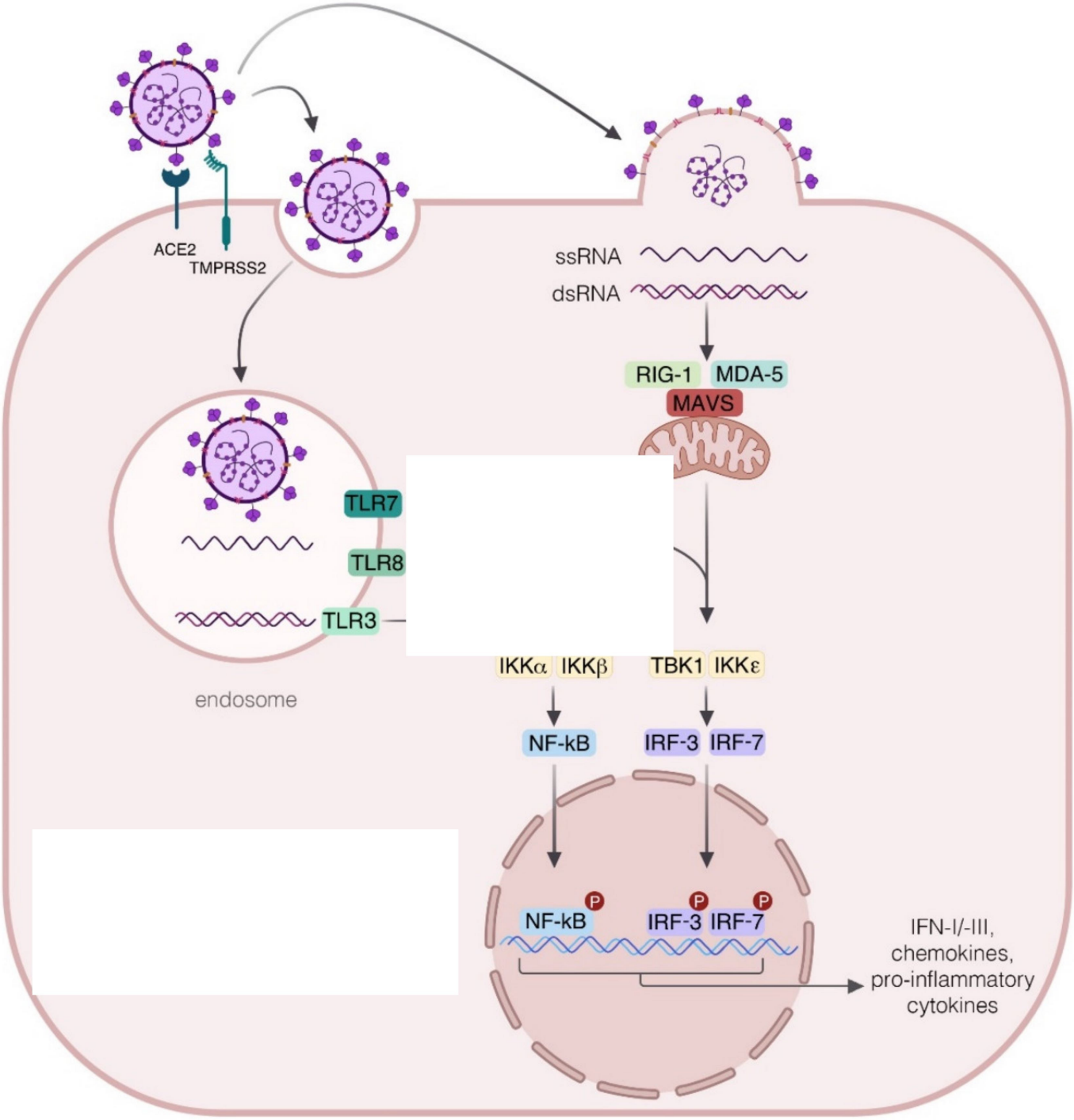


Figure taken from: Ricci D et al, 2021, https://www.mdpi.com/1422-0067/22/13/7017 , Innate Immune Response to SARS-CoV-2 Infection: From Cells to Soluble Mediators

A recent review exposes innate immunity to viral infections (Majdoul and Compton, 2021).

The first line of defense against a virus is nonspecific innate immunity, which manifests itself in the

secretion of antiviral and proinflammatory molecules. Cells recognize the virus by protein and nucleic acid patterns that trigger this innate immunity. In particular, the natural RNA of the virus, different from human RNA, is detected by Toll-like receptors (unlike vaccine mRNA). The recognition of the virus as a pathogen to be eliminated leads to a cascade of metabolic events that may be sufficient to eliminate the virus.
Innate immune cells also contribute to the elimination of the virus: neutrophils, macrophages, killer cells, etc...

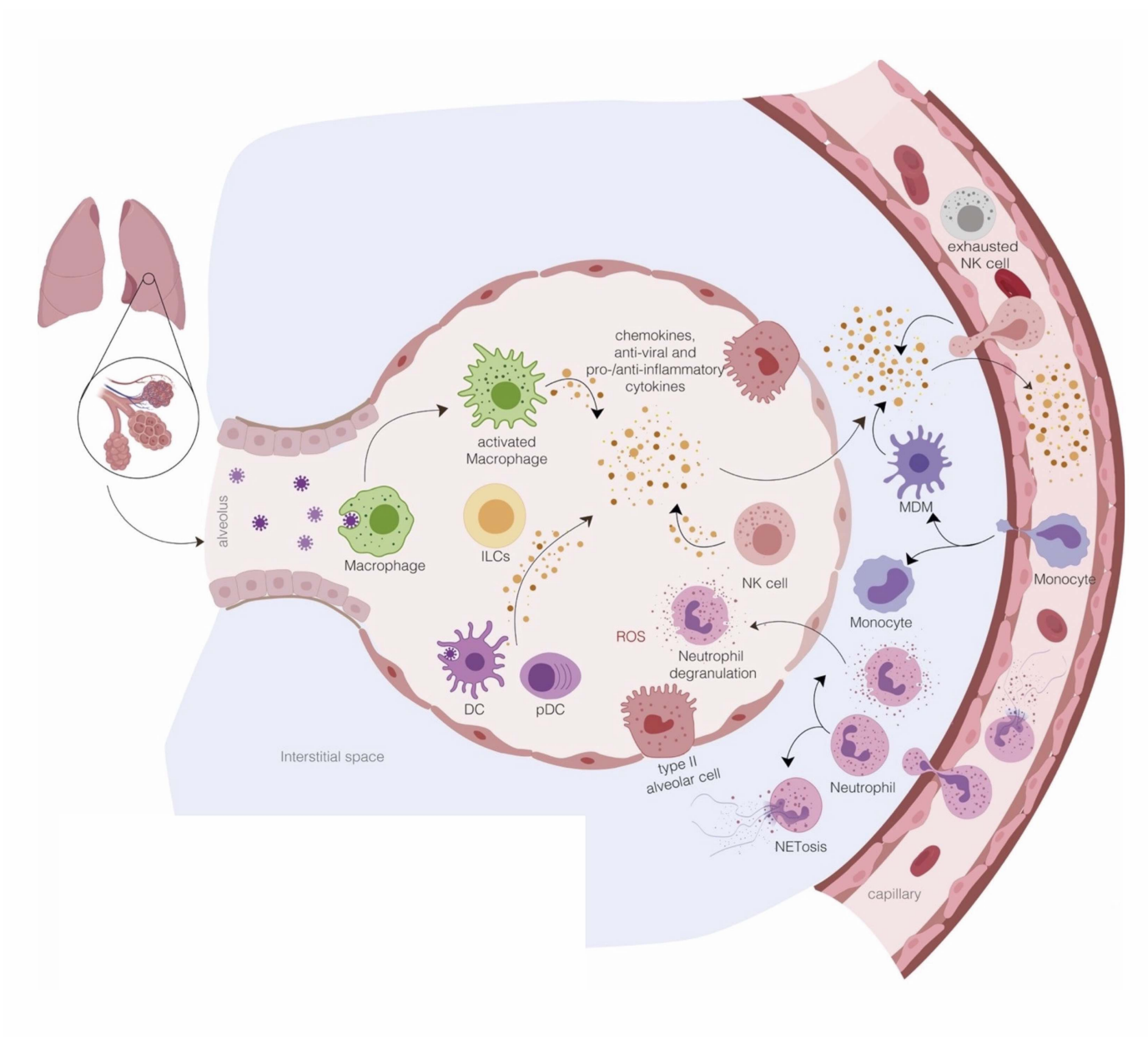


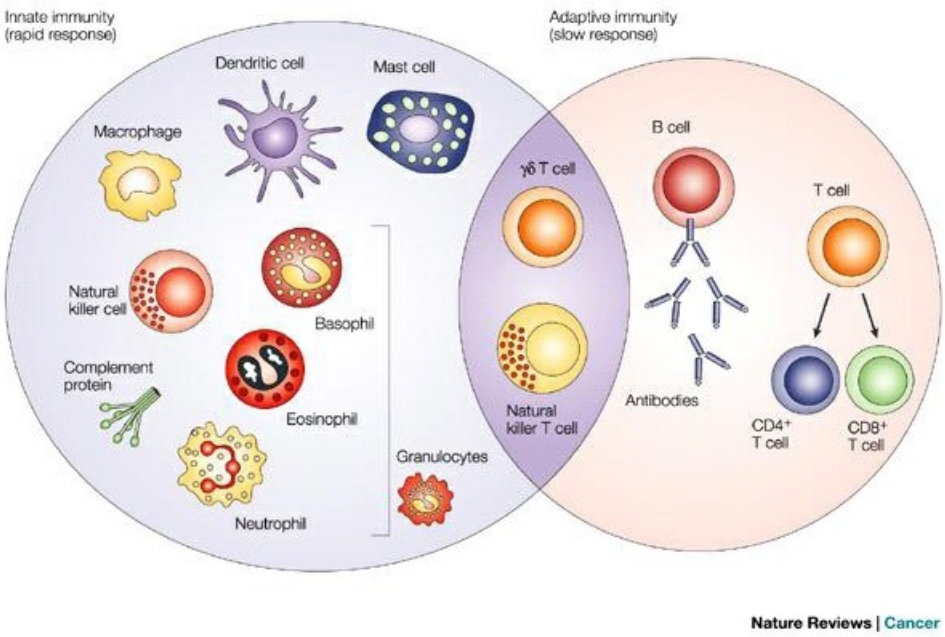
Figure taken from: Ricci D et al, 2021, https://www.mdpi.com/1422-0067/22/13/7017 , Innate Immune Response to SARS-CoV-2 Infection: From Cells to Soluble Mediators

Adaptive Immunity

Excerpts from a review (Cologne, Germany) : The adaptive immune system takes over if the innate immune system fails to destroy the germs. It specifically targets the type of germ that is causing the infection. But to do this, it must first identify the germ. This means it is slower to react than the innate immune system, but when it does, it is more accurate. It also has the advantage of being able to "remember" germs, so that the next time a known germ is encountered, the adaptive immune system can respond more quickly.

Thanks to this memory, some diseases can only be contracted once in a lifetime: The adaptive immune system takes a few days (maximum 14 days for Covid) to react the first time it comes into contact with the germ, but the next time, the body can react immediately. The second infection is then usually not even noticed, or at least it is less severe.

The adaptive immune system relies on T cells, B cells and antibodies in the blood and other body fluids.



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Caption: Cells of innate and adaptive immunity

T cells (called T cells because they were discovered in the thymus) are produced in the bone marrow and then move to the thymus via the bloodstream, where they mature. T cells have three main functions: They

use molecules to activate other cells of the immune system that will start the adaptive immune system (helper T cells). They detect cells infected with viruses or tumor cells and destroy them (cytotoxic T cells). Some helper T cells become memory T cells after the infection has been overcome. They can "remember" the germs that have been defeated and are then ready to quickly activate the appropriate immune system in case of a new infection. T cells have sensing features on their surfaces that can attach to germs. The immune system can produce a type of T-cell for each germ in an infection within a few days. Then, if a germ attaches itself to a corresponding T cell, it begins to multiply, creating more T cells specialized for that germ. Since only the cells that match the germ multiply, the immune response is specific.

B cells are made in the bone marrow and mature into specialized cells of the immune system. They get their name from the "B" in "Bursa of Fabricius", an organ found only in birds.
B cells are activated by T helper cells. They multiply and become plasma cells. These plasma cells rapidly produce very large amounts of antibodies and release them into the bloodstream.
Some of the activated B cells become memory cells and form part of the "memory" of the adaptive immune system.
The different cells of the adaptive immune system communicate either directly or through soluble molecules such as cytokines (small proteins).

Antibodies are glycoproteins (compounds of proteins and sugars or carbohydrates) that circulate in the blood. They are produced by plasma cells (B cells) and are specific to the antigens of the pathogens to which they bind.

SEROLOGY (or measurement of circulating antibodies in the blood)
This is the measurement of specific antibodies produced against SARS-CoV-2, the virus responsible for Covid-19

Reminder: seroprevalence
I recently gave (Banoun, 2021a) some seroprevalence measurements (proportion of the population with antibodies: 1/3 of the population in Kenya in November 2020, 25% in India in January 2021, 23% in France, 50% in the USA, 40% in Madagascar).
Since then, new data have been released:
Czech Republic: 51% HIV positive in Feb/Mr 2021, Estonia: 77% positive as of Sept 27, 2021 (Czech Republic, Estonia, 2021). In Estonia, only anti-N antibodies were measured and therefore this study only takes into account natural immunity (N is not included in the vaccine) and cannot count vaccine-induced antibodies.

Available tests

There are different techniques for antibody detection and not all tests detect antibodies directed against the same SARS-CoV-2 antigens: in general, these are antibodies directed against the S protein (spike), either the whole protein, or only the RBD (receptor binding domain), or against the N nucleocapsid (protein bound to the virus RNA).

The immune responses of individuals who have encountered the virus are very heterogeneous and some may not produce antibodies to any of the virus antigens. In addition, there are numerous mutations in the RBD of the spike, in the whole spike and to a lesser extent in the N protein. Depending on the variant to which the patient has been exposed, the antibodies produced may not be detected by certain serological tests.

The HAS (High Health Authority, France) authorizes 17 serological tests (Covid-19 Santé Gouv.fr, 2021).

The European Commission lists 588 tests for anti-Covid antibodies with the CE mark and 158 without the CE mark (CE, 2021).

As of October 12, 2021, the FDA cleared 89 tests (FDA, 2021).

WHO has made available an international reference standard for the anti-SARS-CoV-2 antibody assay. (WHO, 2020). But it is obtained from convalescent blood collected before June 2020, so before the emergence of variants. The sera are collected more than 28 days after the onset of symptoms but the maximum duration after symptoms is not specified: it will not give any indication on samples taken too long after infection.

The measurement of neutralizing antibodies is performed on strains from 2020 and may not be reproducible with recent variants.

There are very large variations between laboratories testing these pools of sera.

For the low rate pool, very few laboratories using commercial kits gave a quantitative result: the mean and variability could not be calculated.

For all these reasons, it is difficult to rely on the quantitative results calculated from this standard and reported at the end of 2021, especially for the low antibody levels.

Evaluation of serological tests

The performance of these serological tests varies considerably, with some tests falling far short of the sensitivity and efficacy criteria proposed by the FDA. In particular, some serological tests have been shown to have much lower sensitivity at an early stage of infection than at an advanced stage of infection. (Gong F. et al.)

A review article (Liu G et al., 2021) provides a complete description of all serological tests used and the many causes of false positives (especially in patients with autoimmune diseases) and false negatives.

According to recent publications, the level of antibodies depends on the severity of the disease. Convalescents with a mild form of Covid are more likely to have undetectable IgG levels after several months. (Perez-Olmeda M. et al., 2021, Therrien C. et al. 2021), this confirms what was already published in 2020 (for a review, see Banoun, 2020)

Some studies showing the correlation between disease severity and antibody levels: 40% of asymptomatic patients are seronegative, 12.9% of symptomatic patients are also seronegative in early convalescence (Long Q-X et al., 2021).

According to Wu F. et al., 2020, 30% of recovered patients have low antibody levels.

According to Toh Z-Q et al, 2021, a lower proportion of children seroconvert compared to adults [20/54 (37.0%) versus 32/42 (76.2%)]. This was not related to viral load, which was similar in children and adults [mean Ct 28.58 vs 24.14]. Age and sex also did not influence seroconversion or the magnitude of the antibody response in children or adults. In adults (but not children), symptomatic adults had three times higher antibody levels than asymptomatic adults. Evidence of cellular immunity was observed in seroconverting adults but not in seroconverting children (but it is difficult to conclude given the small numbers observed).

According to Liu W et al, 2021, clinical disease does not guarantee seroconversion and laboratories with highly sensitive RT-PCR assays are more likely to detect serological nonresponders. These results provide an explanation for the puzzling variability in seroconversion across cohorts. 36% of their cohort represented serological nonresponders.

According to Masia M et al, 2021, 25% of patients had undetectable antibody titers. Patients who did not seroconvert had higher cycle threshold values for RT-PCR (38.0 vs 28.0), shorter time to viral clearance (3.0 vs 41.0), and were more likely to have SARS-CoV-2 detected only on stool samples. Non-seroconverters also had lower levels of blood inflammatory biomarkers on admission and less disease severity. Serology was performed for anti-S1 and anti-N antibodies.

The result depended on the time between infection and serological testing:

A large percentage of the infected population may have negative serology within months of infection, and the serological response of IgG to SARS-CoV-2 targets is heterogeneous; these targets are: spike protein and N protein (nucleocapsid).

There is heterogeneity of response among patients and not only according to the severity of the infection (Perez-Olmeda M. et al., 2021).

According to an international study of March 2021 (Pelleau S. et al., 2021), one year after symptoms, only 36% of anti-S IgG persist, 31% of anti RBD IgG, and 7% of anti-N IgG persist; IgM and IgA disappear rapidly. A model allows to extend the observation period from 1 to 2 years: After 6 months, only half (55%) of the anti-spike IgG persist, 36% persist 12 months and 16% 24 months. Less than half of the other antibodies detected 15 days after infection persist (whether anti RBD, antiN, IgM antiS, RBD or antiN, IgA anti S, RBD

and N).
One of the best tests according to a French team (Velay A. et al., 2021) would be the Chinese Wantai test measuring total antibodies (IgG, IgM anti RBD of spike), (Wantai , FDA).
It is one of the most sensitive and specific tests according to the manufacturer (Bioscience, https://www.bioscience.co.uk/cpl/sars-cov-2-ab-elisa)

The most used tests detect different antigens with different sensitivities and specificities:
According to a European study, there are substantial differences in sensitivity and specificity between laboratories and between certified and non-certified reagents, with a clear lack of harmonization (Ast V. et al., 2021).
According to a Canadian study, only one commercial test approaches the standards required by Health Canada (Therrien C et al., 2021)

The tests were designed using antigens from the reference strain isolated in Wuhan in 2019. Since then, the virus has mutated and some antigens of the currently circulating variants could induce the synthesis of antibodies that are not recognized by serological tests (problem mentioned by Banoun, 2021b).
Some tests could give false negatives in people infected with distant variants: this possibility is highlighted by the FDA but does not seem to have been evaluated yet (FDA, 23/09/2021).

According to a recent UK report (COVID-19 vaccine surveillance report Week 42), seroprevalence measured by the Roche anti-N test shows a maximum of 25-30% seropositivity depending on region and age.
This report notes the weakening of the N antibody response over time. N antibody levels appear to be lower in individuals who become infected after two doses of vaccination (as explained previously, Banoun, 2021a, vaccination can damage the immune system's ability to respond to infection).
From June to September 2021, the pattern of antibody levels in these cohorts gradually declines, consistent with a decline.
At the start of the vaccination campaign in December 2020, antibody levels were generally in the range of 0.8-1,000 AU/ml, whereas after vaccination, antibody levels generally exceed 1,000 AU/ml. Antibody levels are higher overall in people who have been previously infected; vaccination after infection and reinfection after vaccination are expected to increase existing antibody levels.
The current thinking is that there is no threshold antibody level that provides complete protection against infection, but rather higher antibody levels are likely to be associated with a lower probability of infection.

CELLULAR IMMUNITY

This is the search for memory cells capable of immediately recognizing the virus and triggering a rapid response

According to a French study from the University Hospital of Strasbourg (Gallais et al; 2020), serology (detection of specific antibodies) to detect SARS-CoV-2 infection is absolutely unreliable: there is an absence of antibodies but a strong cellular immunity in pauci or asymptomatic persons. This cellular immunity can be assessed by the ELISpot (interferon-gamma (IFN-γ) enzyme linked immunospot) test: in this study, peripheral blood mononuclear cells are collected and stimulated with a pool of peptides covering not only the spike protein but also the other structural and non-structural proteins of SARS-CoV-2. Then the synthesis of interferon γ by the stimulated T cells is measured.

Contact patients of moderately affected Covid individuals developed Covid symptoms. They were seronegative but showed cellular immunity to SARS-CoV-2.

The serologies were performed with 3 different tests (one of them using the lateral flow technique) and the epitopes tested are the nucleoproteins and the spike protein of SARS-CoV-2. Thus, the cellular response is more sensitive than serology. An asymptomatic contact also develops a cellular response.

The explanation could be that exposure to low doses of virus could induce a brief replication of the virus in these contacts: innate immunity could abort a correct replication of the virus.

Regarding the detection of persons having been infected by Covid-19, the search for antibodies thus leads to an underestimation of exposure.

Virtually all patients tested (healthy, index and contacts) have a response to the spike of HCoVs (common cold coronavirus). This also confirms what has been published as early as 2020 on cross immunity with common cold coronaviruses (Banoun, 2020).

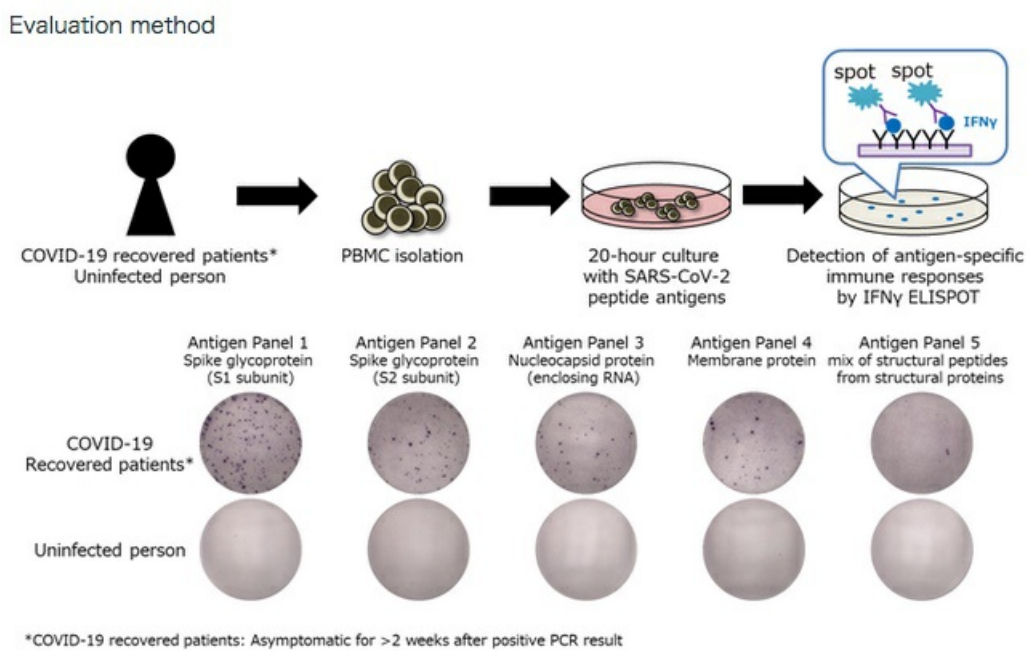

Figure2. Sequence of the ELISPOT method and results of immune response detection

Test description: https://www.kobe-u.ac.jp/research_at_kobe_en/NEWS/collaborations/2020_12_24_01.html

A commercial reagent is available in France: Elispot Cerba (Cerba)
Unfortunately this test is expensive (about 200€) and is not reimbursed by health insurance.
The test consists in measuring the specific cellular response to more than 250 peptides of the SARS-CoV-2 Spike (S) and Nucleocapsid (N) proteins by quantifying the number of T lymphocytes producing interferon γ. Lymphocytes from the patient or the vaccinated patient are isolated and then put in contact with the virus antigens, after 20 hours of incubation, the production of interferon γ is measured. A positive test differentiates immunity related to infection by the virus (anti-S and anti-N positivity) from immunity related to vaccination (anti-S positivity alone).
https://www.lab-cerba.com/home/vous-informer/news/sars-cov-2--un-nouveau-test-dans.html

A British reagent exists and has also been evaluated : T-Spot.Covid from Oxford Immunotec Ltd is available in the UK (https://www.tspotcovid.com), it has been evaluated by Public Health and has a sensitivity of 98.4% and good specificity (there may be a small percentage of false positives by cross-reacting with common cold coronavirus peptides) (Wyllie D et al., 2021) and by Kruse et al., 2021.

Conclusion

Prior to the introduction of the third dose, french health authorities recommended only one dose of vaccine for those already infected, and therefore required pre-injection serology, but this was not mandatory. To date, there is no clear recommendation for a second dose for those already infected.
Serology (measurement of the level of specific anti-SARS-CoV-2 antibodies) gives heterogeneous results depending on the individual, the time elapsed since infection, the severity of the symptoms observed at the time of infection, the reagent used and the laboratory performing the analysis. Low levels of antibodies are difficult to interpret, so it is not sufficient in case of a negative result. It is possible to measure specific anti-Covid cell immunity but this test is expensive and not reimbursed.

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