Coronavirus-19/COVID-19, reflections by an immunologist

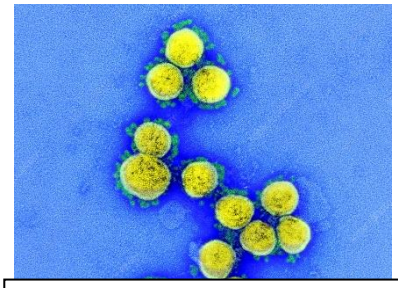

Fig. 1. Transmission electron micrograph of CoV-2 (NIH, Science Photo Library)

Coronavirus-19, also known as SARS-CoV-2 or CoV-2, or CoV-19 is a member of a group of related viruses, the Coronaviridae, and was first discovered in humans causing the acute respiratory infection COVID-19 in Wuhan, China, in December 2019, probably originating in a live wild animal market there. It is endemic in bats and was probably transmitted to humans via an unknown secondary animal host. It is related to SARS-CoV and MERS-CoV that caused outbreaks of severe acute respiratory syndrome causing many deaths in South East Asia, including Japan, and the Middle East respectively. Like the other coronaviruses, the genome is RNA (ribonucleic acid) in the central core combined with a core protein. The core is surrounded by a protein coat which is covered by a membrane. Embedded in the membrane are 3 proteins: a matrix protein, an HE protein, and projecting spikes (S protein) as seen in Figure 1. The spikes give the virus a crown-like appearance, hence the name, and are responsible for attachment to the surface of cells, e.g. lung cells. The spike proteins bind to a receptor molecule (ACE2) on the surface of the lung cells and other cells in the body. This triggers a change in the structure of the S protein on the surface of the virus, causing fusion with the cell membrane and entry into the lung cell. Once inside the cell, the RNA is released and takes over the machinery of the cell to make large numbers of new viruses. The first step is the production, from the viral gene, of an enzyme (RNA polymerase) that makes multiple copies of the viral RNA. This induces the cell to translate the other viral genes and make viral proteins. These are then combined with the viral RNA molecules to make a large number (100s to 1000s) of new viruses which are then released from the cell, resulting in the death of the cell and infection of more cells (Figure 2). The HE protein may be involved in release of completed viruses from the cell.

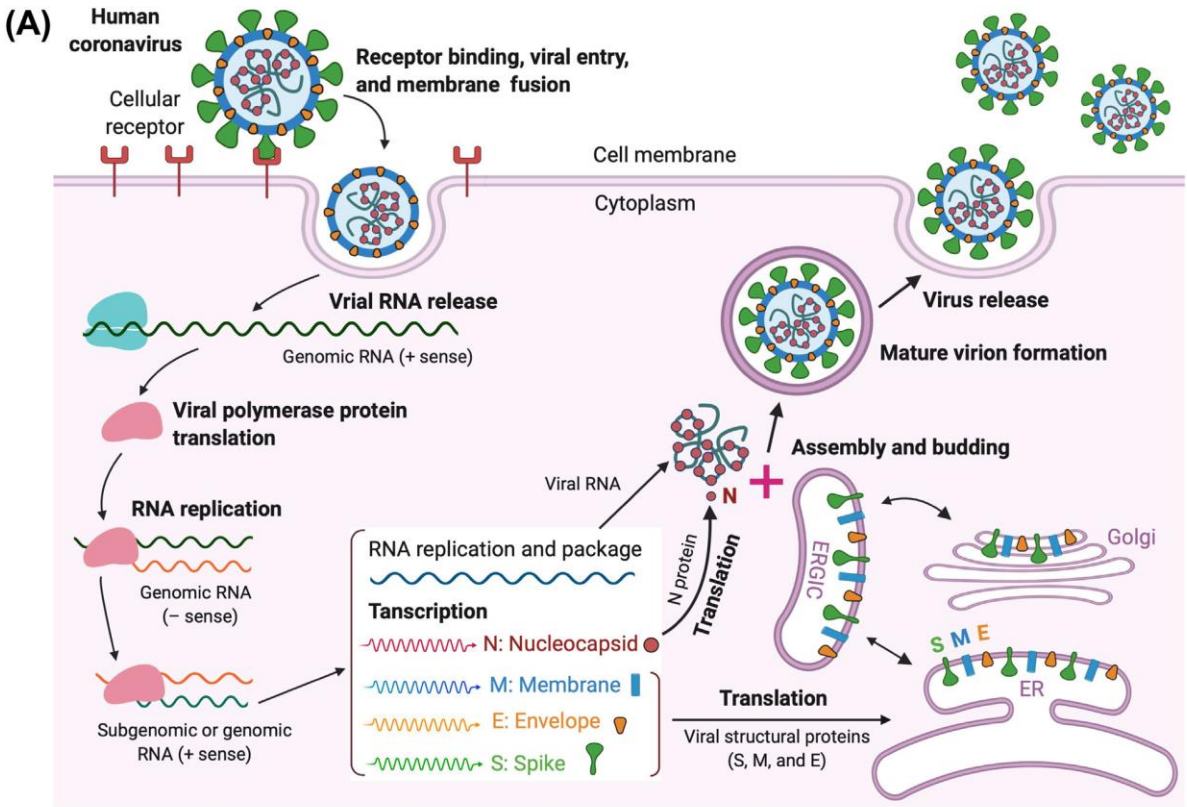

Fig. 2. Mechanism of viral entry, replication and release from a cell (Jiang et al., Trends in Immunology, April 2020).

Different regions of the S protein and the other membrane proteins are potential vaccine targets. Before discussing various approaches to vaccine development it is important to understand how our immune system responds to a virus infection.

A brief overview of the immune response to viruses

There are three wings of the immune response to viruses and other infection: The innate immune response, the B-cell (antibody) response and the T-cell response. This essay is far too short to describe these different aspects of the immune system in detail so I shall summarise their main aspects below.

The innate immune response:-

Briefly, the innate immune response consists of phagocytic cells (literally eating cells) that can gobble up clumps of virus particles, and natural killer cells that can destroy some, but not all, virus infected cells. These cells are present all the time in our body on the lookout for invading microorganisms, but are not very efficient at clearing viruses without the help of the B-cell and T-cell systems.

The B-cell response

Antibodies are made by B-cells and are highly specific for individual viruses by binding to regions of viral proteins (antigens) resulting in destruction of the virus. Antibodies to a virus are made in large quantities during an infection but only after a delay of about two weeks. They can clear viruses that are free in the blood and tissue fluids but cannot attack a virus once it is hidden inside a cell.

The T-cell response

The third wing of the immune response is the T-cell response. Again, this is highly specific for viral proteins and is mediated by cytotoxic T-cells (Tc cells). These recognise small regions of the viral protein and kill infected cells but, again, it takes time for sufficient T-cells to be produced. The antibody and T-cell wings of the immune system respond much more quickly and strongly if they have encountered the antigens before, either from a prior infection by the same virus, or after immunisation with a viral antigen. This is due to the presence of long-lived memory B and T cells specific for the virus and is the basis of vaccination. The idea of a vaccine is therefore to mimic an infection without the harmful effects. In the case of CoV-19 it is not yet clear whether people who have recovered from COVID-19 are protected against further infection by the same virus, although this is usually the case for most, but not all, virus infections.

Current Research on a Vaccine against CoV-19

There are currently (April 2020) at least 79 pharmaceutical and biotechnology companies, research institutes and universities around the world involved in various stages of development of a vaccine against CoV-19 and the field is moving very quickly, so some of the work presented here will be out of date by the time you are reading this essay. Conventional methods of producing a vaccine have been to grow the virus in large quantities and either kill the virus, e.g. influenza and the Salk polio vaccines, or to attenuate the virus rendering it weakly viable but harmless. At least two companies are using these methods but they are fraught with problems for a new, highly infectious and pathogenic virus like CoV-19. Unlike influenza virus, it does not grow on eggs but has to be grown in tissue culture which is difficult and very expensive on a large scale. Also, extreme precautions have to be taken to protect laboratory staff. More modern methods involve various techniques of genetic engineering targeting proteins involved in the mechanism of infection (Figure 2), which has the great advantage that no virus is handled at any stage of production. For example, the gene(s) encoding viral proteins, such as the CoV-19 S protein, can be copied from the viral RNA genome into DNA and inserted into a bacterium or yeast which will make the protein antigen. The bacteria or yeast can be grown on an industrial scale and the antigen purified, a technology that is under development by several organisations. Others are investigating direct injection of RNA or DNA encoding viral proteins into muscle or skin cells which then copy the gene(s) to produce the antigen *in vivo*. Another method is to synthesise small peptides (short strings of amino acids, the

building blocks of proteins) that correspond to antigenic regions of the viral proteins An alternative technology is to insert the relevant gene(s) into a harmless virus such as genetically modified adenovirus, which has only limited reproductive capability. The virus will infect a small number of cells which then produce the protein antigens encoded by the genes. This is particularly good at inducing a T-cell response as fragments of the protein antigen are presented on the surface of the cell in a form suitable for stimulating T-cells. Several organisations are developing anti-CoV-19 using this method.

The usual route for identification of an effective vaccine is to start with animal models, usually mice and/or rabbits and possibly primates such as marmosets. Only then is a potential vaccine used in clinical trials. These would normally start with Phase I with a small number of volunteers to test safety and the immune response, followed by Phase II or I-II, involving a larger number of individuals. If the results are promising, a very large Phase III trial is performed with a placebo control group that is not given the vaccine but may be given an irrelevant vaccine against a different infection. This is to ensure that any apparent protection is real and specific for the virus under investigation.

In the UK, the University of Oxford is embarking on a Phase I-II trial involving 1,110 healthy volunteers. They will be split into two groups: group A will receive the CoV-19 vaccine whereas the second group, the placebo control, will be given a meningitis vaccine. None of the volunteers will know which vaccine they have been given so that it doesn't affect the result. If successful, they will then move to a phase III trial with a much larger number of recipients. A group at Imperial College, London, is also developing a vaccine and hopes to be entering clinical trials soon. Other groups around the world are using similar approaches to development of an anti-CoV-19 vaccine. However, past experience shows that the success rate of vaccines in clinical trials can be as low as 10 – 16%, although that may be improved using modern vaccine technology.

Therapeutic antibodies

Since there is a delay between vaccination and an effective immune response, vaccines are usually only effective prophylactically, though they are essential to eliminate the virus from the population. The same targets for vaccines can also be used to generate monoclonal antibodies for therapy. Monoclonal antibodies are produced by single B-cells and are highly specific for the chosen target. They were discovered by George Köhler and Cesar Milstein at the Laboratory for Molecular Biology, Cambridge, UK, and can be produced in large quantities. They are widely used for diagnosis and therapy of many diseases, from rheumatoid arthritis to various cancers. Several organisations are producing monoclonal antibodies for treatment of Covid-19 patients. Until these are available, serum or plasma containing high titres of anti-CoV-19 antibodies, from people who have recovered from COVID-19, are being tested in patients.

The cytokine storm

One of the causes of death from COVID -19 is the triggering of a cytokine storm. Cytokines are messenger proteins produced by cells of the immune system that enable them to "talk" to each other, much in the way that an army sends messages between the different units in response to an enemy attack. In some circumstances, there is excessive, amplified release of a large number of cytokines, starting with one called IL-6, resulting in severe inflammation and damage to multiple organs. Since this is an over-reaction of the immune response, drugs can be used to dampen it, but these are generally non-specific and suppress the immune response to the virus, which is clearly undesirable. More specific drugs targeting IL-6, including an anti-IL-6 monoclonal antibody, are now available and will almost certainly be used in severely ill COVID -19 patients. Why the cytokine storm occurs in some patients and not in others is unclear.

Conclusions

Several different technologies are being applied to development of a vaccine against Coronavirus-19 with the result that it is very likely that we shall eventually have a safe, effective vaccine but this will take time. The work is proceeding very rapidly but it is essential that this is not at the expense of ,

become available for blocking infection and to prevent the cytokine storm without suppressing the specific immune response.

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