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Topic: The Immune System and COVID-19

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The Immune System

The following is a simplified summary of the immune system. A more thorough discussion can be found in the book *Immunobiology*, which is freely available through the NIH virtual bookshelf at https://www.ncbi.nlm.nih.gov/books/NBK10757/ or in an overview at MedlinePlus at https://medlineplus.gov/ency/article/000821.htm, which is provided by the NIH.

The immune system functions to remove disease-causing germs, recognize and neutralize harmful substances from the environment, and eliminate harmful changes to tissues in the body, such as cancer cells (Janeway et al., 2001). In general, a bacterium, virus, or other microorganism that can cause disease is called a **pathogen**, and the main focus of most immune functions is protection from pathogens. The parts of the foreign body or pathogen that stimulate an immune response are called **antigens**. There can be more than one antigen on a pathogen that interacts with the immune system because they are usually large and have a number of different proteins on the surface. There are two branches of the immune system, the innate immune system and the adaptive immune system.

Branches of the Immune System	
Innate Immune System	**Adaptive Immune System**
• General defense against the initial arrival of a pathogen in the body • Always active	• Activated by the presence of a specific pathogen • Mount a defense that is tailored to the molecular components of the pathogen

In general, the innate immune system will attack all pathogens **to prevent them from starting an infection**, and the components are located in areas where pathogens can enter the body. The adaptive immune system functions to **remove pathogens** that are able to enter the body and infect cells. Both systems interact with each other as well, and there is crosstalk between their functions.

The Innate Immune System

The **innate immune system** reacts in a non-specific way to a pathogen in the body. The innate immune system is mainly localized within areas of the body where antigens and pathogens enter the body, such as the skin, respiratory tract, and gastrointestinal tract. The innate immune system removes potential threats with cells called **natural killer cells** and **phagocytes** that identify foreign bodies and kill or engulf them so they can be removed from the body. The innate immune system also initiates a coordinated immune response by activating cells called **macrophages**. Macrophages release molecules, e.g. cytokines, chemokines, and interferon, which trigger an inflammatory response.

The innate immune system identifies any type of organism or particle that does not display the characteristics that identify it as "self," or part of the host organism, and it does not employ tactics that are specific to the pathogen.

Interferons are a small protein-based molecule that participate in the control of the innate immune response to infections (Ivashkiv et al., 2015). There are numerous types of interferons that are labeled by type (I to III) and specific form (designated by Greek letters). When a cell interacts with a type I interferon, it alerts the cell to a potential infectious threat, and there are changes to the cell that make it more difficult for infectious agents, and especially viruses, to infect the cell. Cells also are primed by interferons to present antigens from the virus for identification by T cells. Interferons also promote the initiation of steps that lead to production of antibodies by B cells and responses to antibodies by T cells.

Overall, the effect of interferons is to prime cells for interaction with the adaptive immune system in response to a viral infection without overstimulating the non-specific inflammatory response from the innate immune system.

The Adaptive Immune System

The **adaptive immune system** targets specific pathogens to clear them from the body. The response is also referred to as **acquired immunity**. The two main cellular components of the adaptive immune system are B cells and T cells, which are also called lymphocytes. In general, B cells function to produce antibodies, and T cells remove pathogens. There are a number of different types of B cells and T cells that perform specific functions, but in this discussion, the general functions will be described for simplicity. **B cells and T cells produce proteins on the cellular surface that specifically interact with only one antigen**. In other words, there is one population of identical B cells and T cells that is equipped to interact with the surface protein of the measles virus, which is called hemagglutinin, and hemagglutinin is an antigen that only that specific population of B cells and T cells respond to.

The response of the immune system from both the innate and adaptive branch is designed to feed back to earlier signals to either reinforce and amplify the response or to quell it if other signals are not present.

This process is meant to keep the immune system from activating when pathogens are not present, which can lead to damage to surrounding tissues and autoimmune conditions. For example, the production of cytokines from macrophages in the innate immune system stimulate T cells that have come into contact with their antigen, but if the T cell's antigen is not present, the T cell does not activate, and if macrophages do not release cytokines because a pathogen has not been detected, the T cells are not further stimulated. Activated T cells can in turn stimulate the function of B cells that respond to the same antigen in order to propagate the immune response and produce cytokines and other immune system messengers to increase the activity of the innate immune system.

What are T Cells?

T cells (or **T lymphocytes**) are cells from the adaptive immune system that function to remove pathogens from the body and coordinate the immune response. T cells produce surface proteins, called the T-cell receptor, that interact with a single antigen. The T-cell receptor is permanently attached to the surface of the cell and is not released into the bloodstream like antibodies from B cells. The T-cell receptor allows the cell to interact with its antigen in order to activate the function of the cell.

T cells also produce a second receptor that contributes to the function of the cell. There are several types of the secondary receptor, but the main types are **CD8** and **CD4**. Those T cells that produce CD8 receptors function as **cytotoxic T cells**, which produce chemicals to kill infected cells displaying the antigen that binds to its T-cell receptor. For example, when a host cell is infected by a virus, the cell is forced to produce viral proteins, which make their way to the cell's outer surface. If a cytotoxic T cell recognizes the antigen on the infected cell, the T cell releases molecules that kill the infected cell and in so doing stops production of new copies of the virus. T cells that produce the secondary receptor called CD4 become **helper T cells**, which produce immune system components that modulate immune responses, e.g. cytokines. Helper T cells can interact with free antigens in the blood stream, antigens that are displayed on the surface of infected cells, or antigens that have been bound by antibodies. The different scenarios where the T cell encounters the antigen can lead to different responses by the helper T cell and differing adjustments to the immune response.

What are B Cells and Antibodies?

Antibodies, which are also sometimes called **immunoglobulins**, are a key component of the adaptive immune system. Antibodies are proteins that have two main functions, neutralization of infectivity of a pathogen and identifying pathogens for other parts of the immune system. Neutralization occurs when an antibody binds to a pathogen and blocks the area required to infect a cell. **In the case of SARS-CoV-2, neutralizing antibodies bind to the spike protein, which is used to bind cells with the ACE2 receptor and begin fusion of the viral surface with the cell's surface.** Antibodies can also hasten the elimination of a pathogen from the body by alerting the immune system of its presence. Binding antibodies can interact with any part of a pathogen, unlike neutralizing antibodies, and when components of the immune system, such as cytotoxic T cells, come across an antibody attached to a pathogen, they digest and/or destroy it.

B cells, which are also called **B lymphocytes**, are the part of the adaptive immune system that produces antibodies. Immature B cells are located in areas where they can come into contact with antigens, such as the spleen and lymph nodes. Numerous immature B cells are available, and each one produces a different antibody on the surface of the cell. The antibodies are randomly generated, and a B cell scans antigens until it finds one that interacts specifically with it. The B cell that interacts with the antigen is then stimulated to mature and divide in order to produce identical copies. The maturation of B cells requires the interaction with helper T cells that interact with the same antigen as well as the molecules released from helper T cells.

Many B cells mature into another type of cell called an **antibody secreting cell**, or **plasma cell**, that produces large amounts of identical antibodies and releases them into the bloodstream.

The category of antibodies that are produced (also called **isotypes**) changes as the infection progresses. Each different isotype of antibody still only interacts with a single antigen, but there are changes in other regions of the antibody that allow it to interact with different components of the immune system and modulate a response. The different antibody isotypes are called **IgM, IgG, IgE, and IgA**. IgM antibodies are the first antibodies produced in response to an infection. They are found mainly in the bloodstream because they are large and cannot easily move into other areas. As the response to the infection progresses, the other isotypes of antibody are produced. IgG, IgE, and IgA antibodies are smaller than IgM isotypes and can move from the bloodstream and into the tissues that are affected by the pathogen. IgG is mainly located in the blood and extracellular fluid within a tissue, and IgA antibodies are mainly found in secretions in the mucous producing parts of the intestinal and respiratory tracts. IgE is present only in small quantities in the blood and fluids in tissues that make up the skin and mucous producing areas. When IgE interact with their antigen and specialized cells, called mast cells, they trigger coughing, sneezing, and vomiting to expel infectious agents.

> **When the amount of the antigen decreases, as occurs when an infection is cleared, the antibody secreting cells are no longer stimulated to produce antibodies**.

The lifespan of an antibody is around **20 days** before it is metabolized through ordinary processes that remove older, damaged proteins from the body, and as the B cells stop producing new antibody, the level in the bloodstream and other tissues will decrease.

When the amount of antigen decreases, some of the B cells transform into a type of cell called **memory B cells**, which are long-lived cells that can quickly respond to another exposure to the infectious agent, starting the process again (Palm and Henry, 2020). Memory B cells do not secrete antibodies, but instead produce them on the surface of the cell. When the antibody interacts with its antigen it is stimulated to propagate and produce large numbers of itself that can then become antibody secreting cells.

While the process described above is the “typical” way B cells react during an immune response, there are some pathogens that elicit a long-lived antibody response. For example, the virus that causes chicken pox elicits an antibody response of more than 50 years, and measles and mumps lead to an antibody response estimated to extend for as long as an individual’s lifetime (Amanna et al., 2007). This extended response has been shown to not be dependent on memory B cells through experiments in animal models that lack memory B cells. In the animals, antibody levels from vaccination remained steady even after memory B cells were removed. Instead, the source of the continued antibody response in the absence of antigen is a special form of plasma cell that lives longer than usual. A typical plasma cell lives for between a few days and a few weeks (Slifka and Amanna, 2020). There are studies that have identified some plasma cells in bone marrow that are present for at least 6.5 years (the length of observation time in the study). Researchers have investigated why some pathogens elicit a long-term antibody response using long-lived plasma cells and others rely on memory B cells to restart a response after antibody levels decline, but specific causes have not been determined. The current hypothesis suggests that the level of response, e.g. higher activation of B cells, leads to a longer antibody response. The speed at which a pathogen induces a disease state has also been implicated in the length of the antibody response, and pathogens that cause illness quickly after exposure are more likely to have a prolonged antibody response.

What are the Basic Steps in an Adaptive Immune Response?

1. Pathogen grows to levels sufficient to elicit an **adaptive immune response**.
2. **Antibodies**, **cytotoxic T cells**, and **helper T cells** specific to the pathogen are produced.
3. **Cytotoxic T cells** eliminate the pathogen, and **neutralizing antibodies** prevent infection of more cells.
4. **Antigen levels decline**, and T cell levels and antibody levels decline due to a lack of stimulation by the antigen.
5. **Memory cells** remain that have a heightened ability to respond to recurrence of infection with the same pathogen.

Ideally, the adaptive immune system responds to a pathogen by enacting a substantial helper T cell, cytotoxic T cell, and neutralizing antibody response to a pathogen, and all contribute to clearance of the initial infection. After the infection is cleared, some of the T and B cells are retained for multiple years as an immunological memory to provide a protective immunity against re-infection.

What Happens when the Immune System Sees the Same Pathogen?

After an infection is cleared, both T cells and B cells transform into **memory cells** that can produce a fast response if there is another exposure to the infectious agent.

> **The rapid response is possible because there are already mature, antigen-specific lymphocytes at the ready.**

Memory cells do not require the presence of the antigen they bind to in order to survive and propagate, unlike regular lymphocytes. This important concept means that **memory cells are not maintained by repeated exposure to infectious virus**. Most memory cells exist in a suspended state until they contact their antigen again, but there is occasional proliferation of the cells in order to keep the population constant.

A subsequent exposure to a pathogen can occur while antibodies are still present in the bloodstream or when there are essentially no antibodies remaining. If there are antibodies from the initial response, they can immediately begin binding to the antigen and divert it to cells responsible for removal. If there is sufficient antibody remaining to clear the infection in this manner, a new immune response will not be activated. If there are not enough remaining antibodies to remove the pathogen, memory B cells and T cells will encounter their antigen and start the response again. When the immune response against a pathogen is reactivated, helper T cells are the first cells observed. B cells require input from helper T cells to propagate, and therefore, an increase in B cell numbers is not seen until after the T cells have been activated.

Memory B cells are mainly located in the same areas as immature B cells, the spleen and lymph system, and come into contact with activated helper T cells and antigen via the blood stream. T cells are able to move through tissues and interact with antigens at the source of an infection. Memory B cells are activated by lower levels of the antigen than immature B cells, leading to a response at a smaller amount of antigen than the first encounter, which also increases the response time. After activation, memory B cells multiply and begin to produce antibodies. The isotype of antibodies produced by memory cells are mainly IgG, IgA, and IgE because they have progressed past the step in maturation where IgM is produced.

Antibody and T-Cell Response to SARS-CoV-2 Leading to Immunity

Initial discussions of immunity to SARS-CoV-2 were mostly focused on the level of antibodies produced in response to infection because antibody levels are relatively easy to measure. Antibodies are proteins and fairly stable under a range of conditions and therefore easy to manipulate. Investigation of T cell immunity requires the propagation of T cells from the blood of donors, which is a more time consuming and delicate process.

A number of studies have reported that the level of antibodies decreases rapidly after the infection with SARS-CoV-2 is over. Different viruses elicit different lengths of antibody responses, and the basis of these differences is not well understood as described above.

If the antibodies to SARS-CoV-2 wane, there may not be sufficient neutralizing antibodies present to prevent an infection, but they can be replenished by stimulating an immune response through memory cells.

While the presence of neutralizing antibodies is an important component to the immune response, specific helper and cytotoxic T cells also mount a defense against a pathogen while activating B cells and the innate immune system.

T-Cell Response to SARS-CoV-2

A number of studies have been published describing the T-cell response to SARS-CoV-2, and they show that there is a strong response in the majority of people evaluated. To measure the T- cell response, it is necessary to obtain T cells from the blood of people who have recovered from SARS-CoV-2 and expose the cells to antigens from the virus. The tests monitor binding of antigens and whether the helper T cells produce cytokines after interaction with the antigens. Because T cells do not prevent the virus from infecting cells like neutralizing antibodies do, T cells that interact with antigens other than the spike protein are still an important part of the response. However, most groups developing vaccines for SARS-CoV-2 are currently using the spike protein as the main antigen, making it important to determine if there are strong responses of T cells to this particular antigen (Grifoni et al., 2020).

Based on previous experience, researchers have found that when an infection with a virus elicits potent T-cell responses associated with protective antiviral immunity it is a strong candidate for rapid vaccine development.

In one investigation of helper T cell response to SARS-CoV-2, researchers produced a pool of potential antigens from SARS-CoV-2 that included 221 targets covering all proteins in the viral genome with a second pool specifically for 253 variations of the spike protein. The response of cytotoxic T cells was tested with an antigen pool that included 12 of the most common antigens from SARS-CoV-2. T cells were obtained from blood samples collected from 40 volunteers. There were twenty unexposed individuals whose blood was collected between 2015 and 2018 by a commercial vendor, and 20 volunteers from the University of California, San Diego Health clinic who had recovered from COVID-19 between 20 and 35 days before. Participants in this study had not required hospitalization for treatment of COVID-19 symptoms. Previous infection with SARS-CoV-2 was established using a combination of PCR-based testing during the active infection period and antibody testing of the collected blood. The antibody testing involved assessment of the presence of the spike protein from SARS-CoV-2, and all participants were found to have IgG antibodies to the spike protein and a large majority also had detectable levels of IgA antibodies to the spike protein.

A general assessment of T-cell levels showed no differences between the unexposed group of participants and those who had recovered from COVID-19. Specifically, there was no evidence of lymphopenia, or a low level of lymphocytes, which has been observed in many people with severe symptoms of COVID-19. There were also no statistically significant differences in the frequencies of helper or cytotoxic T cells between the two groups.

A subset of the participants (samples from ten of the recovered individuals and eleven of the unexposed individuals) were tested for reactivity to the pools of SARS-CoV-2 antigens. **A response of the helper T cells to SARS-CoV-2 spike protein was detected in 100% of individuals recovered from COVID-19.** The pool containing all potential SARS-CoV-2 antigens also elicited a response in all of the participants who had had COVID-19. The proportion of antigen type that contributed to the overall T cell response to SARS-CoV-2 was 27% spike protein, 21% membrane protein (or M protein), and 11% nucleocapsid protein (or N protein). The positions of the proteins on the virus are shown in Figure 1.

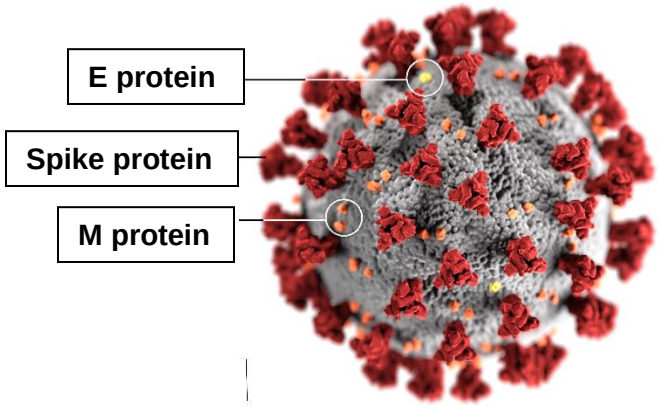

Figure 1. Schematic of the outer shell of SARS-CoV-2. The spike protein is shown in red. The M protein is shown in orange, and E protein is shown in yellow. M protein and E protein (or envelope protein) are proteins within the cellular membrane (gray) that surrounds the virus that assist in formation of the viral particle during production. The N protein is located under membrane and is not visible. (Adapted from https://www.cdc.gov/media/subtopic/images.htm)

The helper T cells also all produced a cytokine in response to the both the spike protein pool of antigens and the whole-virus pool of antigens, showing that the T cells were active.

Approximately 50% of cytokine production from the helper T cells was in response to spike proteins, suggesting that vaccines developed against the spike protein should be able to stimulate protective response.

A response of cytotoxic T cells to the antigens was observed in 70% of the individuals recovered from COVID-19. The researchers were also able to measure a response from the cytotoxic T cells, which secreted cytokines in after being exposed to the antigens from SARS-CoV-2.

A response to SARS-CoV-2 was also observed in between 40% and 60% of the unexposed group of participants. Most of the responses from unexposed individuals were not from antigens of the spike protein. Cross-reactivity between SARS-CoV-2 and seasonal coronaviruses responsible for cold symptoms was suggested as the most likely cause of this response. Accordingly, all of the participants in the unexposed group were found to have antibodies from seasonal coronaviruses, indicating that they had previous exposure to other forms of coronaviruses.

Another study investigated the types of T cells involved in the SARS-CoV-2 response and when they are produced in context of the initial infection (Weiskopf et al., 2020). Blood samples were obtained from ten people who required admission into the intensive care unit for treatment with mechanical ventilation from COVID-19 symptoms. The T-cell responses were compared to a historical group that had not been exposed to SARS-CoV-2. In general, the individuals with COVID-19 were found to have fewer T cells, which has been reported by a number of groups describing clinical symptoms of COVID-19. The percentage of T cells in the blood samples was 44% for the unexposed group and 12% for the group with COVID-19. The ratio of helper T cells and cytotoxic T cells was also found to be different between the two groups, and participants with COVID-19 had fewer cytotoxic T cells.

The T-cell populations were exposed to the same pools of SARS-CoV-2-based antigens as used in the previous report from Grifoni and colleagues. Helper T cells specific for the virus were observed in all ten of the participants while eight were found to have SARS-CoV-2 specific cytotoxic T cells. Helper T cells had a strong response to the spike protein, and the cells were functional based on tests to measure cytokine production. The cytokines that dominated the responses in the group with COVID-19 were IL-6, TNF-alpha, IL-2, and interferon-gamma. Assessment of the level of antibodies while participants with COVID-19 were hospitalized showed that there was an increase in IgG antibodies to the spike protein. The researchers were able to detect the presence of helper T cells and cytotoxic T cells at multiple points during the patient's hospital stay. It was possible to detect SARS-CoV-2-specific T cells in blood samples from patients with ARDS (acute respiratory distress syndrome) in the first two weeks after the onset of symptoms. The amount of helper T cells was found to increase over time while cytotoxic T cell levels did not increase. **The researchers also detected the presence of memory T cells specific to SARS-CoV-2**.

Participants in the unexposed group in this study were found to react to SARS-CoV-2 antigens, presumably from previous exposure to other coronaviruses. During the study, the T cells of two of the ten unexposed participants reacted to antigens from SARS-CoV-2.

A third study also had similar results showing the binding and activation of T cells to SARS-CoV-2 antigens. The researchers mapped which end of the spike protein from SARS-CoV-2 was more likely to interact with the T cells (Braun et al., 2020). The study included 68 healthy donors who did not have a SARS-CoV-2 infection based on PCR-based testing and subsequent retesting with serological tests. There were also 25 participants with COVID-19 from Charité Campus Virchow-Klinikum, Berlin, between March 1 and April 2. The group with COVID-19 had a range of symptom severity with 38.9% with mild symptoms, 27.8% with severe symptoms, and 33.3% with critical symptoms. Additionally, 55.6% of the participants with COVID-19 were treated in the intensive care unit.

The researchers found that 67% of the participants with COVID-19 had helper T cells that reacted to the end of the spike protein that contains the receptor binding domain (RBD), and 83% of the participants who recovered from COVID-19 had helper T cells that interacted with the opposite end of the spike protein that is closest to the surface of the viral particle. Both active T cells and memory T cells were identified.

In the group who had not been exposed to SARS-CoV-2, 35% participants had helper T cells that reacted to the part of the spike protein closest to the surface of the membrane while only 5.8% had helper T cells that reacted to the RBD end of the spike protein. The end of the spike protein that binds to the ACE-2 receptor has been found to have the most variation in amino acid composition between different coronaviruses while the region near the virus surface contains fewer changes. This result confirms the previous observation of a conservation of the sequence at one end of the spike while the other end has variations between types of viruses. As in previous studies, the unexposed participants all had produced IgG antibodies to the seasonal coronaviruses that produce cold symptoms. The antibodies were present even in the participants who did not have helper T cells that cross-reacted with SARS-CoV-2 antigens.

After recovering from COVID-19, researchers have observed that there is often a long period where individuals shed viral RNA (Lee et al., 2020). It has been suggested that the shedding is mainly digested virus rather than whole, infectious virus. Researchers in Singapore were able to analyze the characteristics associated with longer viral shedding in a group of individuals who had a range of symptom severity. It was found that the length of the time period of viral shedding was correlated with the strength of the T cell response at the start of the infection. This characteristic was measured by testing the amount of T-cell specific cytokines in the bloodstream throughout the course of the infection. Individuals who had low levels of the cytokines IL-1beta and IL-17A had prolonged viral shedding, and these cytokines have been identified previously to be key to the activation of anti-viral T-cell responses.

Based on their results, they concluded that a less active T-cell response at the initial phase of infection was associated with prolonged viral RNA shedding, suggesting that early immune responses are beneficial to control viral load.

Age, gender, comorbidities, and disease severity were not associated with duration of viral RNA shedding.

Cross-Reactivity to Other Coronaviruses

The response of unexposed individuals to antigens from SARS-CoV-2 has been observed in a number of studies with the response varying from 20% to 50% (Mateus et al., 2020). The groups included in the studies were from geographically diverse areas, including the United States, Netherlands, Germany, Singapore, and the United Kingdom.

Based on epidemiological data, it is estimated that adults contract a seasonal coronavirus about once every two to three years, and when re-infection occurs, it usually involves low-level and short-lived viral shedding with only mild symptoms of short duration (Braun et al., 2020). Based on knowledge of immune responses, this pattern suggested to the researchers that the response to coronaviruses is mediated by cellular responses (e.g. T cells) rather than a strong antibody-based response.

A specific investigation of the cross-reactivity of human T cells was performed to determine if exposure to seasonal coronaviruses was the cause (Mateus et al., 2020). The T cell samples were collected between March 2015 and March 2018 in order to predate the SARS-CoV-2 pandemic, and the samples were also tested to assure they did not contain antibodies specific to SARS-CoV-2. While the participants did not have antibodies to SARS-CoV-2, almost all the individuals had antibodies from a previous exposure to the three coronaviruses that are most widely spread each year (HCoV-NL63, HCoV-OC42, and HCoV-HKU1). The T cells from the blood samples were exposed to a pool of antigens that encompassed the entire spike protein and a second pool that encompassed antigens found in the rest of the viral genome.

In total, 474 antigens were screened, and 142 were found to interact with T cells from individuals who had not been exposed to SARS-CoV-2. **There were 66 antigens recognized from the spike protein and 76 from the rest of the viral genome**. The T cells in each unexposed-person's sample recognized an average of 11.4 antigens from SARS-CoV-2, and 40 antigens (or 55% of the total) were recognized by more than one person. The region of the virus that had the most frequent (54%) and vigorous responses was the spike protein. Within the spike protein 11% of the antigens fell in the RBD region, and 44% were in the rest of the spike protein. There were no antigens recognized from the M protein of SARS-CoV-2 in the unexposed group even though the protein is part of the immune response observed in people with SARS-CoV-2 infection.

The similarity between the amino acids that form the proteins in the different types of coronaviruses was then investigated. As expected, there was a higher level of similarity observed in the antigens that had been recognized in more than one participant and a lower level of similar amino acids in antigens that were only recognized by a single person or not at all. A threshold was identified where a similarity of 67% or higher between protein in different viruses led to cross-reactivity.

> **Based on their experiments, the researchers found that memory T cells created during a previous exposure to the seasonal coronaviruses exhibit a substantial cross-reactivity to SARS-CoV-2.**

Evaluation of the antigens identified also was performed for T cells from individuals who had recovered from COVID-19. There is an occurrence where exposure to two related viruses can lead to an impaired response of T cells to antigens from the second virus, a process called an original antigenic sin phenomenon. If this occurs, the immune system attempts to fight the infection from the second virus only with T cells targeted against antigens from the first virus rather than using T cells specific to the second virus, leading to a reduced immune response and a more serious disease from the second virus. There were concerns that this was occurring with SARS-CoV-2, but these experiments suggest that new T cells that are specific to SARS-CoV-2 are produced and the increased severity of COVID-19 is not due to a muted immune response occurring from exposure to other coronaviruses.

The cross-reactivity observed with T cells has not been found to occur with neutralizing antibodies produced after exposure to the seasonal coronaviruses. Antibodies to seasonal coronaviruses have been shown to decrease quickly, but the above studies indicate that cellular immunity remains. The biological role of the reactivity to SARS-CoV-2 antigens from exposure to other coronaviruses is not yet known. Some hypotheses include an explanation for the wide range of symptoms and severity of the infection and how children and younger adults seem to be more resistant to infection due to a potential increased exposure to infections in school environments.

However, a review of the available information on SARS-CoV-2 and previous knowledge collected about viral immunology suggests that SARS-CoV-2 T-cell memory likely lasts for years and confers protection from severe disease due to reinfection.

This assumption is being investigated further with studies in primates, which have similar immune systems to humans and would be expected to have a similar response to the virus (Altmann and Boyton, 2020).

Antibody Responses to SARS-CoV-2

Numerous studies have been reported on the longevity of the antibody response associated with SARS-CoV-2 infection, and antibodies against the virus seem to be short-lived. There are two different types of testing to detect if antibodies are present, and a third test that can determine if the antibodies are able to neutralize the virus. The detection assays use antigens from the SARS-CoV-2 as “bait.” Antibodies in the blood bind to the bait antigens that have been immobilized on a surface, and a colored solution is added that binds to the antibody-antigen complexes and can be monitored.

The population-wide serological tests use a point-of-care form of antibody testing that is similar in design to a pregnancy test called a **lateral flow test**. A lateral flow test works by absorbing the liquid to be tested with a wicking action up to a section where the antigens are immobilized and a section to ascertain the test is working correctly (or a positive control). These types of tests are more likely to lead to false positives in part because it can be difficult to interpret a faint line on a test. Typically, the more antibody that is present, the more intense the line on the test is, but quantifying the intensity to the level of antibodies present is difficult and requires a large number of rounds of repeated testing to establish.

More labor intensive, and therefore slower, tests allow for the quantification of the amount of antibody and determination of the isotypes present (e.g. IgG or IgA). These tests are called **ELISA (enzyme-linked immunosorbent assay) tests**. As with a lateral flow test, the antigen of interest is immobilized onto a surface. ELISA tests use plastic plates to immobilize the antigen, and the liquid containing the antibodies is applied to the surface. The plate is then washed to remove any antibody that is not interacting with the antigen, and the antigen-antibody complex is detected with a colored solution, a fluorescent dye, or chemiluminescent reaction. Because the tests are performed on clear, plastic plates the color of the reaction can be measured with a detector that can precisely measure the intensity of the color of the solution. By using a series of known amounts of antigen immobilized on the plate, the characteristics of the binding can be determined with great accuracy. The need for specific detection equipment and multiple reagents means that ELISA tests can only be performed in a laboratory setting, and they are time consuming compared to a lateral flow test. ELISA tests are more accurate, however, and can be used to measure the amount of antibodies present in an individual's blood unlike lateral flow tests.

The third type of testing can determine if an individual has produced **neutralizing antibodies**. There are two ways to test for neutralization. The first is the most difficult, but it directly measures the ability of the antibody to prevent viral infection. Live virus is mixed with antibody, and then applied to a plate of cells. If the virus can infect the cells, the cells die leaving an empty space on the plate, which is called a plaque. If the antibodies prevent the infection of the cells, no or fewer plaques will form. Therefore this type of test is also called a **plaque-reduction neutralization test**. This can definitively determine if an antibody neutralizes the virus, but it must be performed in labs with biosafety protections because it uses live, infectious virus. It also requires growth of cells that can be infected by the virus and incubation of the virus with the cells, which can be a lengthy process. In order to reduce risks with using live virus, tests have been developed that use a **pseudovirus** instead. This is a construct that incorporates a benign virus that is modified to produce antigens from the virus-of-interest. The ability of antibodies to prevent the pseudovirus from infecting cells can then be measured in laboratories with less stringent biosafety protocols. However, since the test is not a direct measure of neutralization with the actual virus, it may not be as accurate as plaque-reduction tests.

The presence of antibodies to SARS-CoV-2 has been studied in areas where significant outbreaks have occurred, such as China, Spain, the United Kingdom, and the United States. Studies with all of the types of antibody testing have been performed on blood from individuals with COVID-19 or who have recovered from the disease. As mentioned above, antibody production to a specific pathogen must be induced by the adaptive immune system, and therefore antibodies are not present in the bloodstream at levels high enough to detect until about two weeks after the initial infection. IgM antibodies appear first, and IgG are produced next. Because of the difference in the time period of production, it is possible to use the isotype to make a rough estimate of the time since the active infection.

China

A study of individuals treated at the Wanzhou People's Hospital in the Wanzhou District in Chongqing Province included 37 asymptomatic individuals with positive PCR-based tests for SARS-CoV-2, 37 people with mild symptoms after a positive PCR-based test for the virus, and

37 people who did not have the virus based on PCR-based testing (Long et al., 2020). The participants who tested positive for SARS-CoV-2 were quarantined at the hospital but did not require treatment for symptoms. The researchers investigated the characteristic of antibodies in the different groups using a variation of an ELISA test and pseudovirus neutralizing antibody tests approximately three to four weeks after initial exposure to SARS-CoV-2.

In individuals who remained asymptomatic throughout their illness, it was found that 81.1% had IgG, and 83.8% of the mildly-symptomatic group had IgG antibodies. At the time of the testing, 62.2% of the group that was asymptomatic had IgM antibodies, and 78.4% of the mildly symptomatic group had IgM antibodies. The amount of antibodies produced was higher in the group with symptoms during the active infection period. Subsequent measurements eight weeks after discharge from the hospital showed that the level of antibodies was still higher in the group that had had symptoms, but the levels in both groups had declined since the previous measurements, and 93.3% and 96.8% of the groups had a decline in the level of antibodies.

The median proportion of the decrease between the measurements was 71.1% with a range between 32.8% and 88.8% in the asymptomatic group, and a mean decrease of 76.2% with a range between 10.9% and 96.2% in the mildly symptomatic group.

The amount of neutralizing antibody levels also decreased in 81% of the asymptomatic group and 62.2% of the symptomatic group with a mean decrease of 8.3% with a range between 0.5% and 22.8% for the asymptomatic group and a mean decrease of 11.7% with a range of 2.3% and 41.1% in the symptomatic group.

Importantly, 40% of the individuals in the asymptomatic group became seronegative, which means that no antibodies could be detected, and 12.9% of the symptomatic individuals became seronegative.

The United States

ELISA measurements of the antibody levels from 34 participants in a study at the University of California, Los Angeles allowed for determination of the reduction in antibody levels and the rate of change (Ibarrondo et al., 2020). Thirty of the participants had a positive PCR-based test for COVID-19, and the remaining four were living with an individual known to have COVID-19, but were unable to get tested due to a lack of available tests and mild symptoms. All but two of the participants had mild symptoms that did not require treatment, and the two requiring treatment had low-flow supplemental oxygen and therapy with an anti-inflammatory agent. Measurements of antibodies that bound the receptor binding domain (RBD) portion of the spike protein from SARS-CoV-2 were taken at two time points for most of the participants and at three time points for three of the participants. The first measurement of antibody levels was taken a mean of 37 days after the onset of symptoms, and the time between the first measurement and the start of symptoms ranged between 18 days and 65 days. The last measurement was taken a mean of 86 days after the onset of symptoms with a range of 44 days to 119 days. **The half-life of the antibody response was approximately 73 days. The loss of antibody was found to occur more rapidly than observed with SARS-CoV-1.**

The United Kingdom

A study performed in London investigated the antibody levels from 59 individuals admitted to Guy's and St Thomas' NHS Foundation Trust in London in addition to six healthcare workers at the same hospital (Seow et al., 2020). The paper was submitted as a pre-print and was not peer-reviewed, but assessments by other experts in the field suggest that the protocols used were acceptable (Howard, 2020). The symptom severity in the participants ranged from asymptomatic to critical. The participants were tested multiple times between one and 94 days after the onset of symptoms. The antibody response was measured using the RBD, spike protein, and nucleocapsid with ELISA testing.

IgG antibodies for RBD were detected in 89.2% of participants, for spike protein in 92.3%, and for nucleocapsid in 93.8%. Production of IgM antibodies targeted for RBD was detected in 92.3% of the participants, for spike protein in 92.3%, and against nucleocapsid in 95.4% of the participants. The number of people with IgA antibodies for RBD and nucleocapsid was lower at 72.3% and 84% respectively, while the number of participants with IgA antibodies against spike protein was similar to the values measured for IgG and IgM (92.3%). The peak of antibody production occurred at between 20 and 30 days after symptoms began, and then the levels declined rapidly.

After 60 days, the levels of IgM and IgA had declined back to the levels at the start of the study, but IgG was still elevated in the majority of participants up to the last measurement at 94 days.

There was a large variation in antibody levels between participants with a correlation between symptom severity and the length of time antibodies could be detected. The magnitude of the response was larger in people with more severe disease. Differences in the severity of symptoms did not correlate with the rate of the antibody production or the timing of the peak of neutralizing antibodies. Anti-inflammatory treatments did not impede an antibody response, and participants treated with anti-inflammatory medications did not have a difference in the rate of antibody production compared with those not treated with the medication.

Pseudovirus neutralizing tests indicated that the average time until neutralizing antibodies were detectable was 14.3 days, and the peak production of neutralizing antibodies occurred at an average of 23.1 days after the start of symptoms with a range between one and 66 days.

When neutralizing antibodies were at their peak neutralization, 7.7% of the participants were found to have low levels, 10.8% had medium levels, 18.5% had high levels, and 60.0% had potent levels of neutralizing antibodies.

When the level of neutralizing antibodies was measured again after 65 days, the number of people with potent responses had declined to 16.7%. At the final time point in the trial, there had been a decrease in the amount of neutralizing antibodies in almost all of the participants.

Some individuals who were asymptomatic had undetectable neutralizing antibodies at the end of the study (39 and 34 days after diagnosis).

There was a coordinated decrease in the overall levels of IgG and the decline in the amount of neutralizing antibodies. Researchers sometimes estimate the neutralizing antibodies based on the level of antibodies to the RBD and spike, but there were some people with high levels of these antibodies that did not have a large neutralizing response, showing that use of IgG levels as a surrogate for neutralizing antibodies can be fraught with complications.

Based on the results of the study, the authors conclude that the level of antibodies of people with confirmed COVID-19 can vary widely, and disease severity enhances the magnitude of the neutralizing antibody response but does not affect the rate of antibody production.

The response to SARS-CoV-1 has been found to be more robust and long-lived, but there were also very few mild or asymptomatic cases, which could further support the correlation between disease severity and antibody response, and the response to SARS-CoV-2 may be more similar to the more mild seasonal coronaviruses that cause cold symptoms. Studies of one of the seasonal coronaviruses showed that antibody levels rapidly declined within a year though they were still higher than before the initial infection.

When re-exposed to the same seasonal coronavirus, infection could be detected with PCR-based testing, but symptoms were not observed.

While the loss of antibodies will not likely affect the immune response to re-infection, it is an important factor for assessment of population studies of viral spread that are based on antibody detection. It also reinforces that the use of “immunity passports” as a means of certifying that people have already had COVID-19 is wrought with complicating factors since timing of antibody testing would need to be soon after infection. The results outlined by Seow and colleagues suggests that IgG antibodies should be targeted for seroprevalence studies rather than IgA or IgM because IgG are longer lasting in many people who have had COVID-19, but the presence of IgA and IgM may be useful to determine timing as they are present only early after the active infection period.

Spain

Researchers from the National Centre for Epidemiology and other institutions in Spain reported a population-based study of antibodies to investigate the extent of the large outbreak that occurred in April and May (Pollán et al., 2020). Residents from across the country were asked to participate, and specific selections were made using random sampling techniques. Between April 27 and May 11, 61,075 participants answered a questionnaire on history of symptoms associated with COVID-19 and received a point-of-care antibody test. Additionally, some participants agreed to donate a blood sample for additional testing with an ELISA-based test. The prevalence of COVID-19 antibodies was found to be 5.0% using the point-of-care test and 4.6% with the ELISA-based test. When the results for participants who took both tests was analyzed, it was found that both tests were positive for 3.7% and at least one of the tests was positive for 6.2%. When the people who had been diagnosed with COVID-19 using PCR-based testing were tested later for antibodies, 87.6% were positive for antibodies with both tests, and 91.8% were positive on at least one test. The number of people who tested positive for antibodies but were asymptomatic ranged from 21.9% to 35.8%.

The sensitivity of the point-of-care tests used was 79.6% overall, and ranged between 61.0% in people who were asymptomatic and 97.2% in those with symptoms of COVID-19.

Additional results, which have not yet been published, from multiple tests during the time frame indicate that the 14% of the participants who had a positive test for antibodies at the start of the study no longer had antibodies at the end of the study. The loss of detectible antibodies occurred most often in those who were asymptomatic (Reuters, 2020).

Vaccine Development

The steps to gather information for approval of a new drug or vaccine are organized into four general phases. Each phase has a specific goal, which is discussed below in detail. In general, however, the early phases of testing focus on safety and the optimum dosing of the vaccine. Because of this, Phase 1 trials can also be referred to as “Dose Escalation Trials”. Phase 2 and 3 studies are used to determine if the vaccine is effective. Vaccines are typically approved after the completion of Phase 1, 2, and 3 trials. It is also possible to combine phases in some trials, such as Phase 1 and 2. If a predetermined number of patients meet the outcome criteria defined in a Phase 2 trial, the vaccine can move on to a Phase 3 trial. A detailed description of each phase is listed in Tables 1 and 2.

Table 1. Description of Early Phases.

Type of Trial	Focus of Research	Summary of Trial Organization
Preclinical and Early Phase 1	To establish the **mode of action** of the new drug in animal models and cell culture.	Includes experiments **in animal models or cells**. Sometimes researchers administer the drug to 1 or 2 people by invitation.
Phase 1, or Dose Escalation	**To determine the highest dose** that can be administered **without causing serious side effects** in people.	Involves **15-50 participants** who are each given a single dose of the drug. With each participant, **the dose is increased** until the side effects occur or levels reach the predicted amount for the drug to have a beneficial effect.
Phase 2	**Provides the first evidence of efficacy in humans.** There is a defined clinical outcome, such as tumor shrinkage. **It is not known if the drug will have an effect in humans.**	Involve **25-100 participants** who are given the dose established in Phase 1. There may be multiple groups to compare the effect of different doses of drug, multiple methods of administration, or the frequency of with which the drug is taken.

How does a Clinical Trial Differ from Medical Treatment?

When you access medical treatment from a provider, the intent of the interaction is to address the needs of an individual patient, and the treatment includes products and procedures accepted by the medical community as both safe and effective. However, while you may receive medical treatment while enrolled in a clinical trial, the intent of the interaction is to answer specific questions about how a drug, procedure, or device affects patients. When you receive an investigational therapy, it is not known if the products and/or procedures will have a benefit to the patient. It is also possible, based on the phase of the trial, that the patient will not know what they are being treated with due to blinding in the study used to reduce bias between the treated group and the untreated group.

Table 2. Descriptions of Phases 3 to 4.

Type of Trial	Focus of Research	Summary of Trial Organization
Phase 3	To determine how the results from Phase 2 trials **compare to current treatments** and to establish if the effect occurs over a wide range of people. It is the **final step before approval by the FDA.** Serious side effects happen less often in Phase 3 trials, but rarer side effects may become evident as more people are exposed to the drug.	Involve **several hundred volunteers** who are **randomly assigned** to receive the new drug or the current standard of care as a control. Even if the patient does not receive the experimental treatment, they will **still receive the best currently available** therapy.
Phase 4	Phase 4 is **not necessary for FDA approval** of the drug. Phase 4 trials attempt to **answer other questions that arise during the Phase 3 trials**. For example, these trials look at the **long term effects** of the drug or whether tumor shrinkage caused by the drug leads to a longer survival time.	Phase 3 participants may be **automatically enrolled** in the subsequent Phase 4 studies to follow **the long term outcomes** of treatment. The drugs used in Phase 4 **can be obtained by prescription** even without enrollment in the study, unlike the other phases.

Vaccines are designed to elicit an immune response that mimics an infection by the virus and leads to development of a long-term antibody response and/or establishment of memory T and B cells. It is important to stimulate a response from both B cells and T cells, and based on preliminary information, stimulation of the T cell response may be stronger during SARS-CoV-2 infections (Seow et al., 2020 and Grifoni et al., 2020). The information collected from both neutralizing antibody production and T-cell activation studies indicates that targeting the spike protein of SARS-CoV-2 should lead to both types of immune response.

Potential SARS-CoV-2 Vaccines that Have been Tested in Humans

The vaccine candidate from **CanSino Biologics** delivers genetic material encoding the spike protein from SARS-CoV-2 using an adenovirus vector (Zhu et al., 2020 and Zhu et al., 2020). The effects of the vaccine were tested after a single injection. Results from the Phase 1 and Phase 2 studies published in the *Lancet* showed that there was a specific antibody and T-cell response to the vaccine with detectable responses by 14 days after inoculation. There was also a statistically significant level of T-cell response and antibody production within 28 days after vaccination for most of the participants. In the Phase 2 trial, older individuals were included as volunteers, and the researchers found that the immune response was smaller for older individuals, but they had fewer adverse reactions that might allow for a second, booster-like dose.

Moderna has developed and tested an RNA-based virus that leads to the production of the spike protein from SARS-CoV-2 (Jackson et al., 2020). The vaccine is administered in two doses 28 days apart. A Phase 1 dose escalation and safety study has been published in the *New England Journal of Medicine*. Neutralizing antibody production was detected, and after the second dose, the levels of antibody production were similar to those observed for people who had recovered from COVID-19. A response from both helper T cells and cytotoxic T cells was also observed. The response from the cytotoxic T cells was only detectable after administration of the second dose.

The Moderna vaccine has also been tested for efficacy in primates (rhesus macaques) using two different doses (Corbett et al., 2020). One of the doses used in the animal testing was lower than that used in the Phase 1 study described above, and the other dose was the same as one of the doses used in the Phase 1 study. There was both an antibody response and a T-cell response to the vaccine in primates. When the animals were exposed to SARS-CoV-2 after vaccination, the researchers were not able to detect evidence of viral replication in respiratory fluids collected from the lungs of seven of the eight animals. There was no viral replication detected in the nasal secretions of the animals vaccinated with the higher dose of vaccine. Only small amounts of viral replication, antigen from the virus, or inflammation was observed in the lung tissue of either of the groups.

The vaccine being developed by **Oxford** and **AstraZeneca** is an adenovirus vector that delivers the genetic material to produce a full-length version of the spike protein. The results from a Phase 1/2 study were published in the *Lancet*. The report described the outcome of a single administration of the vaccine in 543 participants, an initial administration of the vaccine with a second booster dose in ten participants, and a single administration of a placebo vaccine in 534 participants (Folegatti et al., 2020). The researchers observed both a T-cell response and production of IgG antibodies from the vaccine. The T-cell response peaked 14 days after the inoculation, and IgG antibodies rose by day 28. The antibody response after a single dose was not as robust as expected, and administration of a booster 28 days after the initial dose led to production of neutralizing antibodies in all of the participants in this group.

Additional results from the investigation of the Oxford/AstraZeneca vaccine in mice and primates (rhesus macaques) showed both an antibody response and a T-cell response (van Doremalen et al., 2020). After vaccination, there was a reduction in the amount of SARS-CoV-2 in lower-respiratory-tract fluids in rhesus macaques who were exposed to the virus. Symptoms

of pneumonia did not occur in the vaccinated animals. The amount of viral RNA measured in nasal secretions was the same in both vaccinated and unvaccinated animals, suggesting that infection occurs, but the severity of symptoms is decreased.

A group of researchers in China published the interim results of a Phase 1/2 trial investigating the safety and efficacy of a **vaccine containing inactivated SARS-CoV-2 virus** in *JAMA* (Xia et al., 2020). This type of vaccination has been used for prevention of multiple types of viruses in the past, including, for example, polio and influenza. The inoculation was tested in 96 participants at three dose levels in the Phase 1 trial, and safety was compared to an injection of the adjuvant alone. Each dose group received three injections that were 28 days apart. The number of participants that experienced adverse reactions ranged from 16.7% (4 people) to 25% (6 people), and the most common reactions were injection site pain and fever. The reactions resolved without treatment and were mild in nature. There were no serious reactions to the vaccine. In the Phase 2 portion of the trial, there were 224 participants who were randomly assigned to one of a pair of groups. The first pair of groups received two inoculations 14 days apart with the vaccine or two injections of the adjuvant alone (placebo group) 14 days apart. The other pair of groups received two inoculations with the vaccine 21 days apart or two injections with the adjuvant alone 21 days apart. Neutralizing antibodies to the virus were produced in both the Phase 1 and Phase 2 stages of the trial, with no detectable antibodies in any of the placebo groups. Seroconversion (production of antibodies against the virus) was observed 100% of the participants in the low and high-dose groups from the Phase 1 trial and for 95.8% of the participants in the medium-dose group. In the Phase 2 trial, 97.6% of the participants produced antibodies to the virus. When the Phase 2 groups were analyzed separately, seroconversion was observed in 100% of the participants in the group receiving two vaccine injections 21 days apart and in 85.7% of the participants who received the vaccine 14 days apart. Based on the interim results, the researchers determined that a longer period between booster shots produced a higher response. In the Phase 1 trial, a third inoculation was administered and led to higher antibody levels with few side effects, suggesting that multiple boosters are feasible in people without a strong response to the first injections.

The inactivated vaccine has also been tested in mice, rats, guinea pigs, rabbits, and nonhuman primates, such as cynomolgus monkeys and rhesus macaques (Wang et al., 2020). The production of the vaccine was described along with the animals' responses in the journal *Cell*. SARS-CoV-2 was obtained from three hospitalized individuals and examined for characteristics that would make a good vaccine. The most important characteristics identified by the researchers was efficient proliferation and genomic stability (few mutations). One of the strains was found to have these properties, and the researchers grew virus in large containers of cells that are susceptible to the virus. The virus was then inactivated by addition of a chemical, β-propionolactone, that affects the surface of the virus so it cannot fuse with a human cell. Testing confirmed that viral infectivity was eliminated while the structure of the viral particle was maintained. When tested in rabbits, guinea pigs, rats, and mice, the seroconversion rate was 100% after 21 days in all of the animals. A dosing schedule with administration of three doses was measured in cynomolgus monkeys, rabbits, guinea pigs, rats, and mice, and seroconversion occurred in 100% of the animals after 21 days with higher levels of antibody production than with a single administration of vaccine. Further studies reported high levels of neutralizing antibody in rats, mice, guinea pigs, rabbits, cynomolgus monkeys, and rhesus macaques. The vaccine was also able to prevent disease in rhesus macaques after exposure to

SARS-CoV-2 with evidence of both a reduction in viral production and clinical symptoms of illness.

The vaccine being developed by **Pfizer** and **BioNTech** is an RNA-based vaccine that delivers the genetic code for the RBD of the spike protein using two sequential doses (Mulligan et al., 2020). The results of a Phase 1 safety study were published as a pre-print. Information on a T-cell response was not included, but there was a "modest" increase in neutralizing antibody against the RBD detected after the first dose. After the second dose, the level of RBD targeted antibodies were increased to within the range that had been measured in people who had recovered from COVID-19.

Novamax released data from the Phase 1 portion of their Phase 1/2 trial on a protein-based vaccine composed of the full-length spike protein at a press release and later as a pre-print (Zimmer and Thomas, 2020, Novamax, 2020, and Keech, 2020). According to representatives of Novamax, the information is currently being prepared for publication in a peer-reviewed journal. Instead of delivering genomic material from the virus as in most of the previously discussed vaccines, the Novamax vaccine includes a protein from the SARS-CoV-2, which is administered as two separate doses 21 days apart. This type of vaccine has been previously used for hepatitis B and shingles. Novamax also has developed an additive that induces an elevated response to the vaccine, called an adjuvant. The Novamax adjuvant, Matrix-M1, was included in half of the vaccinations given to the volunteers to determine if there was an advantage. The results from the Phase 1 study show that the vaccine was well-tolerated with a good safety profile in 131 participants. There was evidence of both a neutralizing antibody response to the spike protein and a T cell response from the vaccine when participants were evaluated 35 days after the first inoculation. The antibody levels induced were four time higher than the mean from people who have recovered from COVID-19, and the levels of antibody produced from the vaccine including Matrix-M1 were ten times that of the spike protein mixture without the adjuvant.

The vaccine from Novamax has also been tested in mice and baboons to determine its efficacy (Tian et al., 2020). In mice, the researchers determined that the vaccine is able to elicit an antibody response that prevents binding of the ACE2 receptor, neutralizes virus, and prevents infection after exposure to SARS-CoV-2. A T-cell response was also observed with activation of both helper T cells and cytotoxic T cells in both mice and baboons.

A group in Russia has also announced the development of a successful vaccine, but many of the world's experts are skeptical of the amount of testing that has been performed on the potential vaccine (Lowe, 2020). As reported in the trial description at ClinicalTrials.gov, the vaccine is a mixture of two adenovirus vectors that contain the gene for the spike protein, and the design of the vaccine has been found by other experts to be reasonable (Gamaleya Research Institute, 2020). However, the company has claimed that they have regulatory approval for the use of the vaccine, but based on the timeline of the development, it is estimated that the vaccine has only been evaluated in human trials for about two months, which is about the time needed to perform a Phase 1 trial. This suggests that the development is at or near the same stage as the vaccines described above.

Immunopathology of SARS-CoV-2

The concept of immunopathology refers to a disease that is exacerbated by the interaction between the pathogen and the immune system so that the immune system ends up causing damage to the body. This process has been observed with COVID-19 and the severe symptoms that can develop in some individuals during the inflammatory-mediated phase of the disease.

There is probably not simply a single event caused by SARS-CoV-2 infection or simply a single difference in a person's immune response that leads to immunopathology and severe manifestations of COVID-19. Instead, there are likely numerous, small contributions that combine to cause some people to be more likely to become more ill. There may also be several different starting points of the pathway depending on the overall health and genetic components of an individual. Identifying the parts of the immune system that contribute to the effect allows for knowledge of which systems may benefit from treatment. Thus far, researchers have determined that early in the course of infection treatments that target viral replication, such as remdesivir, may be more beneficial while as COVID-19 progresses, anti-inflammatory treatments are better able to prevent extreme reactions to viral infection.

Initial Infection

Coronaviruses have been shown to first infect cells lining the airway, which are called **epithelial cells** (Almaghaslah et al., 2020). Both SARS-CoV-2 and SARS-CoV-1 interact with the epithelial cells mainly through the ACE2 receptor, but MERS and the seasonal coronaviruses use different receptors to initiate contact. The infection of the cells in the upper airways leads to nasal secretions and swelling in the area due to cellular damage from the virus. This damage activates inflammatory molecules associated with the innate immune system that cause cold-like symptoms. New virus that is produced in the upper respiratory tract can then move to the lower respiratory tract, the intestines, kidney cells, liver cells, and T-lymphocytes in some individuals, leading to additional symptoms associated with those systems. Researchers have postulated that SARS-CoV-2 and SARS-CoV-1 may be more likely to infect the lower respiratory tract than seasonal coronaviruses because the level of ACE2 receptors is higher in the cells in the lower respiratory tract, giving them assistance in establishing an infection.

Importantly, coronaviruses have been found to cause the death of T cells through a process called **apoptosis**, which is a process used by cells to die in a manner that does not stimulate the innate immune system through release of the cellular contents. Apoptosis is triggered in cells by infection or serious malfunctions such as cancer. The promotion of T-cell death by coronaviruses disrupts the T-cell response to the infection, making it erratic. The changes in the T-cell response can then lead to malfunctions in the overall immune response, and in severe cases, collapse of the immune system.

Contributions to Immunopathology

The following is a discussion of the processes that have been identified as contributing to the immunopathology of COVID-19. Figure 2 describes the interconnectedness of the processes and how they interact with each other to further disrupt the immune response to SARS-CoV-2.

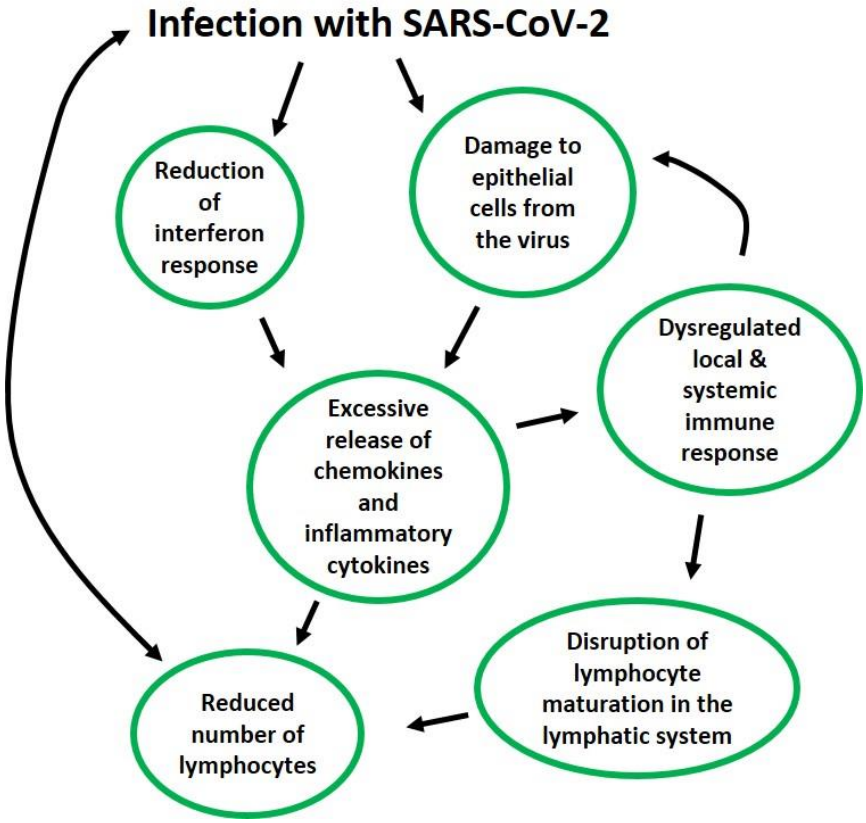

Figure 2. Flowchart of the immunological events associated with SARS-CoV-2 infections. The steps are interconnected and feed back on each other to propagate the effects. (Adapted from Wauters et al., 2020)

Hyper-Inflammatory Response to COVID-19

It has been observed that most people with COVID-19 have **clinical inflammatory syndrome** (Mathew et al., 2020). Clinical inflammatory syndrome involves the marked rise of inflammatory parameters measured in the bloodstream, including serum ferritin, C-reactive protein (CRP) levels, d-dimers, and the erythrocyte sedimentation rate (Prete et al., 2020). There is also evidence from numerous studies that increasing severity is accompanied by high levels of several **pro-inflammatory cytokines,** including **IL-6, IL-1,** and **TNF-α**, and many of these molecules are produced, at least in part, by T cells.

What is the Effect on Lymphocytes from SARS-CoV-2 Infection?

Additionally, hospitalized individuals often have a condition called **lymphopenia**, which is the clinical term for a reduced level of lymphocytes in the blood stream. Lymphopenia technically refers to all types of lymphocytes (T cells, B cells, and natural killer cells), but most often the condition is caused by a reduction in T cells. In SARS-CoV-2 infections, reductions in T cells were observed early in the course of the pandemic, but since then it has been determined that all types of lymphocytes are reduced in number during COVID-19. Lymphopenia can be caused either by the increased death of cells or from movement of the cells to the site of an infection (e.g. the lungs), which removes them from the blood stream so they are not detected in blood tests, but the cells do not die. It has not been determined which of these mechanisms is occurring, but there is preliminary evidence from autopsy studies suggesting that there is not a major deployment of T cells to the lungs, which implicates cell death as the cause.

Lymphopenia and COVID-19 are so interconnected that researchers have been able to use the extent of the condition as a predictor of survival from the disease.

The low amounts of lymphocytes associated with COVID-19 is not permanent as can happen in other viral infections, such as HIV. The level of cells typically returns to normal within weeks of infection. Many hospitalized individuals are found to have lymphopenia when they are admitted to the hospital, which is usually about a week after symptoms first appear, and once in the hospital, levels began to slowly rise almost at once.

The information collected about characteristics of people with COVID-19 points to a pivotal role of T-cell function, or perhaps the dysregulation of T-cell function, contributing to the symptoms associated with COVID-19. Based on the available information, experts have proposed a general scheme of atypical maturation of T cells from exposure to abnormal immune system regulators that leads to severe disease after infection with SARS-CoV-2. A summary of the process is shown in Figure 3.

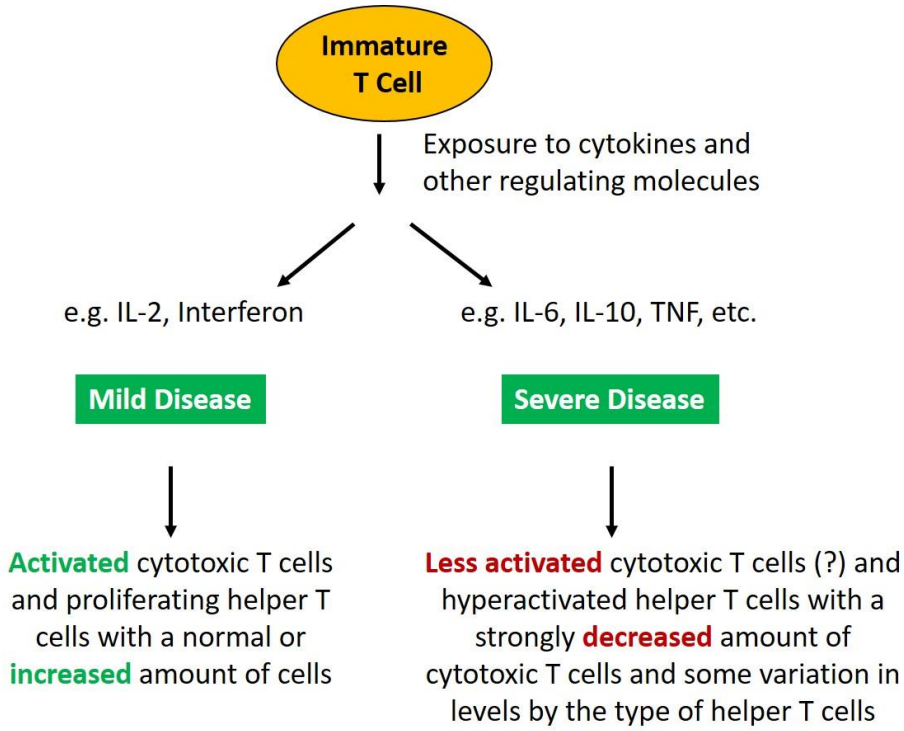

Figure 3. Proposed model of T cell responses during mild versus severe COVID-19 progression. When **mild disease** occurs, T cells have been exposed to immune regulators, such as interferons, that produce an immune response from the adaptive immune system that stops viral replication and clears the virus. When **severe disease** occurs, immune regulators that stimulate a higher innate immune response are more evident leading to an increased inflammatory response, a decreased adaptive immune response, and poorer control of viral replication. (Adapted from Chen and Wherry, 2020)

When immature T cells are exposed to pro-inflammatory molecules such as IL-6, IL-10, and TNF, fewer activated cytotoxic T cells are produced and more over-activated helper T cells are generated. The reduced production of cytotoxic T cells leads to an inadequate response against infected cells, allowing for continued spread of the virus. The overactive T cells produce additional pro-inflammatory molecules, such as IL-6 and transforming growth factor-β (or TGFβ), which is not a normal function of helper T cells. The increased production of pro-inflammatory signals continues the stimulation of the innate immune system, and evidence of hyperactivity in the innate immune system has been observed.

Overall, there are alternative possibilities being investigated, but the proposed process would be expected to lead to a similar immunopathology as has been observed with COVID-19. The contributing factors to COVID-19 remain in question due to frequent discrepancies in published reports over major immunological characteristics. Additional information will be needed to definitively determine how the immune system is contributing to the severe symptoms associated with COVID-19 (Wauters et al., 2020).

What are the Differences in the Production of Interferons in COVID-19?

Researchers have found that the type I interferon response to viral infection plays a large role in the promotion of inflammation in severe COVID-19, and low levels may be indicative of a risk of severe COVID-19. **Several studies have shown that cells infected with SARS-CoV-2 produce almost no interferon** (Hadjadj et al., 2020, Lee et al., 2020, and Blanco-Melo et al., 2020). The lower levels of interferon measured in blood tests from patients were correlated with higher virus levels and worsened (increased) inflammatory responses, such as increased TNF-alpha and IL-6.

Researchers have also compared the production of interferon in the infections with respiratory viruses SARS-CoV-2, influenza, and human respiratory syncytial virus (RSV) (Lee et al., 2020 and Blanco-Melo et al., 2020). **Individuals with COVID-19 had lower lymphocyte levels and higher levels of inflammatory markers in their bloodstream than those with influenza** with evidence of a hyper-inflammatory response in people with COVID-19. Samples of lung tissue from people who had died from COVID-19 showed there were also alterations in the interferon-I response and TNF inflammatory response in the lung. The complete lack of production of interferon that has been observed in people with SARS-CoV-2 and MERS is not observed in RSV and influenza.

> **Overall, the researchers report that the immune response to SARS-CoV-2 was muted compared to RSV and influenza, resulting at least in part from a reduction in interferon.**

One group has proposed that one potential reason that SARS-CoV-2 infection is more serious in older individuals is because the immune response in this group is already reduced compared to younger individuals. The combination of SARS-CoV-2 reducing the immune response and an already suboptimal response could lead to an inability to stop the spread of the virus from the upper respiratory tract to the lower respiratory tract and other sensitive areas (Blanco-Melo et al., 2020). This hypothesis is supported in experiments that compared the outcome of young ferrets with older ferrets. When ferrets at the stage of development near to that of a human teenager are infected with SARS-CoV-2, they show no clinical symptoms and recover within about eight days while older ferrets have severe respiratory symptoms that often lead to death.

Contribution from Toll-Like Receptors

Toll-Like receptors (or TLR) are found on cells of the innate immune system, such as macrophages. They are used as a sensor to alert the cells of the presence of components of viruses (Sallenave and Guillot, 2020). There are multiple types of TLRs, and the different types are used to detect different molecules. For example, TLR3 interacts with double-stranded RNA (a molecule only found in viruses), and TLR7 senses single-stranded RNA (which is the form of genomic information used by coronaviruses). The specific TLR that interacts with SARS-CoV-2 has not been determined, and TLR3, TLR7, and TLR8 have been identified as possibilities. TLR4 has also been found to bind to the M protein of SARS-CoV-2 and may be involved in the immunopathology of the virus (Choudhury and Mukherjee, 2020). Additionally, there is evidence that TLR7, which is expressed in cells of the respiratory system as part of the innate immune

system in the lungs, may be important based on the discovery of two pairs of brothers with mutations in the TLR7 (van der Made et al., 2020).

Researchers in the Netherlands conducted a study to look for a familial link to cases of severe COVID-19. They performed a search for young, severely affected brother pairs to try to identify a single gene that could possibly be X-linked. A mutation found on the X-chromosome (x-linked) can be identified because men are more often affected than women. This occurs because men have only a single X chromosome while women have two. Men inherit their X chromosome only from their mother, making it easier to trace a trait through the generations as well.

Two pairs of brothers were identified who had severe symptoms of COVID-19 that required treatment in the intensive care unit. However, all four men were young and did not have risk factors associated with severe COVID-19, making their severe illness remarkable.

The genomes of the pairs of brothers were sequenced, and in both cases, a mutation in the TLR-7 gene was found that was predicted to cause a complete loss of function of the protein. The mutation was not the same in the two families, but the outcome of the changes in the gene sequence in both cases was expected to be the same. The dysfunction of TLR7 for both mutations was confirmed through laboratory experiments with the men's cells.

The probability of identifying a rare, loss-of-function variants in the gene TLR7 in two unrelated families was calculated to be less than 0.1%, suggesting that the gene has a large contribution to the degree of illness observed in the men.

The researchers examined the mutated cells from both families and found in both cases that the lack of function from TLR7 caused a complete deficiency in production of interferon-gamma in response to stimulation of the TLR-7 pathway. Testing of other routes used by the immune system to induce the production of interferon-gamma indicated that the molecule could still be produced by other cells. Other studies of SARS-CoV-2 have found that the virus leads to a lower response of type I interferons and a resulting reduction in viral clearance.

Woman have been found to have a higher level of TLR7 production due to the presence of two x chromosomes, and increases have been observed in the subsequent production of interferon-gamma as well (Scully et al., 2020). The increased amount of TLR7 has been linked to the increased incidence of autoimmune conditions observed in women, and the researchers speculate that differences in TLR7 may also contribute to the differences in COVID-19 susceptibility between men and women.

From the results, the authors of the study suggest that a reduction in functional TLR7 causes changes in the innate immune system that prevent clearance of the virus, leading to high levels of virus in the cells of the respiratory system.

The high levels of virus would increase the damage caused to the cells by the infection, and the release of cellular components from damage is a pro-inflammatory signal that would increase the response of the innate immune system, causing a potential hyper-inflammatory response such as the cytokine storm associated with COVID-19.

Mutations in TLR7 were found to be extremely rare in the human population based on an evaluation of available sequence databases, suggesting that the protein is important and that changes in the protein sequence lead to serious physiological problems. There has not been a link observed between TLR7 and immunodeficiency reported in published studies, however. Because the mutations are extremely rare, **there is little chance that similar mutations are the cause of severe symptoms in the general population**. However, there is evidence that there may be small differences in the level of TLR7 produced due to minor changes in the genomic sequence that could influence the initial immune response to viral infection in different individuals. A slower response to viral infection combined with other risk factors, such as chronic conditions, could explain differences in response to SARS-CoV-2 infection.

Additionally, with the knowledge of the systems that are central to infection by the virus, it may be easier to develop treatments.

For example, therapies that boost the innate immune system early during infection with SARS-CoV-2 may be beneficial in preventing progression of symptoms, and the use of different types of interferons are already being tested for treatment of COVID-19.

Summary of the Immune System and SARS-CoV-2

- Infection with SARS-CoV-2 leads to both a T-cell response and a neutralizing antibody response that can clear the infection in most people.
- Memory B cells and memory T cells have been observed in people recovered from COVID-19, suggesting there will be long-term immunity even without an extended antibody response.
- The T-cell and neutralizing antibody response to SARS-CoV-2 both utilize the spike protein from the virus, suggesting that vaccines designed around the spike protein would be able to stimulate an immune response in both parts of the adaptive immune system.
- Numerous vaccines have progressed to the point of human trials with evidence of both antibody and T-cell responses, and the types of vaccines at this point of development is expanding to include protein-based and inactivated virus-based vaccines.
- Immunopathology, where an abnormal immune response leads to symptoms of a disease, has been observed after infection with SARS-CoV-2.
- Immunopathological processes include a hyper-inflammatory response, lymphopenia, and a reduced interferon response.
- Identification of these processes allows for the identification of treatments to reverse the processes, and several therapies for reducing the inflammatory response, increasing the amount of lymphocytes, and increasing interferon levels have been used for treatment of other diseases and may be able to be applied to the treatment of severe COVID-19.

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