

Inducing quality immune response to respiratory viruses may not be a simple task

Udaykumar Ranga* and Mangaiarkarasi Asokan

HIV-AIDS Laboratory, Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur (PO), Bengaluru 560 064, India

A few front-runner vaccines for COVID-19 have reported over 90% protection in phase II/III clinical trials, raising hopes. These studies, however, have evaluated only a relative, not absolute, protection. The leading COVID-19 vaccines have been designed to elicit a systemic, not mucosal, immune response. While a systemic immune response may reduce disease severity, only the mucosal immune response can reduce the spreading of a respiratory viral infection. Here, we explain why inducing long-lasting and high-quality immune response to mucosal infections is typically challenging. A few possible solutions are proposed.

Keywords: COVID-19 vaccines, clinical trials, respiratory viral infection, systemic and mucosal immune response.

Respiratory viruses proliferate mainly in the mucosal compartments of the body

CAN vaccines establish a ‘good’ immune response to a respiratory virus? A good vaccine must do several good things – restrict the viral spread in an infected person and a population; prevent the expansion of a respiratory virus to systemic body organs such as the heart or brain; and maintain a protective immune response for a prolonged period, at least for two years or longer. Importantly, a good vaccine should induce a protective immune response in an appropriate body compartment. For instance, a vaccine for the new coronavirus (SARS-CoV-2) must establish a protective immune response primarily in the respiratory tract and the lungs and blood. Before understanding if a vaccine can protect, let us examine if a natural viral infection can establish a protective immune response against a respiratory virus, such as the new coronavirus. The immune system can develop lasting immune responses against some viruses (e.g. poxviruses, measles virus, etc.), but not against certain other viruses (e.g. coronaviruses, rhinoviruses, etc.)¹. Is there something that we must learn from this observation before attempting to make a vaccine for the new coronavirus? Several technical reasons underlie the failure of the

immune system to produce potent and lasting immune responses to respiratory viruses, as discussed here.

Our body may be classified into two major compartments depending on whether they are open to the environment or not. The skin, elementary canal (mouth to the anal pore), respiratory compartment (nose, oropharyngeal cavity, upper and lower respiratory tracts, and the lungs), and the genital tracts are exposed to the environment. Except for the skin, all the other organs are covered with a protective mucus layer; therefore, they are collectively called the mucosal compartment. The most vital quality of the mucosal compartment is the presence of a huge number of microorganisms that are usually not pathogenic and even beneficial. Many kinds of friendly microorganisms live in the mucosal compartments. A healthy human being, for example, carries a microbial load of at least ten trillion (one lack crore) germs in the gut². In contrast, the rest of the body compartments, such as blood, liver, spleen and brain, collectively called the systemic compartment, do not contain microorganisms of any kind. Thus, one part of the body is teeming with hordes of microorganisms; in contrast, the other part is ‘sterile’ – lacks the presence of microorganisms. The contrasting presence or absence of natural microorganisms holds significant implications on two different aspects of protection – the nature of infection of the pathogenic microorganisms and quality of the immune response our body can produce.

Most microorganisms gain entry into the body through the mucosal surfaces which are exposed to the environment. After entry, the destinies of different microorganisms are different; some will remain in the mucosal compartments to establish mucosal infection (e.g. cholera bacterium, rhinoviruses, flu viruses and coronaviruses), some others move to the systemic compartments (e.g. poxviruses, measles virus, rabies virus), while some may infect both the compartments (e.g. tuberculosis bacterium, poliovirus, etc.). The microorganisms that remain in the mucosal compartment have a unique problem and a great advantage. These germs must compete with the natural microbial flora that outnumbers them by a huge margin – not a small problem to overcome. Mucosal pathogenic organisms, such as respiratory viruses, therefore, have evolved only to stay in the host for a short duration, days and weeks – not months or years. However, the

*For correspondence. (e-mail: udaykumar@jncasr.ac.in)

factor that works in favour of mucosal pathogenic organisms is the inherent inability of the mucosal compartments to wage a potent and long-lasting immune response due to a problem called ‘mucosal tolerance’ (see later in the text). The outcome of the infection of the systemic compartment, on the other hand, is different. Since the systemic body compartments do not contain germs under normal conditions, the body will consider any micro-organism, pathogenic or non-pathogenic, a grave danger. The immune system responds vigorously, developing a robust and long-lasting immune response that may last even a lifetime³.

Coronaviruses being respiratory viruses, enter the body through the upper respiratory tract (nasal cavity, nasopharyngeal cavity and the eyes), spread to the lower respiratory tract, and eventually, the lungs, where they grow in numbers. Occasionally, coronaviruses may spread to other body organs, such as the liver, brain, gut and heart. This expansion does not involve systemic body dissemination, unlike more pathogenic viruses, such as measles and poxviruses. Then, why can the mucosal immune system not establish high-quality and long-lasting immune responses? Three primary reasons underlie this inability – the transient nature of the infection, mucosal tolerance and rapid immune renewal.

Respiratory hit-and-run viruses

We may classify viruses, or other types of microorganisms, into two main categories depending on the intimacy of association they establish with the host – ‘hit-and-run’ viruses and ‘hit-and-stay’ viruses. These two categories of viruses are designed differently to achieve things differently. Coronaviruses, flu viruses, rhinoviruses and the like have evolved to avoid intimate and prolonged contact with the host. The pathogenic viruses must compete for space with the commensal microorganisms that naturally colonize the mucosal surfaces. The commensal microorganisms not only outnumber the pathogenic viruses by a vast margin, but also secrete many products that antagonize the pathogenic viruses. Therefore, the primary goal of the hit-and-run viruses is to move from one subject to another as quickly as possible. This is the reason respiratory viruses such as the new coronavirus can cause rapidly expanding pandemics and epidemics. It is not in the interest of a hit-and-run virus to stay with a single host for a prolonged period. For example, the new coronavirus will not remain in an infected person for more than one or two weeks⁴. On the other side, the immune system fails to produce a high-quality immune response against such viruses because the contact between a respiratory virus and the host is minimal. In contrast, hit-and-stay viruses, such as HIV, HPV, HCV and HBV, all infect the host and stay for years or even a lifetime. The immune system can establish high-quality immune responses against hit-and-

stay viruses, although such viruses cannot be removed from the body for other technical reasons. The minimal interaction between the respiratory virus and the mucosal immune system limits the quality of the immune response.

Why do some viruses adopt the hit-and-run strategy? The ultimate challenge of all pathogenic viruses is to avoid and/or overcome the activation of the host immune components. In simplistic terms, at least two different components of the immune system are functional against the invading viruses, and the viruses must evolve strategies to survive these host defence mechanisms. It is not possible for any virus to evade the innate immune responses, such as the interferons, natural antibodies, acute serum proteins, complement, etc. since these host defence components are ever present and activated immediately following viral infection. Therefore, every pathogenic virus must be armed with diverse evasion strategies to outwit the innate immune components. Unlike the innate immune components, the acquired immune elements, the second component of the immune system, comprising IgG and IgA antibodies, helper T-cells and cytotoxic T-cells, are not readily present in the body fluids and need time to develop. At least two to four weeks will be necessary for the immune system to produce quality neutralizing antibodies in sufficient quantities following a viral infection. Therefore, viruses have time and opportunity to evolve diverse evasion strategies to outwit the acquired immune responses. The two kinds of viruses discussed above, the hit-and-run and hit-and-stay viruses, differ radically in how they evade the acquired immune responses. While the hit-and-run viruses avoid the acquired immune responses altogether by leaving the host before such responses can appear, the hit-and-stay viruses develop additional strategies to escape these immune responses since they continue to stay in the host.

A coronavirus designed to stay in an individual host for a brief period, one or two weeks, will need strategies primarily to counter the innate immune components. The virus-specific antibodies (and T cells) produced during the period following viral infection would not be of high quality. Furthermore, the levels of the neutralizing antibodies may not have reached a concentration in the blood necessary enough to neutralize the virus. The virus-specific antibodies must undergo a process of maturation in the form of enhancing the neutralization potential, technically known as ‘affinity maturation’. Additionally, the antibodies must also undergo a different process of maturation called ‘isotype switching’ to produce antibodies of different kinds, including the IgG and IgA varieties. Importantly, for antibody maturation, the presence of the virus and/or viral proteins in the body fluids is crucial. Since the hit-and-run viruses leave the infected subject within weeks just as the virus-specific immune responses begin to appear, the viral antigens will not be available in sufficient quantities. As a result, the

processes of antibody maturation will be compromised. Thus, hit-and-run viruses not only evade the most potent arm of the immune system, the acquired immune responses, but also hamper the maturation of these responses, thus compromising the establishment of effective herd immunity in the population. Thus, several respiratory viruses have adopted an evolutionary strategy that involves leaving the host as soon as possible following infection. While doing so, they do not cause the activation of acquired immune responses and the establishment of within-host long-term immune memory or effective herd immunity. In contrast, hit-and-stay viruses must face the acquired immune responses. To this end, these viruses have developed several additional immune evasion strategies, as reviewed elegantly by Hilleman⁵.

Immune tolerance and immune memory work differently in mucosal compartments

The mucosal surfaces are not only colonized by hordes of harmless microorganisms, but they are also regularly exposed to huge volumes of proteins coming from the food that we eat and the air that we breathe. None of these entities is harmful enough to deserve a severe reaction. The problem is just the opposite. Although these entities are foreign to the body, the mucosal immune system must avoid waging a response against all such benign elements to conserve resources. To this end, the mucosal immune system employs various components and mechanisms to 'actively tolerate' the presence of the harmless foreign entities, which we may call 'mucosal tolerance'. The maintenance of mucosal tolerance also needs resources and energy; therefore, it is an 'active' process. Although the association between the commensal microorganisms and the host is non-hostile, it is only a 'guarded alliance'. The commensal microorganisms are opportunistic and can become dangerous to the host if not controlled using appropriate measures⁶. To this end, the mucosal immune system employs many different elements, including antimicrobial peptides, proteins, antibodies, cytokines and immune cells, to control the commensal microorganisms. Thus, maintaining a symbiotic association with commensal microorganisms is a dynamic process that requires continuous maintenance of mucosal tolerance⁷. Unfortunately, due to mucosal tolerance, the quality of mucosal immune response is compromised even when a pathogenic virus infects a mucosal compartment. Additionally, given the highly sensitive respiratory functions of the lungs and the lower respiratory tract, to avoid self-injury, potent and prolonged inflammatory responses cannot be activated in these organs⁶.

When the immune system encounters a virus, regardless of the compartment, a protective immune response is induced consisting of virus-specific B cells making antibodies and T cells. A protective immune response suc-

cessfully eliminates the virus before much harm can happen. After the virus is removed, the virus-specific immune cells are also allowed to disappear as these cells are not needed any longer. However, the immune system will retain a few immune cells specific to the virus, stored in the local tissues, lymph nodes and the bone marrow – the central storage depot of immune memory. These memory cells can wage a rapid and robust immune response when the same virus infects the body again after some time; this preventive measure is called 'immune memory'. Immune memory will recognize the same virus, not a new virus. Immune memory against a virus can sustain as long as the specific memory cells remain in the local tissues or the bone marrow.

Immune memory of mucosal tissues, however, works differently. Immune memory of the mucosal compartment is sustained predominantly or even exclusively in the local mucosal tissues. Importantly, other secondary lymphoid structures of the body contribute little to respiratory immune responses. Once the secondary lymphoid organs are formed in the local mucosal tissues, these structures can effectively recruit naive B and T cells, activate them, and maintain immune memory mediated by these cells for months even in the absence of the original stimulus⁸. We can understand this difference by taking the example of the antibody types. Polymeric IgA antibodies, necessary to protect the mucosal surfaces, can be generated only in the mucosal tissues. The early IgM type of antibodies changes into IgG type in the systemic compartments and IgA in the mucosal compartments by 'isotype recombination switch'. The environmental niche necessary for a switch of antibodies from IgM to IgA (the Tfh cells, innate lymphoid cells, cytokine milieu, etc.) is available only in the mucosal compartment⁹. Additionally, the dimers and polymers of IgA can be produced only by mucosal epithelial cells¹⁰. Thus, establishing and maintaining memory B cells and T cells in the local mucosal tissues is necessary to sustain mucosal immune memory¹¹, with limited involvement of other secondary lymph nodes. Unfortunately, given the high cell-turnover rate in the mucosal tissues, long-term retention of immune cells specific to any pathogenic virus is less likely.

IgA antibodies are critical for protecting the upper respiratory compartment

The new coronavirus is known to transmit through respiratory droplets. Therefore, the virus is likely to infect the upper respiratory surfaces first, spreading to the lower respiratory tract next and eventually reaching the lungs (<https://www.who.int/news-room/q-a-detail/coronavirus-disease-covid-19-how-is-it-transmitted>). An adequate concentration of polymeric IgA antibodies is required in the upper respiratory tract to protect it from the virus taking the initial hold. The two classes of antibodies, IgA and

IgG, are not uniformly distributed in the body and respiratory compartments. Only a nasal vaccine can induce the production of IgA in the upper respiratory tract¹². A systemic vaccine, in contrast, will generate the production of IgG antibodies in the blood. These antibodies can reach the lungs by 'transudation'. They cannot reach the upper respiratory tract where the coronavirus makes the first contact¹³. The IgG antibodies can also reach the lower respiratory tract, but only in trace quantities. Thus, the IgG antibodies induced by a systemic vaccine, regardless of a high blood concentration, may not stop the virus from taking the initial hold in the upper respiratory tract and prevent viral spread to the lower respiratory tract. Since the IgG antibodies can reach the lungs in high concentrations, they may reduce the disease severity of pneumonia⁷ and prevent dissemination of the virus to other body compartments, such as the brain and heart. However, this potential of the IgG antibodies for COVID-19 needs empirical evidence.

Retention of memory B cells in the local mucosal tissues is crucial

The mucosal immune system establishes and maintains a massive repertoire of memory B cells and plasma cells in the local mucosal tissues to produce dimer and polymeric IgA antibodies to prevent the invasion of the commensal microorganisms. The mucosal tissues must refresh the memory cell repertoire rapidly and regularly as the composition of the microbial population undergoes changes dynamically based on environmental factors. Therefore, the repertoire of memory cells cannot remain stable for long periods in the mucosal tissues. The memory cells are rapidly replaced by freshly produced ones continuously⁹. We may compare this situation to the rush in a temple where the worshippers inside the sanctum sanctorum must make way for new devotees pushing their way into the temple. This phenomenon also destabilizes the memory cell repertoire against a specific pathogenic virus in the respiratory compartment. The flushing out of memory cells is one important reason why the body cannot establish lasting immune memory to several respiratory viruses. The situation is different in the systemic body compartments where memory cells can stay for long durations, even for several decades. A single round of infection with chickenpox virus or a single injection of measles vaccine can establish strong and enduring responses lasting decades³.

Some successful intestinal vaccines and not so successful respiratory vaccines

Despite the challenges of inducing strong and durable mucosal immune responses, vaccines have been developed against enteric infections such as polio, cholera,

typhoid and rotavirus¹⁴. Polio viral vaccines provide an elegant example underlining the importance of mucosal responses – the live-attenuated oral vaccine (OPV) versus inactivated poliovirus vaccine (IPV). Only OPV can generate sterilizing mucosal responses capable of blocking viral shedding in the stools and prevent viral transmission. In contrast, IPV can only induce systemic immune responses, and its efficacy is limited to the prevention of severe disease. IPV-immunized subjects are liable to polio viral infection, and they may spread the virus in the environment. India, representing a moderate-high transmission setting, successfully eliminated polio using OPV. The story of Israel that used the IPV regimen for its national polio campaign is different. Israel suffered a silent outbreak of polio epidemic that peaked around August 2013. Subsequently, it could control the polio outbreak in a few months through the re-introduction of OPV. Notably, only IgA in mucosal surfaces, not IgG in the blood, was identified as a correlate of prevention of polio viral excretion^{15,16}.

Similarly, the best correlate of rotaviral immunity generated by either vaccination, infection, or passive maternal antibody transfer is an IgA response in mucosal surfaces^{17,18}. A recent study of typhoid Vi vaccines identified that prevention of bacterial infection was mediated only by IgA antibodies, and not IgG. IgG antibodies, however, reduced disease severity¹⁹. Thus, the acquisition- and transmission-blocking potency of vaccines against enteric pathogens may be defined as their ability to induce the production of dimeric IgA antibodies on mucosal surfaces²⁰. Polio and rotavirus vaccines have broken oral tolerance and achieved sustained local immunity via multiple doses of OPV. In contrast, the inactivated oral cholera vaccines and polysaccharide typhoid vaccines induce mucosal responses that are relatively short-lived. In summary, a common theme seems to have emerged that while IgA antibodies produced in the local mucosal tissues confer protection against a mucosal infection, IgG antibodies reduce disease severity when the infection breaches mucosal surfaces and spills into the systemic compartment.

In contrast to enteric infections, the development of vaccines against respiratory infections has been much more challenging. *Streptococcus pneumoniae*, the most frequent cause of pneumonia, tends to exist in a commensal state in the nasopharyngeal cavity. Currently available vaccines induce systemic responses that can only prevent invasive disease and pneumonia, but not eliminate the bacterium from the nasopharyngeal cavity by inducing mucosal responses²¹. Likewise, notwithstanding the effort of 60 years, attempts are still in progress to develop a mucosal vaccine for the respiratory syncytial virus to achieve localized viral immunity and avoid vaccine-enhanced respiratory disease²². Influenza viral infection serves as another elegant example of the significance of mucosal protective immune responses. Natural infection

of influenza viruses can establish a prolonged protective immune response. However, the virus employs antigenic drift and shift as a formidable strategy to evade immune response at the population level. In contrast, flu vaccines can effectively prevent a viral challenge with the same viral strain; however, the immune responses are short-lived²³. Although serum IgG antibodies can serve as a surrogate marker for protection, both in the mouse model and natural human infection, IgA antibodies of the mucosa confer protection against the influenza virus^{24,25}.

Efficacy of SARS-CoV-2 vaccines in the non-human primate model and clinical trials

To date, vaccine efficacy results in preclinical models have been published for five major candidate vaccines^{26–30}. All the candidate vaccines demonstrated neutralizing antibody responses and reduced or absent viral titres in the lower respiratory compartment. However, nearly half of the challenged primates showed persistent viral replication in the nasal epithelium. As mentioned above, blood IgG antibodies can translocate to the lungs and partially to the lower respiratory tract, but not to the upper respiratory tract. In addition to neutralizing antibodies, antigen-specific CD8 T cells can also limit viral replication in the lungs. Still, only a subset of these candidates could induce strong CD8 T cell responses in nonhuman primates. Thus, the data of these preclinical studies neither address whether decreasing viral replication in the lower respiratory compartment is sufficient to prevent viral transmission, nor do they establish neutralizing antibodies as the mechanistic correlate of preventing lung pathology.

Vaccine efficacy evaluation: mRNA vaccine trials

Efficacy results from phase II/III trials have been published for four vaccines – the ChAdOx1 vaccine of AstraZeneca; Ad26-Ad5 heterologous prime-boost (Sputnik V or Gam-COVID-Vac) of the Gamaleya Research Institute, and two non-replicating mRNA vaccines of Moderna³¹ and Pfizer-BioNTech³². For the two mRNA vaccines, 90% or higher efficacy has been reported in phase II/III clinical trials. While these data are encouraging, we must interpret these results with caution due to various limitations of study design, data availability and data analysis.

It is vital to bear in mind that the efficacy results are based on imperfect blinding of the vaccines and self-reporting of symptoms by participants (<https://blogs.bmj.com/bmj/2021/01/04/peter-doshi-pfizerand-modernas-95-effective-vaccines-we-need-more-details-and-the-raw-data/>). First, the placebo groups were given saline injections which developed a significantly small number of injection-related reactions compared to the vaccine group, thus

likely causing accidental unblinding between the groups. This lapse may have resulted in a bias in one of the groups reporting a smaller number of infections or adverse events. Second, this issue was further limited by the defined end-point of the study, which was the manifestation of clinical symptoms followed by a confirmatory RT-PCR result for only active viral infections. This limitation resulted in the presentation of only a minority of study participants showing a positive RT-PCR result. Importantly, the analysis ignored a twenty times larger number of study participants who developed clinical symptoms (1594 and 1816 events in the vaccine placebo groups respectively, of the Pfizer-BioNTech clinical study). Had the defined end-point of the studies included both RT-PCR-positive infections and suspected clinical symptoms, the vaccine efficacy projection could have been dramatically different, much lower than the projected 90–95%. Coincidentally, the 3410 suspected cases of the BNT162b2 vaccine trial did not merit a mention in the published report. However, a reference was made to these cases in the Vaccines and Related Biological Products Advisory (VRBPAC) briefing document submitted to the Food and Drug Administration (FDA), USA, and the post-publication reviews (<https://www.fda.gov/media/144245/download>). The VRBPAC document also acknowledges an overlap between vaccine reactogenicity events and COVID-19 symptoms during the post-vaccination period. Third, assessment of the incidence of viral infection was not conducted periodically in the two study groups. Ideally, an unbiased study design must have accommodated RT-PCR testing of all the study participants of both the study arms at weekly intervals to detect the number of viral infections, in addition to considering suspected clinical symptoms in efficacy calculations. Instead, the primary efficacy end-point in both the vaccine trials depended on unsolicited reporting of adverse events during the period beginning one or two weeks after the second dose. Thus, these studies have evaluated only relative, not absolute, protection against viral infection³³. Fourth, primary efficacy was reported during the four weeks following the second dose, when follow-up was also available for most participants. The first week during this period, was reported as a time frame for solicited adverse events (AEs), including local and systemic AEs (88.6% versus 18.8% in the vaccine and placebo groups respectively). The last two (or three) weeks, classified as a time frame for unsolicited AEs and when there were no pre-scheduled clinic visits, included several AEs (6798 and 6085 in the vaccine and placebo groups respectively) in the Moderna clinical study (Table 1). These AEs include fatigue, headache, cough and diarrhoea, all overlapping symptoms with COVID-19. Whether all these cases were tested for SARS-CoV-2 by RT-PCR has not been reported to the best of our knowledge. Further, features that classify an event as AE or COVID-19 symptom, the criteria for testing a trial

Table 1. Glossary of terms

Terms	Meaning
Adverse event	A clinical event that creates discomfort; may or may not require medical intervention.
Antigen	A molecule capable of being bound by the immune response generated in the host.
Correlate of protection	Refers to a specific aspect of the immune response that is associated (either positively or negatively) with protection from infection or disease and can predict the outcome of vaccination. Absence of this factor may or may not impact the effect of vaccination.
Disease	Infection of the body leading to manifestation of clinical symptoms such as fever, cough, headache, nausea, vomiting, etc.
Immunogen	A subset of antigens that is capable of inducing an immune response in the host.
Infection	Presence of replicating microbes in the body.
Mechanistic correlate of protection	Refers to the specific factor(s) that is responsible for providing protection from infection or disease. Absence of this factor significantly reduces the impact of vaccination.
Microbe	Any microorganism.
Mucosal compartment	The body parts that are in contact with or present a barrier to the outer environment. This includes the eyes, respiratory system (nose, respiratory tracts), alimentary canal (mouth, gut) and reproductive system (vagina and uterus).
Mucosal vaccine	A vaccine administered to mucosal sites such as nasal, oral or ocular (eyes).
Neutralizing antibodies	A subset of antibodies that binds to specific sites on the antigen and prevents its function. Examples of functions blocked by neutralizing antibodies include prevention of entry of virus particles into target cells, or preventing cell death induced by toxins.
Pathogen	A subset of microbes that causes disease in a susceptible host.
Systemic compartment	Body compartments that can be accessed only after breaching either the skin or the mucosal compartment. This includes blood, tissues and organs.
Systemic vaccine	A vaccine administered by breaching the skin to access either dermis (intradermal), tissue between dermis and muscle (subcutaneous), muscle (intramuscular) or blood (intravenous).

volunteer for SARS-CoV-2, and the number of negative PCR tests in trial participants (especially among those who have been classified as AEs) have not been reported. Lastly, vaccine efficacy in the long term is unclear for either of the vaccines and the measurement of this critical data has been hampered by the hurry to administer the vaccines to participants of the placebo group under ethical considerations.

ChAdOx1 vaccine trial

Efficacy of the ChAdOx1 vaccine has been reported from a pooled analysis of four studies spanning three countries³⁴. Symptoms that qualified the trial participant for an illness test and a swab test were pre-specified. One out of the four studies also included a weekly nasal swab RT-PCR test for asymptomatic infection. In contrast to the two mRNA trials, the ChAdOx1 study variably utilized either a conjugate meningococcal vaccine or saline as a placebo across different studies and sites, and accordingly, reported comparable incidence of reactogenicity between the vaccine and placebo groups. The efficacy of the vaccine itself has been much debated and confounded by differences in vaccine dose, varying efficacy rates across study sites and wide confidence intervals for the primary end-point of symptomatic COVID-19. Vaccine efficacy was superior in a group that received only a half-dose of the vaccine at priming (3.8%, CI -72.4 to 46.3) compared to the group that received the standard dose (58.9%, CI 1.0 to 82.9). While the pre-clinical study used mucosal

vaccination, the vaccine doses in the clinical trial were administered intramuscularly.

Ad26-prime, Ad5-boost Sputnik V vaccine trial

An interim analysis of the Sputnik V trial reported 91.6% efficacy in reducing symptomatic COVID-19 cases, beginning three weeks after the priming dose³⁵. This study broadly suffers from the same limitations as those of the mRNA trials in that vaccine efficacy analysis was based on self-reporting of symptoms followed by PCR confirmation of SARS-CoV-2 infection.

What should be the form of an ideal vaccine for SARS-CoV-2?

We expect to find at least five different attributes in a SARS-CoV-2 vaccine. First, a vaccine must reduce the chances of reinfection. Most subjects who have been administered a complete regimen of vaccination should be resistant to reinfection by dominant viral strains in circulation. In practical terms, a protective immune response may not or need not necessarily prevent a second round of viral infection. However, a protective immune response must block the spread of the virus within the body from the initial portal of entry to other organs that could be the main target of the virus. Second, most importantly, a vaccine must reduce disease severity. In a coronaviral infection, protective immune responses must prevent dissemination of the virus from the upper respiratory tract,

the portal of entry, to the lungs, and other body organs such as the heart, brain, etc. Third, a vaccine must prevent viral transmission. A vaccinated subject should not only be resistant to reinfection and protected from disease, but must also not serve as a source of viral transmission to other susceptible subjects. Fourth, a vaccine must ensure protection against variant forms of the virus that are likely to emerge with the progression of an epidemic. The induction of broad-range immune response against genetic variants of the virus emerging during an epidemic is crucial. The problem of genetic variation is indeed significant for RNA viruses. Despite the limited magnitude of genetic variation, several variant strains of SARS-CoV-2 have been identified worldwide that appear to be more infectious than the canonical viral strains^{36,37}. Lastly, a vaccine should not cause side effects beyond an acceptable safety limit in diverse populations and ethnic groups. An additional property of a coronaviral vaccine may also be critical. Since SARS-CoV-2 predominantly represents a respiratory infection, the induction of mucosal immune response, especially in the respiratory tract, may be crucial. In summary, an efficient vaccine must elicit a protective, broad-range, local and long-lasting immune response with low risk.

There are currently nine COVID-19 vaccines authorized for public use (<https://www.raps.org/newsand-articles/news-articles/2020/3/covid-19-vaccine-tracker>), more than 60 vaccines at different levels of clinical evaluation, and nearly 200 other candidate vaccines at various levels of development (<https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines>). Can any of these satisfy all the requirements of an ideal vaccine for COVID-19 mentioned above?

The protective efficacy of a vaccine is conditional on at least three critical components of its design and administration. First, what goes into the vaccine determines the specificity of the immune response. Second, in what form the vaccine is administered defines the nature of the immune response. And, lastly, into which body compartment the vaccine is delivered marks the nature of protection.

RNA viruses are endowed with a natural propensity to generate genetic diversity even in the absence of selection pressure. The variant viral strains remain a minority until the viral population encounters an AE, such as the immune response, a drug or vaccine-induced immunity. In the face of an AE such as the above, the majority viral population will disappear unable to proliferate, and the minority variant viral population will occupy that space dominating the epidemic. A vaccine must induce a broad-level immune response to minimize the emergence of resistant variant viral strains. The breadth of the immune response induced by a vaccine is directly proportional to the number of viral antigens it contains. A COVID-19 vaccine containing only the spike antigen of the virus is expected to induce a relatively narrow immune response

than the one that contains the spike antigen and an additional viral antigen such as M, N or E antigen. Thus, more the number of antigens in a vaccine, broader is the immune response and less are the chances of viral escape. However, the presence of several antigens in a vaccine can also be a safety concern. Many viral proteins contain multiple functions; some of these are toxic to the cells since they directly subvert the protective immune responses of the host. Therefore, more need not necessarily be better. One must carefully select only a minimal number of viral antigens necessary to induce a broad-level protective immune response in the absence of side effects. Antigen selection for vaccine development requires a thorough and basic understanding of the antigenic and toxic properties of all the viral proteins.

The majority of vaccine candidates incorporate the spike protein of SARS-CoV-2. The assumption is that the antibodies induced against the spike protein of the vaccine today must prevent the attachment of the virus to the target cells tomorrow, should a real infection happen. However, reducing disease severity by preventing viral spread to the lungs and other body organs is the other attribute crucial for vaccine-induced protection, as discussed above. Cell-mediated immunity and non-neutralizing antibodies perhaps play a role (apoptosis-induction, phagocytosis, complement-mediated lysis, etc.) as crucial as that of neutralizing antibodies in preventing viral spread to the lungs and different organs. Neutralizing antibodies must typically target a viral protein expressed on the surface of the virus particle and required for viral entry, such as the spike protein, not the one located inside the viral particle, such as the nucleocapsid antigen. However, there are no such restrictions for the induction of cell-mediated immune responses which target and destroy a host cell harbouring the viral infection. Hence, an ideal COVID-19 vaccine must encode a few viral proteins capable of inducing both neutralizing antibodies and cell-mediated immune responses. The probability of viral escape against such vaccines is smaller.

Unfortunately, the correlates of protection (the nature and mechanisms of protective immune responses) are not yet understood for COVID-19. Vaccine studies and trials have not addressed this question. Clinical trials of the approved vaccines were designed to assess only symptomatic (associated with viral replication in the lower respiratory tract and lungs) and not asymptomatic (associated with the lack of symptoms) infection. As demonstrated by the pre-clinical studies, reduction of symptomatic COVID-19 is associated with the presence of systemic immune responses (either antibodies or T cells) and reduction of viral titres²⁶⁻³⁰. The spread of viral infection to the lungs and body organs could be prevented by direct antiviral activity (neutralization, phagocytosis, complement-mediated lysis, etc.) and killing of infected cells mediated by antibodies, NK cells or T cells. Immune

responses directed against the spike protein and other viral proteins can activate such mechanisms to restrict viral spread in the body. In support of this hypothesis, in a hamster model, vaccines encoding the M protein and nucleocapsid have been shown to protect against severe disease. A strong negative correlation between the pre-challenge titres of nucleocapsid antibodies in the serum and post-challenge lung pathology scores has been reported³⁸. Thus, the inclusion of the more abundant nucleocapsid protein in a vaccine formulation is more likely to be effective in the long-term control of the pandemic due to limited sequence diversity and by preventing viral escape through antigenic diversity.

A nasal vaccine is essential to protect the lungs and the respiratory tract

Unfortunately, most current COVID-19 vaccines have been designed to induce systemic, not mucosal, immune response. Can systemic vaccination protect against a respiratory viral infection? The answer to this question could be affirmative or negative, both, depending on what we need.

A mucosal, not systemic, vaccine is necessary to protect a mucosal compartment because that is how the immune system works. The presence of virus-specific antibodies and T cells in the respiratory compartment, not in the blood, is relevant to protection against a respiratory viral infection^{24,39}. A vaccine must reach the right compartment to be effective. Little or only moderate cross-protection exists between the mucosal and systemic compartments or even among different mucosal subcompartments¹⁴. A mucosal vaccine administered as nasal drops or powder can elicit superior quality protection in the upper and lower respiratory tracts and the lungs than those administered by any other route⁴⁰.

A potential solution is the combined use of nasal and systemic vaccines together to produce both IgA and IgG antibodies collectively, notwithstanding the limitation of costs and logistics. Both IgA and IgG antibodies are necessary to block viral infection at the portal of entry, minimize the spread of the virus to the lower respiratory tract and lungs, and prevent the dissemination of the virus to other systemic body organs. However, for reasons described above, the mucosal immune system cannot establish strong and enduring immune responses against microorganisms, harmful or not. Since the mucosal immune system cannot generate high-quality immune responses, mucosal vaccines also may not induce potent immune responses against pathogenic microorganisms. There have been many attempts to break mucosal tolerance using various adjuvants – chemicals that boost the efficacy of vaccines⁴¹ – such attempts have mostly been unsuccessful. A vaccine regimen that can induce protective immune responses to reduce disease severity and

elicit the production of IgA antibodies to lessen viral transmission must be identified experimentally. The combination of vaccines that must be used for immunization, the order of their administration, the number of booster immunizations needed, types of adjuvants included, the safety issues, etc. must be determined in appropriately designed clinical trials.

In summary, developing a vaccine for the new coronavirus may not be technically challenging, unlike for viruses such as HIV and HCV. HIV hides the envelope protein from neutralizing antibodies using cryptic conformational epitopes. Coronaviruses do not intend to stay in the host for a long duration; therefore, they need not bother about virus-neutralizing antibodies and hide their surface spike proteins. The induction of virus-neutralizing IgG antibodies in the blood, therefore, may not be technically difficult using a vaccine format of any kind, especially given the advances in stabilizing the spike antigen in the neutralization-sensitive prefusion-closed conformation⁴². Keeping the safety matters aside, the question, arises whether the induction of mucosal polymeric IgA antibodies is more relevant for protection of the respiratory tract. Systemic vaccines are not likely to induce the needed mucosal immune response, and mucosal vaccines are not likely to elicit long-lasting immunity. Therefore, we need some creative thinking and hope that the virus would naturally establish herd immunity. Natural infection can establish herd immunity; however, such protection comes only at the cost of mortality, morbidity, loss and suffering. Establishing herd immunity using a vaccine, not a virus, is desirable. Systemic vaccines may provide partial protection against respiratory viruses, but expecting sterilizing immunity may be unrealistic and over-optimistic.

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