The protective immunity induced by SARS-CoV-2 infection and vaccination: a critical appraisal

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Abstract

Understanding the interactions of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) with humans is deeply grounded in immunology, from the diagnosis to pathogenesis, from the clinical presentations to the epidemiology, prevention, and treatment. However, the difficulty of capturing the complex and changeable array of immunological concepts and incorporating them into the strategies of control of the SARS-CoV-2 pandemic poses significant hindrances to establish optimal public health policies. The contribution of immunology to the control of the pandemic is to shed light on the features and mechanisms of the protective immunity elicited by SARS-CoV-2 infection and vaccines. Do they induce effective protective immunity? How? For how long? What is the effect of vaccination on individuals who were previously infected? To appropriately answer these questions, it is necessary to get rid of the outdated notion of a naïve, static, and closed immune system, which leads to misconceptions about susceptibility, specificity, immunological memory, and protective immunity. The present essay discusses these issues based on current immunological concepts.

Keywords

Introduction

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection has spread throughout the world at an astonishing rate, leading to overall sanitary, social, and economic disruption, and above all, causing an enormous burden of suffering and lost lives. Such a global threat calls for the global collaboration of all fields of human expertise. Immunology is but one of these fields, albeit quite a central one. Immunology provides insights into some of the core issues concerning why some infected subjects remain asymptomatic, while others develop a mild and self-limited disease, and a minority suffers a severe and lethal disease.

Furthermore, the understanding of the pathogenesis of the disease and the rationale for its treatment, as well as the conceptual basis for the development of effective vaccines, are grounded on immunological knowledge.

However, the complexity and changeability of the immunological concepts make it difficult to be captured by the professionals involved in the control of SARS-CoV-2 infection. Consequently, some profoundly ingrained misconceptions hinder optimal control measures. For example, the assumption that, since SARS-CoV-2 is a novel human pathogen, the whole world population would be susceptible to the infection and, if no interventions were made, estimates of 7 billion infections and 40 million deaths would occur [1]. The supposition of universal susceptibility to novel pathogens is a misconception. Notwithstanding the novelty of the pathogen, a proportion of the exposed individuals always resist its infection. That happens due to the individual's natural resistance and the occurrence of cross-immunity induced by related or unrelated residents and environmental microorganisms [2, 3]. At its first contact with the organism, the novel pathogen finds a fully functioning immune system composed of an array of cells capable of recognizing its components and reacting to them. Therefore, a pathogen never encounters an absolutely naïve immune system. Conversely, it is inserted into a highly dynamic setting of ongoing cellular and molecular interactions and provokes a perturbation of this network. Since the immune system is ruled by autopoiesis, it subsequently returns to a steady state, although at a different threshold. This flexibility allows it to cope with the pathogen while maintaining homeostasis. Another critical aspect of susceptibility is that it is not an all-or-nothing phenomenon but a continuum, which ranges from total susceptibility to complete resistance. It comprises a spectrum of intermediate states, which contributes to the different clinical presentations of the infection, spanning from asymptomatic to mild, moderate, severe, and fatal disease [4].

This essay discusses the features of protective immunity induced by SARS-CoV-2 infection and vaccination based on the current immunological concepts.

The concepts of protective immunity, specificity, and immunological memory

Protective immunity is an inclusive concept that implies some degree of advantage for the individual or the collectivity. At the individual level, it means the immune system's ability to resist infection or reinfection or attenuate an infectious disease or its clinical presentation. At the population level, it is known as herd or collective immunity and referred to as the indirect protection from infection, which is conferred to susceptible individuals when a sufficiently large proportion of immune individuals exist in a community [5].

The classical paradigm of immunology posits that protective immunity depends on two features of the adaptive immune system. The first is specificity, the ability of lymphocytes to discriminate self from nonself components and react to the latter [6, 7]. The second is immunological memory, which refers to the long-lived specific lymphocytes' ability to promptly and efficiently respond to a reencounter with an immunogen [8, 9]. However, these deep-rooted concepts were challenged by recent data, and currently, it has been established that some adaptive immune responses can display no specificity or memory, while some innate immune responses can exhibit both [10-15]. Furthermore, innate immune cells can be long-lived, and adaptive T and B lymphocytes can have innate features [16-18].

The concept of specificity has been significantly widened by demonstrating that the adaptive immune cells react, with different degrees of affinity, to the specific antigen and an array of other molecules, including environmental and resident microorganisms [19-21]. Indeed, the crucial role of the microbiome, virome, mycobiome, and probably, archaeome in the development, activation, and modulation of innate and adaptive immune systems have been recently acknowledged [22-25].

The classical paradigm of immunological memory posits that, at its first contact, the infectious agent (or the vaccine) finds an immune system populated by naïve lymphocytes, which had never been exposed to it before. The result of this encounter will be activation, proliferation, and differentiation of the cells into short-lived effector lymphocytes and long-lived memory lymphocytes. The latter would usually be kept in a quiescent state, waiting for the subsequent encounter with the same infectious agent (or vaccine), and would mediate more rapid, intense, and efficient responses. However, such a view involves two outdated conceptions.

The first concerns the assumption of the immune system's naïvety, which some robust evidence has dismissed. The immune system is shaped, organized, activated, and modulated since the fetal life, initially by the microbiota, infectious antigens, immunoglobulins, and lymphocytes from maternal origin [26-29], and later by the myriad of constant antigenic stimuli, both from environmental and resident microorganisms, as well as from self-antigens [30-33]. Infectious agents contain several antigens, which are, in their turn, composed of a significant number of epitopes, each of them capable of inducing different immune responses, with different kinetics, dynamics, and outcomes, which may cross-react with those induced by several other infectious agents. Therefore, at its first contact, a pathogen neither finds a naïve and static immune system nor induces a single immune response that gives rise to effector and memory cells. Instead, it encounters a highly complex and dynamic immune system already formed and organized, with many immune responses in action, involving various cells at diverse activation and differentiation stages. These cells interact in different molecular landscapes under the effects of stimulatory and inhibitory signaling pathways, resulting in stimulation or inhibition of several immune responses. Immunological memory has to be understood within this context [16]. Recent findings show that effector CD8 T cells dedifferentiates into long-lived memory cells [34], and T memory cells possess a propensity to convert to another cell type by plasticity when active regulatory programs are lost [35, 36].

The second misconception of the classical paradigm of immunological memory is that it always enhances protective immunity. Infectious agents contain a great variety of epitopes capable of inducing different immune responses. The occurrence of protective immunity depends on the epitope's immunogenicity, its frequency, immunodominance, and relevance for the infectious agent and the type and interactions with reactive immune cells. For example, regulatory T memory cells are not expected to provide protective immunity as helper T memory cells [9, 37] or that enhancing-antibody producing B memory cells positively contributes to protection [38].

A frequent caveat on the interpretation of the phenomenon of immunological memory is taking the part for the whole when one infers that blood memory cells, the population usually studied, represent the entire repertoire of immunological memory cells. Memory cell repertoire includes a variety of phenotypically and functionally diverse populations of cells disposed of in different compartments, which comprise subsets of memory T lymphocytes (effector memory, central memory, tissue-resident memory, peripheral memory, and stem cell memory) [8, 9] and memory B lymphocytes (T-cell dependent and independent memory, isotope-switched and unswitched, mutated and unmutated memory B cells, distributed as bone-marrow central memory and circulating long-lived plasma cells) [39, 40]. The memory cell pool is under constant transcriptional and epigenetic constraints by an array of antigenic stimuli, cellular interactions, cytokines and chemokines, hormones, neuropeptides, and several other factors [41-44]. It is, therefore, a very dynamic system subject to frequent changes that, in certain circumstances, may suffer cell apoptosis, leading to loss of memory [21]. Moreover, recent evidence shows that immunological memory extends beyond conventional B and T lymphocytes and includes natural killer (NK) cells and other innate lymphoid cells, as well as myeloid cells such as monocytes and macrophages [16]. These findings have widened the spectrum of immunological memory and added more difficulty to understanding how the memory repertoire is organized and functions.

Besides specificity and memory, protective immunity is influenced by the context in which the immune response occurs. It includes factors associated with the individual (genetic background, epigenetic makeup, age, hormonal and emotional influences, and the status of the immune system at the moment of the antigenic stimulation), factors depending on the antigenic stimulus as such (immunogenicity, antigenic load, interactions of the different immune responses induced by diverse epitopes), as well as on the virulence factors and escape mechanisms used by the infectious agent [45-50].

Finally, it has to be stressed that the detection of specific antibodies or lymphocytes that react to epitopes of an infectious agent is not a trustful indication of protective immunity. For example, it has been demonstrated that the synthesis of immunoglobulin (Ig)G antibodies to SARS-CoV-1 can persist from six years [51] to up to 12 years after infection [52], and T memory lymphocytes can be detected from six [53] up to 11 years post-infection [54]. However, these observations do not mean that protective immunity lasts for this time

since antibodies and memory cells can be directed toward irrelevant components of the pathogen (e.g., those that do not imply a reduction of infectivity).

Protective immunity induced by the SARS-CoV-2 infection

The remarkable adaptability of SARS-CoV-2

To reach pulmonary cells and give rise to productive infection, SARS-CoV-2 has to overcome eight host defense barriers: mucus and mucociliary clearance, surfactant proteins, respiratory tract microbiota, the antimicrobial peptides defensins and lactoferrin, nonspecific secretory IgA antibodies, respiratory epithelial cell interferons, innate immunity, and adaptive immunity. For succeeding, the virus has developed strategies of survival that include impairment of interferon production; hiding immunogenic motifs; avoidance of viral RNA detection by cell sensors; triggering host cell death by apoptosis, necroptosis, or pyroptosis; induction of lymphocyte exhaustion and depletion; and virus mutation. Besides, SARS-CoV-2 uses strategies to take advantage of cell resources for its benefit by manipulating the process of autophagy, inhibiting the ubiquitin-proteasome system, and hijacking mitochondria functions.

Although destruction is the visible face of the relationship of SARS-CoV-2 with humans, it is not its aim. Destruction of the counterpart is harmful to both sides. If the virus destroys its host, it destroys itself, and if the host causes early elimination of the virus, immunity is not achieved. Therefore, immunity should be viewed as a strategy of adaptation, not destruction. Adaptation depends on tradeoffs between the pathogen and the host to improve mutual fitness and survival. Gene sequences of SARS-CoV-2 isolated from different geographic areas confirm that genetic changes of the virus are continuously arising, which ultimately promote adaptation [55, 56].

On the other hand, the virus constrains the host to produce hundreds to thousands of proteins involved in antiviral defense and virus replication and shedding [57]. These molecular changes are signatures of adaptation. Adaptation to the host is a frequent strategy of survival of infectious agents. Human coronaviruses NL63, HKU1, OC43, and 229E are now part of the human respiratory virome [58-61] and induce cross-reactive T [62-67] and B memory cells to SARS-CoV-2 [68-72].

The role of antibodies in protective immunity

It is well documented that SARS-CoV-2 infection induces potent immunity that is critical in clearing a primary infection. The protective immunity depends on the coordinated action of the three compartments of the adaptive immune system: B-cells, and CD4+ and CD8+ T cells [73]. Neutralizing antibodies have been considered as the correlate of protection in SARS-CoV-2 infection. They bind to the virus and directly block its ability to infect cells, usually by inhibiting the interaction between the viral spike (S) protein and the cellular angiotensin-converting enzyme-2 (ACE2) receptor. Several data demonstrate that most individuals infected with SARS-CoV-2 develop neutralizing antibodies that peak within 3-5 weeks of infection and decay afterwards [74-79], but keeping titers relatively stable for months [80] and increasing their avidity over time, an indication of underlying immune maturation [81]. The pattern of the initial rapid decay of neutralizing antibodies is due to the short half-life of the immunoglobulins and antibody-secreting cells. However, these cells are further replaced by a population of long-lived memory B cells [82, 83] that are remarkably stable and mature up to 6 months after SARS-CoV-2 infection and may provide long-lasting protection [69, 84]. Indeed, antibodies direct to SARS-CoV-2 S protein remain detectable for at least 11 months after infection due to the presence of long-lived bone marrow plasma cells [85].

Neutralizing antibody levels and kinetics vary with the clinical presentation of SARS-CoV-2 infection. Individuals with severe or moderate disease tend to have an earlier appearance of neutralizing antibodies at higher levels than those with mild illness [86-89]. Conversely, asymptomatic individuals frequently produce low levels or no neutralizing antibodies at all [86, 87]; paradoxically, the same pattern showed by those who die at early times post-onset of symptoms [89]. In addition, the higher neutralizing titers of moderate and severe subjects decrease following a two-phase pattern, with an initial rapid decline that significantly slows after day 80 [90].

An intriguing point about the protective effect of SARS-CoV-2 neutralizing antibodies is their positive correlation with the severity of clinical illness [87, 89, 91-98]. Different possibilities have been suggested for explaining this apparent paradox. It could be due to the higher viral loads of patients with severe SARS-CoV-2 infection [89, 99], to their more prolonged exposure to a greater abundance of viral antigens [94], or even to the phenomenon of antibody-dependent enhancement of the immune response [96]. Alternatively, the lower representation of regulatory CD4+ T cells in patients with severe disease [100] could explain an excessive antibody production. This matter is still an open question.

Another category of antiviral antibodies bridges viral components to immune cells expressing Fc receptors (FcRs). They use different strategies to eliminate the virus: antibody-dependent cell-mediated cytotoxicity (ADCC) that is most commonly carried out by NK cells, and antibody-dependent endocytosis (ADEn), performed by macrophages, monocytes, and neutrophils. While NK cells involved in ADCC detect and destroy cells expressing virus components in their membrane, phagocytes involved in ADEn endocytose and destroy virus components complexed with IgG antibodies. Both Fc-dependent and neutralizing antibody functions are characterized and quantified by *in vitro* cell systems using plasma samples. Therefore, their biological importance in protective immunity is solely inferred.

Robust evidence shows that SARS-CoV-2 induces antibodies capable of engaging in ADCC or ADEn in most infected individuals, from asymptomatic to those with mild, moderate, or severe disease [101-105]. Fc-dependent antibody levels and functions are lower in asymptomatic individuals than those in symptomatic cases [105]. In mild to moderate patients, S-specific antibodies capable of Fc-mediated antiviral activity remain readily detectable in almost all donors up to four months post-infection, even in those whose neutralizing antibody responses have waned to undetectable levels [102]. By the second week of infection, those with severe disease presented higher levels of antibodies than those with moderate disease. Notably, while the individuals who survived severe disease successfully class-switched to IgG antibodies, with high-affinity FcR binding capacities, individuals who did not survive showed less robust switching and exhibited a delayed evolution of high-affinity FcR binding antibodies and Fc effector function [101].

Part of the long-lasting memory B cell pool is formed by pre-existing cross-reactive seasonal coronavirus-specific (HCoV-HKU1 and HCoV-OC43) clones, contributing to the early production of anti-SARS-CoV-2 neutralizing antibodies [68, 69, 106]. These cross-reacting antibodies are particularly prevalent in children and adolescents, are predominantly of the IgG class, and target the S2 subunit of the virus S protein, whereas SARS-CoV-2-induced neutralizing antibodies present in higher titers are either of the IgG, IgA, or IgM classes and target both S1 and S2 subunits [71]. Besides these findings, another point challenges the idea that SARS-CoV-2 primes only naïve cells at its first encounter with the immune system: the IgG antibodies' precocious appearance, sometimes preceding IgM antibodies during SARS-CoV-2 infection [107-109]. This pattern is compatible with a secondary immune response, suggesting a previous contact of the immune system with cross-reacting antigens. These facts are compelling and lead to the conclusion that, even at its first encounter with the immune system, a novel pathogen such as SARS-CoV-2 elicits various immune responses toward its diverse epitopes, some with features of secondary responses due to the occurrence of cross-reactivity with microorganisms from the microbiota and the external environment.

The role of T lymphocytes in protective immunity

Notwithstanding which effects antibodies may play in protective immunity to SARS-CoV-2, they can be overcome by other viral clearance mechanisms since mild infections can resolve before seroconversion [99, 110] or in its absence [111, 112]. Moreover, 10-20% of symptomatically infected people have little or no detectable antibody [113, 114]. And, more surprisingly, agammaglobulinemia patients, unable to produce antibodies, may present no symptoms or mild clinical manifestations when infected with SARS-CoV-2 and recover from the infection [115, 116]. These observations point to the importance of the participation of T cells in the protective immunity to SARS-CoV-2 infection. Indeed, it has been shown that most of the convalescent subjects have undetectable antibodies but presented robust T-cell immunity against SARS-CoV-2 [117-119].

It is well established that T cells play a central role in protection against SARS-CoV-2 virus infection [120], and their early activation is associated with viral clearance and mild disease [121]. Different from neutralizing

antibodies that peak within the first few weeks after the symptom onset and decay afterward, T cell responses to SARS-CoV-2 infection are generally more stable [122, 123]. It has been shown that 100% of convalescent individuals present specific CD4+ T cells, while 70% of them exhibit CD8+ T cells [64]. Interestingly, an association has been found between the proportion of CD8+ T cell responses and mild disease, suggesting a potential protective role of these cells [124]. In severe disease, CD4+ T cell responses are more frequent than those of CD8+ T cells [124], with increased proportions of cytotoxic follicular helper cells and cytotoxic T helper cells [100].

Antigen-specific CD4+ T cells expressing memory markers, as well as interleukin (IL)-2, interferon-γ (IFN-γ), tumor necrosis factor (TNF), and CD154 (CD40 ligand), were markedly increased in COVID-19-recovered individuals compared with healthy donors [63, 64, 125]. However, the relative frequency of these cells decreased 22% to 32% six months after infection [126]. In addition, they overwhelmingly show a T helper (Th)1 pattern, considered a beneficial profile, as severe patients show reduced IFN-γ and a shift to a more Th2 profile [64, 127]. Although SARS-CoV-2-specific CD8+ T cells are also detected six months after infection, their responses are far more variable and generally less robust than those of CD4+ T cells [126].

A key yet unsettled aspect of the protective immunity to SARS-CoV-2 infection is the role of cross-reactive T cells, found in 40% to 60% [64] to 100% [128, 129] of the unexposed healthy individuals and that react to different virus proteins as S, membrane (M) [65, 118], and nucleocapsid (N) proteins [62]. Several research groups have identified the common cold coronavirus (HCoV-OC43, HCoV-HKU1, HCoV-NL63, and HCoV-229E), presenting different homology degrees with SARS-CoV-2, as responsible for the cross-reactivity with SARS-CoV-2 [62, 65, 118, 129, 130]. The common cold is the most frequent infectious disease in humans; adults have two to five episodes per year, and school children from seven to ten [131]. Human coronaviruses account for 8-15% of all cases [132].

The presence of cross-reactive T cells in a considerable fraction of the general population could explain the different COVID-19 clinical outcomes. In addition, it can affect the dynamics of the pandemic, impact the epidemiological models of herd immunity, and influence the design and performance of SARS-CoV-2 vaccines [63, 65]. Furthermore, cross-reactive lymphocytes could help mount a faster, more robust, and persistent immune response upon exposure to SARS-CoV-2 and thereby limit disease severity [67, 128].

Real T-cell cross-reactivity implies that a single T-cell receptor recognizes more than one distinct peptide-major histocompatibility complex (MHC) ligand. That was demonstrated to occur in memory T-cells from SARS-CoV-2 infected individuals. Their receptors cross-react to epitopes of SARS-CoV-2 and seasonal human coronaviruses [133]. However, the low functional avidity of the cross-reactive TCRs for SARS-CoV-2 [128, 129, 133] makes them less susceptible to intrinsic negative control mechanisms [128] and argues against a significant role in infection [129]. Nevertheless, the finding that CD4+ cells isolated from unexposed healthy donors can differentiate towards the Th1 lineage in the presence of SARS-CoV-2 proteins and produce IFN-γ [130], a potent antiviral and immunomodulatory molecule, is highly suggestive of their functionality.

The efficacy of post-infection immunity

The question that emerged during the COVID-19 pandemic is whether the SARS-CoV-2 infection protects against reinfection and for how long. Eight months after the pandemic emerged, the first case of reinfection by a phylogenetically distinct variant of SARS-CoV-2 was reported [134] and was rapidly followed by many other cases worldwide [135]. While the known number of confirmed cases of reinfection represents a tiny fraction of the vast number of cases of COVID-19, it may nonetheless represent a small fraction of the persons who have been reinfected [136]. The more obvious reason for this unaccounted but undoubtedly significant sub-notification is that most individuals who became infected or reinfected did not have access to confirmatory laboratory tests or health services and were not included in the official datasets [137]. Furthermore, to characterize true reinfection and distinguish it from the recurrence of the initial infection, viral genome sequencing of virus samples collected in both situations has to be performed and compared [135, 137], which is not a trivial task.

Notwithstanding those evident limitations and aware of the possibly tremendous number of unnotified cases, the risk of reinfection has been calculated in different settings. A study of 25,661 healthcare workers in England reported that a previous infection provides an 84% risk reduction for reinfection, with a median protective effect observed seven months following primary infection. Notably, a variant of concern known as B.1.1.7 did circulate during the final part of the observation period, causing about 50% of all infections, but did not seem to affect reinfection rates [138]. Another investigation, comprising a cohort of 150,325 users of the United States health system that excluded healthcare workers, concluded that people previously infected by SARS-CoV-2 showed 78.5% protection and 83.1% against symptomatic infection [139]. Interestingly, the authors found that the protective effect increased over time, suggesting that the viral shedding or ongoing immune response may persist beyond 90 days and may not represent actual infection. The most extensive published study included a cohort of approximately 4 million Danish people and found that previous SARS-CoV-2 confers protection of 80.5%, which decreased to 47.1% in those aged 65 years and older [140].

The variability of the features of reinfected people does not allow the definition of a clear pattern of those susceptible to reinfection. An analysis of 23 cases of reinfection found that the mean interval between the two episodes was 15 weeks; the mean age was 44.5 years; 56.5% were male; in 17/23 (74%), no comorbidity was observed in the first episode; in 10/23 (43.5%), the second episode was milder, in 4 cases there was no difference in severity, in 7 cases the next episode was more severe, and in 2 cases both episodes were asymptomatic [141]. Another study encompassing 16 proved cases of reinfection showed that it occurred across demographic spectra; 8/16 (50%) were between 20 and 30 years old, and in 75% of the cases, the initial infection was either asymptomatic or caused mild disease [135]. Reinfected people usually were not immunocompromised and, when tested, they present anti-SARS-CoV-2 antibodies [136].

Different possible explanations can be posed for explaining reinfection. The first is the waning of protective immunity induced by primo-infection since reinfections occurred after a mean interval of nearly four months when neutralizing antibodies, considered by some as correlates of protection, present low levels [142, 143]. However, a study of 12,541 healthcare workers found that the presence of anti-S or anti-nucleocapsid IgG antibodies was associated with a substantially reduced risk of SARS-CoV-2 reinfection in the ensuing six months [144]. Another non-exclusive possibility is that reinfection was due to a new variant of the virus or an extremely high virus load, capable of escaping previous immunity. Detection of reinfection is most straightforward when the virus belongs to a different clade or lineage, as this provides clear evidence of infection by a different virus. Among 16 published reinfection cases, 5 (31%) had a distinct clade or lineage detection between initial infection and reinfection, 8 (50%) were infected with the same clade but had differences of > 2 substitutions/month between them, and 3 cases (19%) presented different D614G alleles [135].

The short time since SARS-CoV-2 emerged does not allow a definitive answer about the duration of the infection-induced protective immunity. However, the finding that infected individuals develop long-lived memory B cells [82, 83] that are remarkably stable and mature up to 6 months after SARS-CoV-2 infection [69, 84, 123], together with the demonstration of long-lived CD4+ and CD8+ memory T cells [63, 64, 123, 125], allows anticipating the occurrence of a somewhat lasting protective immunity. Still, it will likely reduce the frequency and severity of COVID-19 instead of leading to sterilizing immunity and prevention of reinfection.

The antibody-dependent enhancement of the infection

The finding that SARS-CoV-2 reinfection is sometimes more severe than the infection suggests the occurrence of antibody-dependent enhancement of infection (ADE). The ADE phenomenon facilitates virus entry into host cells and, consequently, enhances the infection [145]. The occurrence of ADE has been demonstrated in coronavirus infections, including middle-east respiratory syndrome coronavirus (MERS-CoV) [146], SARS-CoV-1 [147, 148], and SARS-CoV-2 [149, 150]. ADE ensues when antibodies binding to the virus particle fail to efficiently neutralize the virus, either because they bind to viral epitopes other than those involved in cell attachment and entry or due to the presence of sub-neutralizing concentrations of antibodies. The complex virus-antibody (IgG, IgM, or IgA), with or without complement (C), is internalized into those cells expressing Fc or C receptors, such as lymphocytes, monocytes, macrophages, dendritic cells, granulocytes,

and endothelial cells. This process contributes to the augmentation of viral infection and the functional alteration of infected cells, ultimately exacerbating viral pathogenesis and disease severity [151, 152]. The ability of SARS-CoVs to invade cells that do not express ACE2, but Fc or C receptors, utilizing ADE has been demonstrated *in vitro* in human B cells, using sera from immunized animals, as well as from convalescent subjects [153], and in monocytes and macrophages, by using antibodies specific to the viral S protein [148, 154]. Active replication of SARS-CoVs in monocytes, macrophages, and lymphocytes has been demonstrated *in vitro* and *in vivo*, although not usually at a sustained higher level [155]. However, the virus's simple presence in the host cells' interior can alter cell function and viability [155-159], even in the absence of replication. ADE possibly plays a role in critical pathological mechanisms of SARS-CoV infection, such as lymphopenia, cytokine storm, and lung involvement [38, 156, 157]. The second peak of the throat and nasal virus load that occurs after the 9th day post-infection when antibodies to SARS-CoV-2 start to be detected [160], and the association between seroconversion and the worsening of the clinical conditions [161, 162] are highly suggestive of the occurrence of the ADE phenomenon.

Protective immunity induced by SARS-CoV-2 vaccination

Anti-SARS-CoV-2 vaccines were developed at an unprecedented speed and display an impressive efficacy in protecting from severe disease. However, several crucial questions remain under scrutiny. For example, 'what mechanisms of protective immunity do they induce, and how to measure them?'; 'how comparable is post-vaccination immunity to post-infection immunity?'; 'what is the breadth of their effects on virus's genetic variants?'; and 'what is the duration of their effects?'

What is vaccine efficacy?

Many different endpoints are used in vaccine research to define efficacy. Outcome data from randomized controlled trials are often presented as a proportional reduction in disease between vaccinated and control participants to calculate the decline attributable to the vaccine. In the case of SARS-CoV-2, an efficacious vaccine might prevent infection, disease, or transmission. Alternatively, vaccines that do not affect the clinical course, but reduce the transmissibility of SARS-CoV-2, could still be valuable interventions on a population level [163]. Therefore, there are different vaccine 'efficacies', and the one so far attained by all SARS-CoV-2 vaccines is protection from severe disease and death in all settings, although the prevention of asymptomatic transmission and mild-to-moderate disease occurs less constantly [164].

The features of SARS-CoV-2 vaccines

By April 2021, there were over 200 SARS-CoV-2 vaccine candidates in various stages of preclinical and human testing, and five in phase IV trials: Pfizer/BioNTech + Fosun Pharma (BNT162b2); Moderna + National Institute of Allergic and Infectious diseases (mRNA-1273); AstraZeneca + University of Oxford (AZD1222); Sinovac Research and Development Co. (CoronaVac); and Janssen Pharmaceutical (Ad26.COV2.S), by Johnson and Johnson. These vaccines that have been deployed worldwide use three different formulations: mRNA that contains the sequence encoding pre-fusion S protein encapsulated in lipid nanoparticles (Pfizer/BioNTech and Moderna), DNA encoding S protein delivered by non-replicating adenovirus vectors (AstraZeneca and Jansen), and formaldehyde-inactivated virus (CoronaVac) [165, 166].

Each vaccine formulation uses different mechanisms for eliciting immune responses. For example, in RNA-based vaccines, the mRNA molecules captured by local innate immune cells are released from the lipid nanoparticles into the cytosol and sensed by toll-like-receptor (TLR) and retinoic acid-inducible gene (RIG)-I receptors. That elicits type I interferon secretion to the extracellular matrix, creating a milieu that favors Th1 responses over Th2. In the cytosol, mRNAs are directly translated by ribosomes into polypeptides processed by the proteasome system leading to peptide presentation onto MHC-I on the cell surface. Peptide presentation onto MHC-II may occur by antigen-presenting cells after uptake of extracellular proteins or cell debris containing protein [167]. This simplified model conveys several gaps that pose difficulties to understanding RNA-based SARS-CoV-2 vaccines. Since all cells express low-density lipoprotein receptors, the same used for capturing lipid nanoparticles [168], the consequences of the uptake of nanoparticles

containing encapsulated mRNA by different local cells are unknown. After all, antigen-presenting cells are minimally represented among cells at the site of intramuscular injection. In this context, it has been shown that cells that have endocytosed mRNA in lipid nanoparticles secrete extracellular vesicles containing mRNA, which is possibly an alternative mechanism for delivering the molecules [167].

Notwithstanding how mRNA vaccines deliver the immunogen, protein production is detectable at the intramuscular injection site for up to ten days after vaccination [167]. Immunogen-laden dendritic cells further migrate to draining lymph nodes where interactions with T and B lymphocytes occur, leading to the formation of germinal centers and resulting in the generation of antibody-producing plasma cells and long-lived memory B cells. B-cells produce an array of epitope-specific antibodies with different functions, including neutralizing antibodies. These antibodies are usually in similar levels or higher than those found in sera of humans who recovered from infection and are lower in 65-85 years-old people [167]. After the first immunization with mRNA vaccines, neutralizing activity was detected in less than half of the subjects. However, all showed increased antibody titers and neutralization after the second immunization [169] and, when applied to previously SARS-CoV-2-infected individuals, can induce robust humoral and T-cell responses after a single dose [170-173], even when this happens one year after the infection [174]. Activated T cells are predominant of the CD4+ Th1 type and produce IFN-γ, TNF, and IL-2 cytokines when restimulated with SARS-CoV-2 peptide pools [175], but CD8+ T cells are also induced [176].

The DNA vaccines delivered by non-replicating adenovirus vectors (AstraZeneca and Jansen) elicit effective immune responses and emulate the natural infection through the transgene products expressed. Vaccination of non-human primates with a DNA expressing different forms of SARS-CoV-2 S protein developed humoral and cellular immune responses, including neutralizing antibody titers at levels comparable to those found in convalescent humans and macaques infected with SARS-CoV-2 [177]. After vaccination, all animals were challenged with SARS-CoV-2. The vaccine encoding the full-length S protein resulted in > 3.1 and > 3.7 log10 reductions in median viral loads in bronchoalveolar lavage and nasal mucosa, respectively, compared with viral loads in sham controls [177].

The inactivated SARS-CoV-2 vaccines (e.g., CoronaVac) use the whole virus particles and, therefore, are supposed to elicit immune responses to all virus components. The vaccine triggers local activation of innate immune cells, production of type I interferons, activation of B cells that produce neutralizing antibodies, and CD8+ T cells with cytotoxicity to virus-infected cells [165].

The quest for correlates of protection

To date, all provisionally approved SARS-CoV-2 vaccines elicit humoral and cellular immune responses, including virus-specific binding antibodies, neutralizing antibodies, and virus-specific CD4+ and CD8+ T-cell responses. However, the measurement of vaccine efficacy is challenging, as efficacy trials need to include large cohorts to be followed for a long time. To circumvent that difficulty and allow comparisons between vaccines, correlates of protection (CoP) are used. An immune CoP is an immunological marker that reliably predicts protection against disease or infection after natural infection or vaccination [178]. The current unavailability of reliable CoPs [178-180] poses a significant hindrance in developing and deploying vaccines against SARS-CoV-2.

Neutralizing antibodies are sometimes considered as CoPs for assessing post-infection and post-vaccine protection due to the frequently positive correlation of their titers with the recovery from infection or efficacy of vaccines [181, 182]. However, this interpretation may be misleading. As previously discussed, SARS-CoV-2 infection and disease can be resolved without seroconversion, demonstrating that antibodies are not essential for recovery. Indeed, Th1 lymphocyte responses, cytotoxic lymphocytes, innate immune factors, trained immunity, and local mucosa immunity contribute to protection against SARS-CoV-2 infection and disease [179, 180, 183]. In addition, the antibody repertoire that participates in protective immunity probably includes those capable of mediating Fc-dependent functions as complement activation, phagocytosis, and antibody-dependent cellular cytotoxicity against SARS-CoV-2 [103, 184].

Moreover, convalescent plasma [185, 186] and monoclonal neutralizing antibodies [187, 188] have relatively limited effects on COVID-19 evolution. Finally, neutralizing antibodies comprise a heterogeneous array of antibodies of different isotypes, affinities, kinetics, functionality, and specificities [104, 105, 184, 189, 190] making it unrealistic to assign a single effect to them.

The variants of concern

The emergence of SARS-CoV-2 genetic variants of concern caused both surprise and concern. The surprise accounts for the unanticipated speed that variants of concern have arisen in a virus known for its slow mutation rate [191, 192], estimated from global phylogenies to accumulate approximately two mutations per month [164]. Within 28 days, from December 2020 to January 2021, three major variants of concern have emerged. The concern with the new variants arises from their potential to cause substantial additional mortality by affecting the efficacy of vaccination and disrupting the pandemic control programs [193]. Are these concerns justifiable?

Natural selection usually determines the fate of a newly arising mutation. However, other potential mechanisms such as chance events, host shifts, persistent infection in immunocompromised hosts, or mutations affecting the proofreading function could also drive viral evolution. Mutations confer a competitive advantage for viral replication, transmission, or escape from immunity. Therefore, mutated variants increase in frequency and become dominants [194]. By April 2021, three SARS-CoV-2 variants were considered of concern: B.1.1.7, B.1.351, and P.1. The B.1.1.7 variant (23 mutations with 17 amino acid changes) was first described in the United Kingdom on December 14, 2020; the B.1.351 variant (23 mutations with 17 amino acid changes) was initially reported in South Africa on December 18, 2020; and the P.1 variant (approximately 35 mutations with 17 amino acid changes) was reported in Brazil on January 12, 2021. By February 22, 2021, the B.1.1.7 variant had been reported in 93 countries, the B.1.351 in 45, and the P.1 in 21. All three variants have the N501Y mutation, which changes the amino acid asparagine (N) to tyrosine (Y) at position 501 in the receptor-binding domain (RBD) of the S protein. The B.1.351 and P.1 variants both have two additional RBD mutations, K417N/T and E484K. These mutations increase the binding affinity of RBD to the ACE2 SARS-CoV-2 receptor [195]. The major concerns stemming from the emergence of the new variants are their effects on viral transmissibility, disease severity, reinfection rates (i.e., escape from post-infection immunity), and vaccine effectiveness (i.e., escape from vaccine-induced immunity) [195].

Since its emergence, the B.1.1.7 variant largely replaced the circulating local lineages and became the predominant variant in the UK. This variant presents higher transmission, contributes to increased incidence, hospitalizations, and pressure on the health system [194]. Epidemiological studies and mathematical modeling suggest that it spreads 56% faster than other lineages and results in higher nasopharyngeal viral loads than the wild-type strain [194]. Retrospective observational studies estimate a 35% (12% to 64%) increased risk of death associated with the B.1.1.7 variant, indicating that it is not only more transmissible than pre-existing lineages, but can also cause more severe disease [194, 196]. Plasma samples from vaccinated individuals show a modest reduction in the neutralization capacity toward the B.1.1.7 variant [197]. Sera from individuals vaccinated twice with the Pfizer-BioNTech BNT162b2 vaccine, longitudinally tested up to 6 weeks after vaccination, were able to neutralize the virus comparably to the D614G reference virus [198]. The AstraZeneca/Oxford AZD1222 vaccine showed efficacy against the B.1.1.7 variant, although sera from vaccinated individuals exhibited reduced neutralization activity [199].

One month after its detection, the B.1.351 variant had replaced the circulating viruses in the region and became the dominant variant, suggesting higher transmission rates. Similar to the B.1.1.7 variant, B.1.351 shares the N501Y mutation, located in the RBD of S protein, which confers an increased binding affinity of S RBD for the ACE2 receptor, raising the viral transmission rate. However, in addition to the N501Y mutation, this variant accumulates two additional mutations in the same RBD (K417N and E484K) that could play a pivotal role in the interaction with the receptor leading to immune evasion [194]. Indeed, the B.1.351 variant showed marked resistance to neutralization by convalescent plasma (9.4-fold) and sera from individuals who have received mRNA vaccines (10.3-12.4-fold) [200]. In addition, a clinical trial in South Africa evaluating a two-dose regime of the AstraZeneca/Oxford AZD1222 vaccine did not show protection against

mild-to-moderate COVID-19 due to the B.1.351 variant [201]. However, the Novavax NVX-CoV2373 vaccine exhibited the expected response [202]. In addition, a mass vaccination campaign in Qatar showed that the Pfizer-BioNTech BNT162b2 mRNA vaccine presented approximately a 20% reduction efficacy associated with the B.1.351 variant [203]. No evidence of increased virulence or disease severity related to the B.1.351 variant has been reported [194].

The P.1 variant that was circulating in Manaus, Amazonas State, Brazil, in December 2020, soon became the dominant circulating virus in the country and later spread to 26 countries. It contains a unique constellation of lineage-defining mutations, including E484K, K417T, and N501Y [204], which are associated with increased affinity to ACE2 receptors [205], and resistance to neutralization by monoclonal antibodies [98, 206]. The P.1 variant caused a 2.6-fold increase of SARS-CoV-2 transmission and was considered the most transmissible among the variants of concern by April 2021 [207]. The possibility that CoronaVac protects against the P.1 variant has been suggested [208].

Non-specific vaccines?

The prevailing paradigm in vaccinology assumes that vaccines only protect against the target infection. However, epidemiological vaccine research has generated observations that contradict this assumption and suggest that vaccines have significant nonspecific effects on overall health in populations. These include the observations that several live vaccines reduce the incidence of all-cause mortality in vaccinated compared with unvaccinated populations far more than can be explained by protection against the target infections [209]. It is now established that several live-attenuated vaccines targeting infections like tuberculosis, measles, and polio induce protective innate immunity capable of affecting other pathogens [210]. Innate immune responses, triggered by a family of pattern recognition receptors, induce interferons and other cytokines and activate myeloid and lymphoid immune cells to provide protection against a wide range of pathogens [210]. Live-attenuated vaccines can induce a long-lasting and robust activation of innate immune cells by reprogramming their epigenetic makeup and thus endowing them with the capability to affect heterologous pathogens. Bacillus Calmette-Guérin (BCG) [211-213], measles, mumps, and rubella (MMR) vaccine [214] have been suggested to indirectly protect against SARS-CoV-2 infection by activating the innate immune system. Furthermore, a negative association was found between SARS-CoV-2 infection rates and previous vaccination with polio, *Haemophilus influenzae* type-B (HIB), MMR, varicella, pneumococcal conjugate (PCV13), Geriatric Flu, and hepatitis A/hepatitis B (HepA–HepB) vaccines [215].

Protective immunity as an adaptive strategy

The prevailing paradigm in immunology and vaccinology assumes that ideal protective immunity aims to keep the organism free of the target infectious agent. Hence, an effective immune response acts on some crucial components of the infectious agent to avoid the infection's success. However, this concept is now challenged by the proposal that protective immunity aims to facilitate the permanence of infectious agents in the organism with minimal harm and provide mutual adaptation. That means transforming pathogens into microbiota and, thus, promoting symbiosis ("living together") and coadaptation. Through this state of coadaptation, conditions are created for promoting coevolution [216, 217]. Therefore, the immune system acts to integrate microbes into the animal-cell community [218, 219]. Amazingly, the individual's immune system is in part created by the own microbiota [220, 221].

All living beings, from bacteria [222, 223] to plants [224] and animals [225], not only harbor infectious agents as they depend on them for living and evolution [226]. That means all living beings are holobionts [219]. Microbes of all kinds live around us, on us, within us, and even being a part of our DNA. Human cells are vastly outnumbered by microbial cells, and their symbiosis implies that millions of unique microbial genes work in conjunction with human genes. Humans' first exposure to microorganisms occurs during the fetal period through contact with the maternal microbiota [227]. Environmental viruses, bacteria, archaea, and fungi are selected to form the individual microbiota during and after birth.

The human gut microbiota performs critical roles, including the biosynthesis of vitamins K, biotin, folate; steroid hormones; and neurotransmitters (e.g., it is the major source of serotonin in the organism). It also metabolizes dietary components, short-chain fatty acids, drugs, and carcinogens; influences energy biogenesis, neurologic signaling, bone density, and vascularization; provides the immune system maturation, activation, and regulation [228, 229]; and critically promotes protection against pathogens [219].

Although the microbiome shows a degree of plasticity depending on the diet and host physiology, it exhibits remarkable stability, resilience, and diversity [230, 231]. However, the more intriguing feature is its uniqueness [232, 233]. Each person's microbiota is so unique that its characterization is used as a forensic tool for identifying criminals [234]. That indicates that the selection of the microbiota's components is a genetically restricted process. As the only genetically restricted system in the organism is the immune system, the obvious conclusion is that the selection and maintenance of the microbiota depend on this system.

How does the immune system act permissibly rather than restrictively in the selection of the microbiota components? That involves mutual trade-offs between the microbe, the immune system, and target cells with gains and losses of each active part. These interactions are better understood with the gut microbiota, the largest and best known of the organism. It can be inhabited by some of the over 150,000 different identified species [235], together with a massive number of viruses, archaea, and fungi. These communities are coevolving with the immune cells over millions of years [236]. A central strategy of the immune system to maintain its homeostatic relationship with the microbiota is to minimize the contact between microorganisms and the epithelial cell surface, limit cell invasion and microbial translocation [25]. This segregation is partially achieved in the gastrointestinal tract by the epithelium, the mucus layer, antimicrobial peptides, and secretory IgA.

Segregation from the immune cells also interests the microbiota. To pursue that, microbiota bacteria produce molecules that promote the integrity and function of the intestinal epithelium, inhibit the production of proinflammatory cytokines, and induce the production of anti-inflammatory IL-10 [237, 238]. Furthermore, specific microbiota components promote Foxp3+ regulatory T (Treg) cell accumulation, supporting B cell class-switch and production of IgA [25]. Two kinds of secretory IgA are produced: T cell-independent (innate IgA) and T cell-dependent (adaptive IgA). While innate IgA can exclude microorganisms from the gut, those are not as potent as adaptive IgA in shaping a mutualistic relationship with the microbiota [239]. That happens because IgA molecules can coat microbiota bacteria and help them anchoring to the epithelial surface and, therefore, promoting colonization [240, 241].

On its part, the immune system plays a fundamental role in shaping and preserving the microbiota ecology, and both engage in a 'homeostatic dialogue' to reinforce each other's function [25]. Innate immune cells participate in this dialogue. Microbe-derived sphingolipids recognized by invariant natural killer T (iNKT) cells regulate their proinflammatory ability [242]. The adaptive immunity collaboration to the 'homeostatic dialogue' relies mainly on Treg cells. These cells regulate effector T-cell responses to gut bacteria, secrete anti-inflammatory and pro-tolerogenic cytokines such as IL-10 and transforming growth factor β (TGFβ) [243], and efficiently restrain aberrant type 2 or type 17 responses [244].

The microbiota is kept in the organism by the active and sustained action of the permissive immunity deployed by the immune system. Conversely, restrictive immunity is elicited when outside pathogens check homeostasis, which occurs in eventual circumstances. Over time, if the pathogen's contact continues, it may be incorporated into the microbiota through permissive immunity. It is important to keep in mind the velocity microbes adapt to new environments. Their growth rates usually vary between a few minutes to some hours. Thus, within 24 hours, they can give rise to several new microbe generations, speeding up adaptation.

A corollary of the concept that the primary goal of protective immunity is to eliminate pathogens from the organism is that the immune response is directed toward an antigen that is a crucial component of the pathogen. In SARS-CoV-2, this essential component is the S protein that the virus uses to enter host cells. Therefore, it is assumed that the protection against SARS-CoV-2 relies on neutralizing antibodies to S protein to impede the virus' invasion. Most SARS-CoV-2 vaccines emulate this concept and use S protein as their primary immunogen. Although the short-term results of vaccination are promising and show a

reduction of the severe infection a few weeks after immunization [245], the long-term effects are unknown and may be disappointing. There are robust experimental data about SARS-CoV-2 epitopes, including their mapping [246], identification of those recognized by T cells [247-250], and the immunoglobulin-binding epitopes [249, 251-253], the definition of epitope immunodominance and immunoprevalence [249, 250, 254], demonstration of their conformational dynamics [255], and detection of epitope mutations [251, 256]. Thus, it can be anticipated that the next generation of SARS-CoV-2 vaccines should contain a selection of multiple immunodominant epitopes from different virus structures and encompassing a maximum of known and predictable variants. It can also be anticipated that they will continue to follow the restrictive model of protective immunity. However, there would be very useful if the following vaccine formulations also contain epitopes capable of speeding up the trajectory of SARS-CoV-2 to become a component of the respiratory microbiota, as have already happened to other coronaviruses.

Conclusions

The difficulty of non-immunologists to capture the vast array of current immunological information and concepts and incorporate them into the strategies of controlling the SARS-CoV-2 pandemic represents significant hindrances for establishing realistic and reliable public health policies. Some misconceptions are so deeply rooted that they are hardly perceived, though their deleterious consequences are appreciable. The primary source of difficulty is the old notion of a naïve, static, and closed immune system. This idea assumes that, at its first contact, the infectious agent (or the vaccine) would find an immune system populated by naïve lymphocytes, which have never been stimulated before. The results of this encounter would be activation, proliferation, and differentiation of the cells into short-lived effector lymphocytes and long-lived memory lymphocytes. The latter population would be kept in a quiescent state, waiting for the subsequent encounter with the infectious agent, when it would mediate a more rapid, intense, and efficient immune response. Robust experimental data surpassed this conception. Throughout life, since the fetal period, the immune system is maintained under continuous constraints induced by the antigens from environmental and resident microorganisms, self-antigens, and regulatory molecules from the nervous, endocrine, and immune systems. Therefore, the immune system must be considered an open system in constant communication with the external and internal environments. Such openness is responsible for the system's high dynamicity, whose cells are under continuous activation, differentiation, regulation, counter-regulation, and destruction. This current view of the immune system's organization and functioning, together with the erosion of the functional limits between adaptive and innate immune systems, has dramatically challenged the classical concepts of specificity, susceptibility, immunological memory, and protective immunity.

It is expected that the incorporation of the current concepts of immunology to the control of SARS-CoV-2 infection sheds light on some present misconceptions and contributes to reducing the burden of the COVID-19 pandemic.

Abbreviations

ACE2: angiotensin-converting enzyme-2

ADCC: antibody-dependent cell-mediated cytotoxicity

ADE: antibody-dependent enhancement

ADEn: antibody-dependent endocytosis

CoP: correlate of protection

COVID-19: coronavirus disease-19

IFN-γ: interferon-γ

Ig: immunoglobulin

IL: interleukin

MHC: major histocompatibility complex

mRNA: messenger RNA

NK: natural killer

RBD: receptor-binding domain

S: spike

SARS-CoV-2: severe acute respiratory syndrome coronavirus-2

Th: T helper

Declarations

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Author contributions

As the sole author, I am responsible for the conception and preparation of the manuscript.

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The author declares that there are no conflicts of interest.

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