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Review

The next frontier in vaccine design: blending immune correlates of protection into rational vaccine design

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Despite the extraordinary speed and success in SARS-Cov-2 vaccine development, the emergence of variants of concern perplexed the vaccine development community. Neutralizing antibodies waned antibodies waned and were evaded by viral variants, despite the preservation of protection against severe disease and death across vaccinated populations. Similar to other vaccine design efforts, the lack of mechanistic correlates of immunity against Coronavirus Disease 2019, raised questions related to the need for vaccine redesign and boosting. Hence, our limited understanding of mechanistic correlates of immunity – across pathogens - remains a major obstacle in vaccine development. The identification and incorporation of mechanistic correlates of immunity are key to the accelerated design of highly impactful globally relevant vaccines. Systems-biology tools can be applied strategically to define a complete understanding of mechanistic correlates of immunity. Embedding immunological dissection and target immune profile identification, beyond canonical antibody binding and neutralization, may accelerate the design and success of durable protective vaccines.

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Introduction

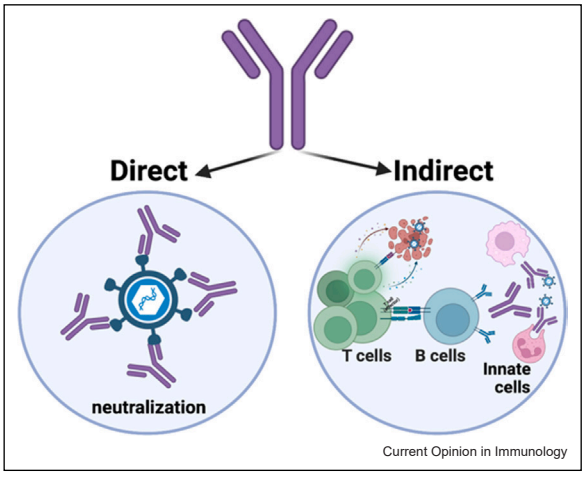
Antibodies represent the primary correlate of immunity for most licensed vaccines [1]. However, antibodies may either directly contribute to antipathogen immunity or simply mark the presence of other mechanisms of immunity (Figure 1). While the measurement of antibody levels has been instrumental for the development of several clinically approved vaccines, emerging data suggest that the measurement of binding or neutralizing antibodies alone may be insufficient for the development of highly effective, durable vaccines for more complicated pathogens.

Vaccine technology has evolved considerably since Edward Jenner demonstrated immunity to a disease (smallpox) after an immunizing event (cowpox inoculation), which eventually led to the eradication of disease [2] (Figure 2). Nearly a century later, Louis Pasteur developed pathogen-specific attenuation techniques that saved the life of a young child with the use of a live-attenuated rabies vaccine [3], paving the way for the identification of pathogens and development of immunization strategies against a range of diseases such as cholera and tetanus using whole-cell killed vaccines and toxoid candidates (Figure 2). The application of Koch's postulates [4], and discovery that attenuation and even split vaccines could drive sufficient immunity to protect populations, led to a novel opportunity to simply identify the pathogen or essential pathogen components and then produce vaccines [5]. Linked to the development of advanced tools to identify pathogens, perform advanced culturing, develop safer inactivation, the identification of vectors, and the explosion in synthetic chemistry accelerated successful vaccine development for many target pathogens over the next 50 years [6] (Figure 2).

However, for many pathogens, such as the Human Immunodeficiency Virus (HIV), malaria, tuberculosis, and so on, traditional attenuation or protein/carbohydrate delivery has shown limited success [7], motivating the field to dig deeper into pathogen-evasive mechanisms, with a focus on pathogen antigen structure. Deeper analysis of the 3-dimensional structure of pathogen antigens, and how they may evade vaccine-induced antibodies, has enabled the development of more sophisticated vaccines, however, even with a keen understanding of the pathogen target, vaccines to influenza [8,9], HIV [10], malaria [11], and so on continuously fail to confer broad immunity arguing that understanding the pathogen is just not enough to develop vaccines against more evasive pathogens (Figure 2).

The Coronavirus Disease 2019 (COVID-19) pandemic revolutionized vaccine development, marking a moment in

Figure 1



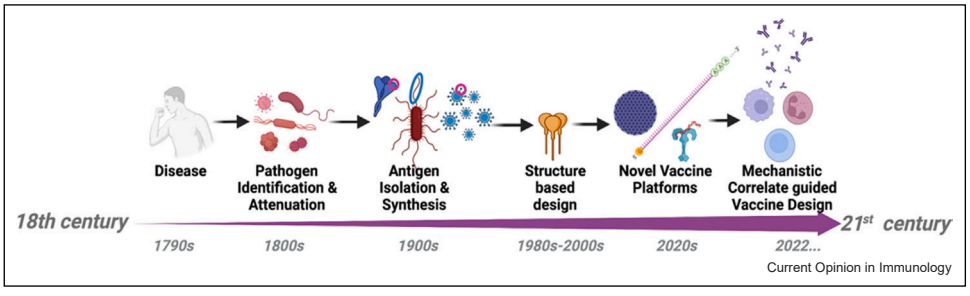
Antibodies as markers of immunity. Vaccine-induced antibodies may both act directly to prevent infection via neutralization or mark the presence of a robust T-cell or functional antibody response that may confer protection against disease through distinct immunological mechanisms.

vaccine history when dozens of new vaccines, platforms, adjuvants, and combinations of vaccines were tested nearly simultaneously for real world efficacy [12]. The remarkable vaccine successes were both a testament to the rapid ability to share antigen sequences [13], the identification of stabilizing mutations [14] based on structure-based antigen design that led to improved antigen immunogenicity, as well as to the availability of novel nucleic acid platforms, novel cost-effective protein production platforms [15], and the use of highly immunogenic adjuvants [15]. Guided by the dogma that neutralizing antibodies are the correlate of immunity against respiratory pathogens, robust protection against COVID-19 was observed in the first wave of phase-III vaccine trials [16]. However, the emergence of variants of concern such as Omicron, which are able to easily subvert the neutralizing antibodies induced against the original viral strain, illustrated our imperfect understanding of correlates of protection (CoPs) in the context of COVID-19. Guided by this belief that neutralizing antibodies were the CoP against COVID-19, vaccine developers rapidly explored the need to redesign vaccines that could provide protection against this evolving pathogen. Instead, the majority of vaccines continued to provide protection against severe disease and death, suggesting that alternate vaccine-induced mechanisms were key to providing protection against COVID-19, and that neutralizing antibodies were a surrogate of protection. However, in the absence of a defined mechanistic correlate of protection against COVID-19, the world continues to chase variants, rather than designing and deploying vaccines with a firm understanding of immunity to SARS-CoV-2.

Importance of correlates of protection in vaccine design

Defining mechanistic correlates of protection (mCoPs) has been contentious since their conceptualization, due to the need to distinguish between surrogates versus mechanisms of immunity [17]. While a mCoP is the specific functional immune mechanism that is believed to confer protection (required opsonophagocytosis for vaccine-induced neutralizing tetanus-toxin antibody-mediated protection), a nonmechanistic correlate of protection may contribute but may not explain mechanistically how the pathogen is controlled or cleared (total IgG antibody levels against pneumococcal vaccination). For example, following *Streptococcus pneumoniae* vaccination, a subset of the total pneumococcal-specific IgG antibodies (CoPs) contains opsonophagocytic antibodies that are mCoPs [18]. However, because total IgG correlates with opsonophagocytic antibodies following vaccination, the mCoP is not directly measured. However, in some instances, mCoPs are not directly associated with surrogates [19] and thus more sophisticated assays are required to guide vaccine development. For example, no simple surrogate of broadly neutralizing antibodies exists for HIV. Thus, sophisticated lentiviral envelope panels have been developed, including dozens of viral strains from across the globe, to evaluate vaccines [20]. However, for many pathogens, such as *Mycobacterium tuberculosis (Mtb)*, the absence of mCoPs has led to the development of both antibody and T-cell-inducing vaccines [21–23] that have

Figure 2



Timeline and evolution of vaccine ingenuity and CoPs. Major landmarks in the history of vaccine design.

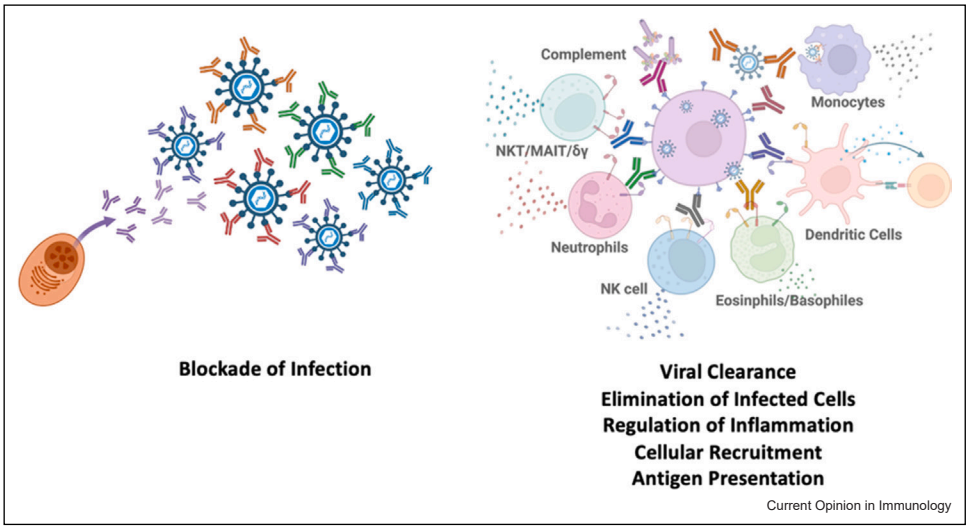
repeatedly failed to confer protection. Thus, in the absence of mCoPs, vaccine development is significantly compromised, lacking a defined endpoint immune target immune profile known to drive antipathogen activity. mCoPs are therefore required to inspire the development of vaccines able to provide longer-lived and broader protection. Thus, while a great deal of effort has been invested over the past 2–3 decades in understanding pathogen-derived antigen structure, vaccinology has taken the immune system for granted, assuming that neutralizing antibodies were sufficient to confer protection. Instead, collectively, the data suggest that understanding both the pathogen and the host response to infection is key to the design of the most effective vaccines.

Protection through more than just antibody blocking

Antibodies contribute to pathogen control and clearance via more than just the simple blockade of infection (Figure 3). For diseases such as anthrax, protection from infection correlates with levels of antitoxin-neutralizing antibodies [24]. However, when neutralizing antibodies were administered to mice lacking Fc receptors critical for mediating immune complex clearance via opsonophagocytosis, protection was lost. These data argue that although neutralization is a strong surrogate of protection against anthrax disease, protective antibodies required the additional capability of clearing the bacterial toxin. Likewise, licensure of the meningococcal serogroup-C conjugate vaccine, new formulations of *Haemophilus influenzae* type b, and *Streptococcus pneumoniae* conjugate vaccines were all based on sero-epidemiological data that established a presumed antibody threshold as a CoP associated with risk reduction of disease [25–27]. However, mechanistic studies highlighted the importance of antibody-mediated pathogen elimination, rather than antibody blockade of infection alone, as a key mechanistic correlate of immunity [28–32]. All three bacteria however have different levels of CoPs among pathogenic serotypes as well as between colonization, localized infections, and invasive disease [33], suggesting that the quantification of antibody titers alone does not fully capture mCoPs of immunity.

For childhood vaccines against well-known viruses, including those included in the MMR (measles, mumps, and rubella) vaccine, historical dogma has focused on the role of neutralizing antibodies as mCoPs. Specifically, microneutralization titers of above 1000 mIU/ml and 10 IU/ml have been defined as protective against measles and rubella, respectively. Conversely, CoPs for the mumps virus, on the other hand, remain largely undefined [24,34]. Antibody titers that can reduce plaques by 50% or a 1:8 dilution in hemagglutination inhibition have been proposed as protective levels against mumps in pediatric cohorts [35]. However, emerging data suggest that additional arms of the immune system may also play a critical role in conferring protection against mumps [36,37]. In fact, following MMR vaccination, vaccine efficacy to mumps wanes rapidly compared with immunity to measles and rubella [38]. Interestingly, the vaccine-induced mumps-specific response is largely directed at the nucleocapsid protein, which is not a neutralizing antibody target, thus, neutralizing antibodies represent a minor fraction of the vaccine response that wanes more rapidly over time, rendering individuals susceptible to infection [38,39]. Yet, in the setting of several recent mumps outbreaks,

Figure 3



More than just antibody binding. Beyond their ability to bind and block viral infection (left), antibodies can leverage the immune response to clear the pathogen by providing specific instructions, including the induction of cytokine secretion, phagocytosis, cytotoxicity, complement activation, antigen presentation, and so on (right).

epidemiologic studies suggest that low neutralizing antibodies are not a definitive marker of susceptibility to infection [39], pointing to the potential importance of other immune arms as mCoPs. Whether nucleocapsid antibodies, cellular immune responses, memory B cells, or other arms of the immune system are needed to confer long-lived protection against mumps remains unclear, but could provide key insights for the redesign of mumps vaccines that may provide equivalent immunity to that induced to measles and rubella. In the setting of recent mumps outbreaks, a third dose of the mumps vaccine clearly reduced viral spread and disease [40], via the augmentation of neutralizing antibodies. However, as these boosted antibody titers will likely wane, two options exist: 1) to continue to intermittently boost vulnerable populations or 2) capitalize on the clear population-level evidence of immunity to mumps in the absence of robust neutralization and define the mCoPs to develop vaccines that may be able to confer immunity globally for life.

The yellow fever vaccine (YFV), arguably the most effective vaccine of the modern era due to its longevity in conferring protection against clinical infection, is thought to confer protection via neutralizing antibodies alone [41]. However, emerging data suggest that CD8 + T-cell responses may also play a critical role in protective immunity due to their robust induction at the time of immunization [41]. However, the presence of both robust neutralizing and cytotoxic T cells following vaccination does not automatically indicate their critical role as mCoPs. Instead, the elimination of either neutralizing antibodies or CD8 + T cells both resulted in compromised intracranial clearance of the vaccine viral strain, through an interferon-γ- (IFN-γ) and perforin-mediated mechanism [42], pointing to the potential critical mechanistic collaboration between neutralizing antibodies and T cells. Mechanistic experiments would suggest that neutralizing antibodies represent the first line of defense aimed at blocking infection. However, if transmission occurs, CD8 + T cells play critical second-line defense as the mechanistic players in controlling and eliminating the virus post transmission [43]. Thus, the power of the YFV vaccines lies in its ability to drive both arms of the immune response, that are vital to protection against this highly lethal virus.

In the setting of malaria vaccines, the dogma that neutralizing antibodies and T cells are critical for protection has been disrupted by new studies that have begun to point to alternative immune mechanisms of protection against parasitic infection. Specifically, while antibodies alone can clearly provide protection against sporozoite infection [44], vaccine trials using the current most advanced malaria vaccine, RTS'S, suggest that antibody-binding titers are an incomplete predictor of immunity [45,46]. While the added induction of T cells, via vectored vaccination, conferred limited additional protection to previous antibody-driving vaccine platforms [47], deeper analysis of antibody-mediated mechanism(s) of action identified the critical role of opsonophagocytic and cytotoxic antibodies as key correlates of immunity against malaria infection in controlled human challenge (CHIM) [48]. Specifically, across 3 different RTS'S CHIM trials [45,49–52], ADCC and phagocytic antibodies were enriched among individuals able to resist sporozoite infection compared with those that became infected, linked to in vitro evidence of Natural killer (NK) cell, monocyte, and neutrophil mechanistic restriction of parasitic infection of human liver cells. Moreover, recent evaluation of a reticulocyte-binding protein homolog 5.1 [RH5.1]/AS01B vaccine, aimed at limiting the blood stage of malaria, has also suggested that vaccine-induced antibodies may prevent erythrocyte invasion by the merozoite via the combined role of IgG-mediated neutrophil phagocytosis [53,54]. These data collectively point to the critical role of distinct antibody-effector mechanisms in the mechanistic control of different stages of the malaria parasite life cycle. Thus, vaccines able to leverage these novel antibody mechanisms may be the ultimate 2-step immunity required for complete protection against malaria.

Yet, beyond antibody interactions with the innate immune system, the interest in the role of the innate immunity following vaccination has exploded [55]. While previously regarded as a nonspecific defense against pathogens, the recent discovery that vaccines may program innate immune cells to respond more rapidly and effectively to infection has inspired a new area of vaccinology. First developed by French scientists Albert Calmette and Camille Guérin, aimed at driving immunity to *Mycobacterium tuberculosis*, the **Bacillus Calmette-Guérin** (BCG) vaccine has recently been shown to epigenetically and metabolically alter myeloid cells to respond more rapidly to non-*Mtb*-specific innate stimuli after exposure to the vaccine [56]. This 'innate training' drove the accumulation of myeloid cells with enhanced antimicrobial activity, permitting these 'trained' cells to produce cytokines more rapidly upon exposure to pathogen-derived antigens for longer periods of time [57], providing a potential explanation for the enhanced heterologous immunity observed to unrelated pathogens in children immunized with BCG at a global level [56].

Thus, collectively, mounting data points to a variety of immunological mechanisms, beyond simple neutralizing and binding antibodies, as key signatures of immunity. Thus, the integration of our emerging more sophisticated understanding of immunity may provide the critical insights to guide next-generation highly efficacious and durable vaccines.

Challenges that have emerged and the need for a paradigm shift

Upon infection, the immune system functions as a coordinated set of networks of cells and proteins, able to provide instructions and arm the host to fight the incoming

pathogen [58]. As such, understanding immunity to a pathogen requires more than just the simple characterization of the pathogen. In fact, depending on the site of pathogen entry (lung, gut, skin, blood, etc.) and pathogen-evasive mechanisms, the key to immunologic protection requires a fine balance between the host and the pathogen. However, most correlates of protection, to date, have been defined based on a single-dimensional model, evaluating the ability of vaccines to elicit antibodies able to bind or block infection. This approach ignores the multitude of immunological mechanisms that collectively respond to infection, working in concert to provide complete protection from disease. The neurotropic viruses such as Japanese encephalitis and tick-borne encephalitis have CoPs that are thought to be antibody-dependent with neutralization titers of 1/10 for Japanese encephalitis and a level of 125 ELISA units for tick-borne encephalitis, respectively. However, the delineation of the immune pathways, both in the blood and in the central nervous system (CNS) sites, is still lagging and the need for understanding the immune basis of CNS trophism and thereby CoPs at the mucosal level and in the CNS.

Moreover, the immune system has evolved a number of compensatory, complementary strategies to enhance the likelihood of resisting infection. Thus, for many pathogens, for which vaccines do not exist, this simple single-dimensional model has been insufficient to inspire the design of protective vaccines. Instead, defining the underlying mechanisms of pathogen clearance may offer a targeted approach to inspire the design of vaccines that will have the greatest likelihood of providing protection.

Along these lines, vaccines against most enteric pathogens do not provide complete protection, and instead offer largely partial efficacy [59–61], attributable to our incomplete understanding of the mechanism(s) by which the immune system controls pathogens within the gastrointestinal tract. Immunological dogma has oversimplified enteric vaccine design, suggesting that high titers of neutralizing IgA antibodies are the key to protective immunity. However, emerging correlates point to a critical role for IgG responses, rather than IgA responses, in protection against Shigella [62]. Likewise, a critical role for complement-fixing antibodies, rather than IgA levels, has also been proposed for protection against invasive nontyphoidal salmonella [63,64]. However, the interplay of the mucosal immune response, mucus biology, and the microbiome likely plays a critical role in shaping the ability of the immune response to discriminate between pathogens and commensals [65]. Innate-like lymphoid cells, epithelial–immune cell interactions, metabolic communication between the host and the microbiome, as well as mucins [66] all appear to play a critical part in determining pathogen colonization and disease. However, vaccine-design approaches have yet to harness the full spectrum of these host–microbiome–immune cell interactions.

Emerging data point to a critical role of the microbiome on shaping vaccine-induced immunity, even in the setting of vaccines that are administered intramuscularly. For example, systems-biology-level analysis pointed to a critical role for microbiome-derived Toll-like receptor (TLR5) signals as critical adjuvants in shaping the intramuscular influenza-specific immune response [67], arguing for an intimate, though distant, interaction between the gut and secondary lymphoid induction of immunity. Moreover, even for orally administered vaccines, beta-microbiome diversity was associated with rotavirus immunogenicity, where specific bacterial taxa were associated with enhanced rotavirus vaccine-response boosters and rotavirus shedding [68,69]. However, the precise opportunities to leverage the interplay of the mucosal immune response, mucus biology, and the microbiome remain unclear.

However, given the heterogeneity of exposure, disease, and difficulty in sampling in relevant immunity in field trials, more recent efforts have focused on the development of human-challenge studies that enable controlled exposure to the pathogen and the dissection of mechanistic correlates of immunity [49,70]. For example, while serum vibriocidal antibody titers of > 1:320 were previously proposed as CoPs against cholera [24], recent CHIM studies point to a critical role of mucosal IgA and memory B cells as mechanistic correlates of immunity against the bacteria [49]. Specific secretory IgA in serum, saliva, or urine can potentially serve as a predictor of the release of specific IgA at intestinal surfaces after intragastric immunization. However, recent data point to a lack of correlation in IgA levels across these compartments to the cholera toxin pointing to a potential difference in IgA coordination in the context of specific antigens, dosing, or vaccine platforms [71]. Thus, the precise relationship between systemic and mucosal IgA remains incompletely understood. Yet, recent deep immunological analysis of samples collected during a *Salmonella enterica* serovar typhi (*S.* typhi) CHIM study revealed that a common Vi-polysaccharide and Vi-conjugate vaccine correlate in human-challenge participants, pointing to Vi IgA, neutrophil phagocytosis, and avidity as key correlates of immunity linked to reduced disease severity, collectively pointing to a critical role for high-avidity IgA recognition and activation of neutrophils as key functional mCoPs in the control and clearance of *S* typhi [61,72••].

Beyond human challenge, recent studies in nonhuman primates have revealed unexpected correlates of immunity following BCG vaccination [73,74]. Specifically, while BCG vaccination is thought to confer limited protection against *Mtb* via the induction of Th1-dependent IFN-γ and Interleukin (IL-17) immunity, disarming the capacity of the bacteria to take up residence in macrophages [75], the recent administration of BCG intravenously (IV-BCG) resulted in nearly sterilizing immunity against *Mtb* in nonhuman primates [76]. While IV-BCG resulted in a large expansion of polyfunctional T cells in the lung [74], this

route of immunization also raised large quantities of antibodies against the bacteria in the lung [75], marked by high levels of highly avid IgM responses that were able to limit bacterial growth in macrophages in vitro pointing to the importance of the route of immunization as a mechanism to tune the quality and location of the immune response. Given our emerging appreciation for the potent complement fixing and antimicrobial activity of affinity-matured IgM response [77••], these controlled animal-challenge data, using alternate routes of immunization, offer new insights into an unexpected and novel immunologic axis for protection against *Mtb*. Additionally, intravenous administration of attenuated sporozoites, as a vaccine, also led to 40% protection against sporozoite challenge in CHIM studies, and a > 1000-fold decrease in parasite burden was observed in the liver of mice following IV vaccination, further suggesting that the route of immunization may point to the unexpected correlate of immunity and reflect a new approach to tune the quality of the immune response across pathogens and across organ system [54,78,79].

In the absence of human-challenge studies, controlled animal studies provide an opportunity to define mCoPs. Moreover, in settings where animal models also do not exist, deep immunological profiling in epidemiologic studies has provided critical mechanistic insights on mCoPs [59,60]. These examples highlight the need to explore correlates of immunity beyond the canonical mechanisms that have been proposed in the past. Thus, the application of new immunological tools, that probe more than just the specificity and magnitude of the adaptive immune response, coupled to controlled exposure studies, offers a unique opportunity to define mCoPs to guide vaccine design. With the explosion of new immune profiling tools, linked to controlled protection studies, the opportunities to guide vaccine design with mechanism are at our fingertips. The COVID-19 pandemic has illustrated the urgent need for enhanced experimental medicine trials aimed at defining both the immunogenicity profiles of novel vaccine platforms/adjuvants in humans, but also to rapidly define mechanistic correlates of immunity and to rapidly and iteratively improve vaccine regimens and durability across populations (children, the elderly, and immunocompromised populations) [80–82]. Moreover, the linkage of these types of studies with sophisticated nonhuman primate studies provides a means to mechanistically and systematically define unexpected correlates of immunity and guide vaccine development, as has been shown recently in the setting of BCG vaccination [66,67] that has begun to show promise in providing protection against Mtb- sustained infection [67]. Specifically, while BCG vaccination is thought to confer limited protection against TB disease progression, recent revaccination studies have shown that BCG is able to induce Th1-dependent IFN-γ and IL-17 immunity that may be key to disarming the capacity of the bacteria to take up residence in macrophages in adolescents.

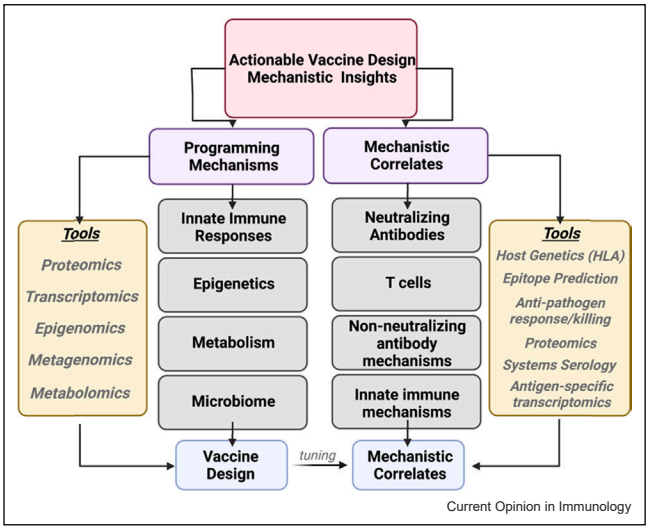
Using immunology to guide vaccine design

Immune profiling tools have exploded, enabling the analysis of host genomics, single-cell transcriptomics, proteomics, metabolomics, cell phenotyping, and so on. However, it was not until the COVID-19 pandemic that we have seen them all applied simultaneously to understand correlates of immunity and the impact of these tools in informing vaccine immunity. Specifically, the use of proteomics and transcriptomics predicted disease severity, just days after infection [83,84], but also helped explain differences in vaccine immunogenicity [83,85]. Transcriptomics also revealed the unexpected role of mRNA vaccines in driving nonspecific epigenetic remodeling of innate immune cells, pointing to the power of mRNA in innate immune training [84]. Antibody profiling tools provided key insights into targets of neutralizing antibodies [86], mechanisms of viral escape of immunity [86], and additional antibody functions of potential importance in the control of disease [87]. Yet, despite this flood of information that has allowed us to understand vaccine-induced immunity across populations, with relation to age, sex, and comorbidities, defining the actionable insights for vaccine design is of utmost importance. Exploiting these tools to comprehensively understand innate, adaptive, and tissue-resident immunity induced by individual vectors, mRNA platforms, adjuvants, interval of immunization, routes of immunization, and so on, may provide the critical means to rationally designed next- generation vaccines.

Beyond canonical mechanisms of protection, emerging data point to a critical role for complement in driving immunity across pathogen type. The immune basis of protection against many pathogens relies on complement to enhance antibody-mediated neutralization, phagocytosis, and lysis. Many viruses (including dengue virus, West Nile virus, and Nipah virus) have evolved mechanisms for evasion or dysregulation of the complement system to enhance viral infectivity and even exacerbate disease symptoms [88]. The complement system has multifaceted roles in both driving rapid and direct innate immune activation to drive viral and bacterial clearance, but also plays a critical role in arming and activating the adaptive immune system, via both intracellular and extracellular mechanisms [89,90]. Moreover, given our emerging appreciation for the importance of adjuvants in shaping the ability of antibodies to leverage complement [89,90], opportunities have begun to emerge to selectively tune the critical role of this ancient and potent arm of the antipathogen immune response.

Moreover, our rapidly growing appreciation for the immunomodulatory role of adjuvants has led to an explosion of novel molecules that shape the quality of the cell T-helper and antibody functional response. Specifically, the use of an array of adjuvants in HIV gp140 immunization [91,92] SARS-CoV-2 receptor-binding domain [93] resulted in the generation of qualitatively

Figure 4



Strategic use of OMIC technologies to guide vaccine design. OMIC technologies can both help us understand the signals that program effective immunity and provide resolution on the functional immune mechanisms involved in protection against the pathogen. Learning to classify these tools provides strategic insights on how they may be used to rapidly and effectively develop best-in-class vaccines.

distinct T-cell cytokine profiles and antibody-effector functions, pointing to the important role of diverse adjuvants that each trigger innate immunity in distinct manners, in tuning immunity in a manner that may lead to improved pathogen control [91,92,94]. In a similar vein, emerging data suggest that distinct vectors also have the capacity to shape the immune response, by triggering distinct pattern-recognition receptors [93]. For example, distinct immune responses were observed following AstraZeneca (ChAdOx1-S), Sputnik (rAd26 and rAd5), and Johnson & Johnson (Ad26. COV2) vaccines [81,82,95,96], highlighting the added opportunity to leverage distinct vectors to drive optimal immunity to elicit protective immune responses.

Yet, despite this explosion of multi-OMIC platforms that provide an unprecedented depth of information related to the host immune response to vaccination and/or infection, understanding how to use these tools in an 'actionable' manner is key to accelerating vaccine design. Some tools provide deep insights on the mechanism(s) by which vaccines program immunity, explaining differences in vaccine platforms, adjuvants, dosing effects, impact of interval of immunization, or even host variation (Body Mass Index (BMI), age, etc.) in vaccine response. For instance, adjuvants may play a role in enhancing cellular immune mechanisms in the development of more effective influenza vaccines for older adults. Conversely, other OMIC tools provide information on the effector mechanisms that contribute directly to pathogen control/clearance [97]. Binning OMICs/immunology tools into these clear categories provides a first level of discrimination for 'actionable' tool application (Figure 4). Thus, as we move forward into a new era of vaccine development that takes both the complexity of the pathogen and host response into consideration, the integrated use of deep immunological profiling, coupled to machine learning and artificial intelligence, offers a new path to development of effective vaccines. Designing both for the target (antigen design) as well as ensuring the induction of highly efficacious mechanistic immunity (mCoPs) will revolutionize vaccine development and design. Moreover, because mCoPs may vary with age, organ system, comorbidity, and so on, understanding shifting mCoPs across populations will further aid in even the potential customized design of vaccines for all populations.

Conclusions

The cost of vaccine failure far outweighs the cost of mechanistic studies to define mCoPs. While vaccine development has entered a new era, with fast flexible platforms coupled to antigen design, understanding the nature of protective immunity represents the final frontier for the development of highly effective vaccines. For some diseases, particular emerging pathogens or those for which there are no animal models, will remain a challenge for the development of mCoPs. However, as we define mCoPs against several respiratory, enteric, encephalitic, and so on pathogens, rules of immune engagement will become clear, and can be used to guide de novo 'blind' vaccine design against newly emerging pathogens based on infectious route and life cycle. Learning how different platforms and adjuvants tune the immune system, then will allow for the rapid

development of highly effective vaccines to pathogens and beyond in the future.

Conflict of interest statement

The authors declare the following financial interests/personal relationships that may be considered as potential competing interests: GA is a cofounder and/or equity holder in SeromYx Systems Inc. and Leyden Labs. GA receives research funding from Pfizer, Sanofi, BioNtech, BMS, Gilead, Medicago, and GSK. GA's interests were reviewed and are managed by Massachusetts General Hospital and Partners HealthCare in accordance with their conflict-of-interest policies. CB has no conflicts to disclose.

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