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REVIEW

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The contributions of T cell-mediated immunity to protection from vaccine-preventable diseases: A primer

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ABSTRACT

In the face of the ever-present burden of emerging and reemerging infectious diseases, there is a growing need to comprehensively assess individual- and population-level immunity to vaccine-preventable diseases (VPDs). Many of these efforts, however, focus exclusively on antibody-mediated immunity, ignoring the role of T cells. Aimed at clinicians, public health practioners, and others who play central roles in human vaccine research but do not have formal training in immunology, we review how vaccines against infectious diseases elicit T cell responses, what types of vaccines elicit T cell responses, and how T cell responses are measured. We then use examples to demonstrate six ways that T cells contribute to protection from VPD, including directly mediating protection, enabling antibody responses, reducing disease severity, increasing cross-reactivity, improving durability, and protecting special populations. We conclude with a discussion of challenges and solutions to more widespread consideration of T cell responses in clinical vaccinology.

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Introduction

Vaccines are among the most impactful public health interventions in history and are projected to save 51.5 million lives from 2021 to 2030.[1] There remains, however, a substantial global burden of communicable diseases, as evidenced by the COVID-19 pandemic and the recent increase in measles cases in settings that had previously achieved elimination. Together, both emerging and reemerging infectious diseases continue to pose threats to global public health.[2–4]

There are many reasons to develop a thorough understanding of immunity to vaccine-preventable diseases (VPD). An individual's level of immunity is indicative of their susceptibility to infection and severe disease.[5] On a population level, this knowledge facilitates implementation of effective routine vaccination programs, in terms of how vaccines are used (i.e., number and timing of doses) and resource allocation.[6] Having a baseline understanding of population immunity is a critical tool for public health as it enables VPD outbreak management as well as pandemic preparedness.[7] For special populations, such as individuals who are immunocompromised, pregnant, or older, in-depth characterization of immunity can guide (re-) vaccination strategies and post-exposure prophylaxis.[8] Finally, understanding the immune response induced by current vaccines is critical to guiding the development of next-generation vaccines.[9]

Despite the importance of developing a thorough understanding of both individual- and population-level immunity to VPD, many vaccine-related research and public health efforts focus exclusively on antibody-mediated immunity.[10] While antibodies are the central mediators of infection prevention and are relatively easy to measure through rapid high-throughput assays, they alone do not provide a complete picture of the immune response to infection and vaccination.[5] The immune system is composed of many cell types, including lymphoid cells (e.g., B cells, T cells, and NK cells) and myeloid cells (e.g., dendritic cells, macrophages, and neutrophils). Each cell type has distinct functions in preventing or resolving infections. Here, we focus on T cells, which both promote antibody responses and play several key roles in the protection against VPD, yet are often ignored in the consideration of vaccine-induced immunity. Aimed at clinicians, public health practioners, and other colleagues who are central to effective human vaccine research but may not have formal training in immunology, we provide a literature review of how vaccines induce T cell responses, and use classic examples to highlight how T cells contribute to protection from VPD. We also discuss challenges and opportunities for more wide-spread incorporation of the assessment of T cell-mediated immunity in vaccinology.

Understanding Tcell-mediated immunity in the context of vaccination

Both CD4 T helper (T_H) cells and CD8 cytotoxic T cells can be elicited in response to vaccination.[11] CD4 T_H responses are generated when an antigen-presenting cell, such as a dendritic cell, takes up antigen at the site of vaccination and migrates to

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a lymph node, where it presents antigen to the CD4 T cell in the context of MHC class II molecules.[12] Following activation, CD4 T_H cells differentiate into various subsets with effector functions that depend on the type of pathogen that was encountered.[13] For example, extracellular bacterial infections induce differentiation into T_H17 CD4 T cells, which recruit neutrophils and macrophages to the site of infection, thus promoting phagocytic killing of bacteria.[14] In contrast, intracellular pathogens, such as viruses, induce differentiation into T_H1 CD4 T cells, which are characterized by the expression of interferon gamma (IFNγ) and promote the CD8 T cell responses that are needed to resolve intracellular infections.[15] A subset of CD4 T cells also differentiate into T follicular helper (T_{FH}) cells, which are required for the activation of B cells that have encountered their cognate antigen in a secondary lymphoid organ.[16] In contrast to CD4 cells, CD8 cytotoxic T cells are primarily activated by intracellular antigens found in the cytosol of nucleated cells. Antigens are processed into short peptides and presented to the CD8 T cell by MHC class I molecules.[17] Activation of CD8 T cells requires help from CD4 T cells.[18–20] Importantly, CD8 T cells can also be activated through cross-presentation, whereby extracellular antigens are processed and presented to CD8 T cells in the context of MHC class I molecules.[21] Once activated, CD8 T cells acquire cytotoxicity and migrate to the site of infection, where they induce apoptosis of infected cells, and secrete pro-inflammatory cytokines, such as IFNγ and tumor necrosis factor (TNF), to help resolve the infection.[22]

Following the primary immune response, generation of a memory T cell response is crucial for the success of vaccination. Once the initial stimulus is eliminated, most T cells undergo apoptosis, but a subset survives as long-lived memory cells.[23] In both the CD4 and CD8 compartments, memory T cells can be sub-divided based on their trafficking patterns: central memory cells circulate through secondary lymphoid organs, effector memory cells circulate in the blood and can migrate to the site of infection upon reactivation, and tissue-resident memory cells (T_{RM}) reside in the nonlymphoid tissues without recirculating.[24] Regardless of the subset, when memory cells encounter their cognate antigen, they can respond rapidly, without the need for further differentiation.[12,25] Key attributes of functional memory T cells include their ability to proliferate, induce the activation of other cells like macrophages and B cells, recruit other cells through the secretion of chemoattractants, and kill infected cells.[11] In a vaccine setting, factors such as the amount of antigen, the site of delivery, and how the antigen is presented to the immune system can control the type of response, as well as the quality and longevity of the memory T cell response. Accordingly, the type and location of the desired immune response must be taken into account in vaccine design and formulation.[11]

Which types of vaccines induce protective T cell-mediated immune responses?

While all vaccines, except polysaccharide vaccines (discussed below), elicit some degree of a CD4 T_H cell response, the manner in which antigen is presented to the immune system determines the type of CD4 T cell response and the vaccine's ability to induce a CD8 T cell response.[26] Live vaccines are robust inducers of T cell responses because they closely mimic natural infection.[23] In fact, live vaccines such as the smallpox and yellow fever vaccines are so efficient at eliciting T cells responses that they have been used as models to better understand the biology of T cell memory in humans.[23,27] Because live attenuated vaccines result in productive infections characterized by transient viral replication, antigen is presented to the immune system in the same way as in a natural infection – namely, intracellular antigen is presented to T cells, thus inducing both a robust CD8 T cell response[28,29] and a balanced T_H1/T_H2 CD4 T cell response.[30,31] Importantly, the replicating nature of live attenuated vaccines results in a high dose of antigen being presented to the immune system for a prolonged period, thus allowing for thorough activation of the innate immune responses that are critical for the induction of sustained T cell responses.[27]

In contrast to live attenuated vaccines, inactivated and subunit vaccines are less potent activators of T cell responses.[26] A clear example of this is the comparison between inactivated and live attenuated seasonal influenza vaccines. In both children[32] and in a human tonsil organoid model,[33] the live attenuated vaccine induces a T cell response of greater magnitude and quality than the inactivated vaccine. Compared to live attenuated vaccines, inactivated vaccines are poor inducers of cellular immunity because they result in lower availability of antigen over a shorter period of time and the vaccine cannot infect cells, so little or no antigen is available in the cytosol to be processed and presented through the classical MHC-I pathway.[34] For both inactivated and subunit vaccines, failure to induce T cell responses has been addressed in some cases by increasing the number of doses administered or through the use of adjuvants.[35,36] Importantly, certain adjuvants (e.g., ASO1) engage T cell responses by promoting cross-presentation, such that extracellular antigens can be presented through MHC-I to activate CD8 T cells.[26,37]

For newer vaccine technologies, such as the COVID-19 mRNA and viral vector vaccines, the ability to generate a robust cell-mediated immune response is largely dependent on the antigen delivery system (i.e., the lipid nanoparticle or viral vector), which allows the antigen to gain access to the cytosol, mimicking a natural infection.[38] For the mRNA vaccines, once the mRNA is released into the cytosol, host machinery is used to translate the mRNA into antigen. This endogenous antigen can be processed and presented in the MHC-I pathway, eliciting a CD8 T cell response, or exogenously expressed to be taken up by professional antigen presenting cells and presented via the MHC-II pathway to induce a CD4 T cells response.[39–41] Similarly, the adenovirus-vectored COVID-19 vaccines deliver viral DNA intracellularly, resulting in the endogenous production of spike protein.[42] Unlike mRNA vaccines, however, the recombinant viral genome delivered by adenoviral vectored COVID-19 vaccines must enter the host cell nucleus and undergo various cellular processes to be expressed, and some of the viral vector formulations lack mutations to stabilize the resulting spike protein.[38] Although viral vector vaccines share some characteristics with live attenuated vaccines, to date, all of the licensed

formulations are replication-deficient, meaning they do not cause a productive infection. More work is needed to understand the implications of antigen processing on T cell immunogenicity for these newer vaccine technologies.[43]

How are T cell-mediated immune responses measured?

Vaccine-induced T cell responses can be measured in whole blood assays or by isolating peripheral blood mononuclear cells (PBMC) from whole blood.[44] While whole blood samples usually must be used within a few hours of sample collection, isolated PBMC can be cryopreserved and biobanked for later use.[45] In both cases, samples are typically stimulated *in vitro* with the antigen of interest to activate the population of antigen-specific T cells.[44] Once stimulated, the responding T cells can be characterized via immunophenotyping (i.e., using flow cytometry to determine the expression of various markers on the cell surface), or by measuring functional read-outs, including proliferation, cytokine production, or cytotoxic potential.[46] The most commonly used functional read-out in vaccine development and evaluation is the IFNγ ELISpot, which quantifies the number of IFNγ-producing (T_H1) T cells in response to *ex vivo* antigen stimulation.[47] First described in the early 1990's,[48] IFNγ ELISpots are high-throughput, sensitive, and reproducible, making them attractive for large-scale clinical research.[47] IFNγ ELISpots, however, measure a single parameter and do not assess the quality or functional potential of the response.[11] Driven by the increasing availability of high-parameter flow cytometry and efforts to develop vaccines against HIV, malaria, and tuberculosis, there is now a growing appreciation of the critical importance of assessing the quality of the T cell response, in addition to enumerating antigen-specific T cells.[11] In this regard, poly-functional T cells, meaning T cells that secrete multiple cytokines (typically IFNγ, interleukin 2 (IL-2), and/or TNF), can be enumerated via intracellular cytokine staining and have been shown to have the greatest functional capacity in terms of providing co-stimulation, degranulation, and cytolytic activity.[49,50] Alternatively, the COVID-19 pandemic renewed interest in flow cytometry assays that measure the expression of activation-induced markers (AIM) to broadly quantify T cells responding to restimulation without focusing on specific cytokines.[51]

How do T cell responses contribute to protection from vaccine-preventable diseases?

T cells can play both leading and supporting roles in protecting individuals from VPDs. In the sections below, we use select well-characterized examples to highlight six key roles of vaccine-induced T cell responses (Figure 1).

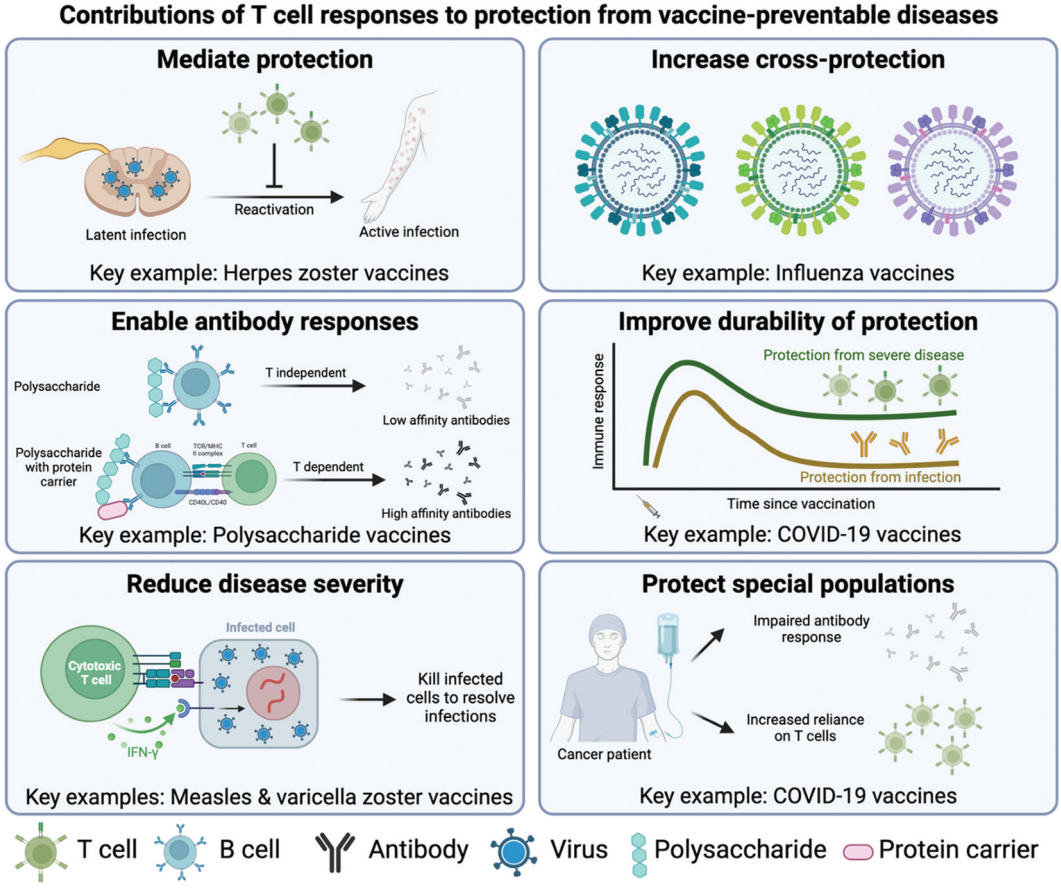

Figure 1. Contributions of T cell responses to protection from vaccine-preventable diseases. T cells play both central and supporting roles in protection from vaccine-preventable diseases. We show a graphical representation of six of these roles, along with key examples for each. Figure created with Biorender.com.

T cells mediate vaccine-induced protection

There are certain vaccines, such as the herpes zoster (HZ) vaccines, for which the T cell response is the primary mediator of protection. HZ is caused by the reactivation of a latent primary varicella zoster virus (VZV) infection, which can lead to debilitating postherpetic neuralgia (PHN) and other neurological complications.[52] Unlike most other vaccines, which aim to prevent primary infection, HZ vaccines must prevent viral reactivation in a latently infected host.[52] Based on epidemiological studies and clinical observations in older and immunocompromised populations, it has long been thought that T cell-mediated immunity confers protection against VZV reactivation.[53] Direct evidence supporting these observations came from a large, prospective observational cohort in Japan, which found that cell-mediated immunity was inversely correlated with the incidence of HZ, the severity of HZ, and the incidence of PHN.[54–56] In contrast, antibody titers were not significantly associated with any clinical outcomes.[55,56] Further reinforcing the role of T cells in protection from HZ is the remarkable and persistent efficacy of the adjuvanted glycoprotein subunit vaccine,[57,58] which has been attributed to a T_H1-mediated response.[59,60] Together, there is clear evidence that T cells are the primary mediators of protection for HZ.

The switch from whole-cell pertussis (wP) to acellular pertussis (aP) vaccines provides an example of how antigen composition can dictate the type of T_H response, resulting in a meaningful impact on vaccine effectiveness. wP vaccines were introduced in the mid-twentieth century and significantly reduced the incidence of pertussis and its associated mortality.[61] By the 1970s and 1980s, the high rates of local reactions, fever, and febrile seizures associated with certain wP vaccine formulations eroded public trust in these vaccines in many high-income countries.[61] For this reason, and other programmatic considerations, many high-income countries replaced the wP vaccines with less reactogenic aP vaccines, which contain various combinations of up to five pertussis antigens rather than the whole killed organism.[61] The uptick in pertussis cases in many regions in the 2010's, however, led many to investigate the comparative immunogenicity of the two different pertussis vaccine types.[62] Initial reports showed that the aP vaccines had similar or superior antibody responses and short-term efficacy compared to the wP vaccines,[63–65] but many studies have since demonstrated that a key distinction between the two vaccines lies in the type of T cell response induced. While the wP vaccines induce a T_H1/T_H17 polarized T cell response, the aP vaccines induce a T_H2 response.[66–68] This polarization is dependent on the type of vaccine received in infancy, which results in imprinting of immune memory and thus has long-term implications, regardless of the number and type of booster vaccines received.[69,70] Importantly, evidence from animal models suggests that IL-17$^+$ and IFNγ^+ T_{RM} cells (i.e., a T_H1/T_H17 response) recruit neutrophils, which have bactericidal activity, to the nasopharynx and lungs, thus preventing *Bordetella pertussis* colonization and transmission.[66,71–73] Collectively, this evidence provides a plausible hypothesis linking the type of T cell response induced by aP vaccines to waning vaccine immunity and the increase in the number of pertussis cases.[74] In the context of the changing epidemiology of pertussis and the search for improved vaccine candidates, it is therefore now evident that cell-mediated immunity must be considered in all immunological investigations as a potential correlate of protection.[62]

T cells enable antibody responses to vaccination

For other vaccines, although T cells are not the central mediators of protection, they are crucial to enabling protective antibody responses. A classic example is the substantially improved efficacy of infant glycoprotein conjugate vaccines relative to polysaccharide vaccines for encapsulated bacteria, such as *Haemophilus influenzae type b* (Hib), *Neisseria meningitidis*, and *Streptococcus pneumoniae*.[75] These encapsulated bacteria are a major cause of meningitis and pneumonia in infants, resulting in significant morbidity and mortality, particularly in low-resource settings.[76,77] Vaccines targeting the polysaccharide capsules of these bacteria were first licensed in the 1970s and 1980s but did not elicit a durable antibody response and were not efficacious in children under 18 months of age.[75] The lack of efficacy in infants is attributed to the Tcell-independent nature of the immune response generated by polysaccharide vaccines.[75,78] Because of their repetitive structure, polysaccharides can directly activate B cells to differentiate into plasma cells that produce antibody, primarily of the IgM isotype.[79,80] In the absence of help from T cells, few IgG$^+$ memory B cells are produced, the plasma cells are short-lived, and the resulting antibodies are of lower avidity.[81,82] Importantly, these defects are most pronounced in infants, for whom B cells are incapable of forming a productive response without the help of T cells.[83,84] The failure of polysaccharide vaccines to protect the most vulnerable populations led to the development of protein-conjugate vaccines, where the polysaccharide antigen is conjugated to a protein carrier, such as diphtheria or tetanus toxoid.[75] The presence of the protein carrier engages T cells, thus generating a T-dependent response to vaccination.[85,86] With T_H cells providing the necessary co-stimulation, germinal centers are formed, resulting in the production of long-lived plasma cells that produce class-switched high-affinity antibodies and memory B cells.[85,86] The impact of this T cell help is clear, particularly in infants, for whom vaccine immunogenicity and efficacy were drastically improved by the switch from polysaccharide vaccines to protein-conjugate vaccines.[87–89]

T cells reduce the severity of disease

A key function of T cells is their ability to kill infected cells and contribute to clearing established infections. There are therefore many VPD for which T cells are not directly involved in the prevention of infection but play a pivotal role in reducing the severity and duration of disease. Much of what is known about this function of T cells is derived from animal models and studies of various immunocompromised human populations. For example, in a nonhuman primate model of primary VZV infection where different lymphocyte subsets were depleted using monoclonal antibodies to investigate their roles in clearing an established infection, loss of B cells had no impact on the severity of disease, while loss of CD8 T cells

resulted in increased viral loads and a longer duration of infection.[90] Depletion of CD4 T cells had the greatest impact on disease severity, leading to a significantly elevated viral load, longer infection, disseminated disease, and impaired antibody and CD8 cell responses.[90] Accordingly, in humans, several case studies have reported severe VZV disease courses in children with active HIV who have low CD4 counts.[91–93] In contrast, children with low or absent immunoglobulin (i.e., hypogammaglobulinemia or agammaglobulinemia) have normal VZV disease courses.[94,95] Similarly, the importance of T cell immunity in promoting clearance of measles infections has been shown in several nonhuman primate models[96–98] and in children with HIV, who have prolonged measles virus shedding.[99] These findings are in line with the ability of measles virus to spread within a host through direct cell-to-cell contact, thus evading neutralizing antibodies and requiring CD8 T cells to effectively clear infection.[100] In summary, for measles, primary VZV, and other viral infections,[101–103] T cells are critical for promoting viral clearance and resolution of infection.

T cells increase cross-protection

For pathogens with large antigenic diversity, T cells contribute to protection by expanding the breadth of vaccine-induced immunity. For example, current inactivated influenza vaccines rely primarily on neutralizing antibody responses to the highly variable hemagglutinin protein. These neutralizing antibodies cannot effectively recognize mutated versions of the surface glycoproteins, however, necessitating updated seasonal influenza vaccines each year.[104] In contrast, T cell responses are cross-reactive, meaning that the response induced by one viral strain may be effective against others – making influenza vaccines that elicit robust cross-protective T cell responses a 'holy grail' for next generation vaccine development.[105] CD8 T cells are cross-reactive because they are elicited by the more conserved internal proteins of the influenza virus, rather than the highly variable external proteins that elicit antibody responses.[106–109] In addition to the more conserved nature of the internal proteins, cross-reactivity is conferred by the mechanism through which T cell receptors bind peptides presented by MHC molecules, whereby only a small number of peptide residues directly interact with the T cell receptor, allowing for amino acid variability in regions that are not in direct contact with the T cell receptor.[110] This has been shown specifically for the influenza nucleoprotein, where even when mutations do occur, variants tend to adopt similar conformations, allowing them to be cross-recognized by the T cell receptor.[111,112] The public health benefit of cross-reactive CD8 T cells against variant influenza viruses was elegantly demonstrated in a prospective cohort study in the UK during sequential waves of the 2009 H1N1 pandemic.[104] In this cohort, individuals lacking detectable antibodies against the pandemic H1N1 virus at baseline demonstrated preexisting cross-reactive T cells induced by previous seasonal influenza viruses. Moreover, the frequency of these cross-reactive T cells was inversely associated with the severity of influenza illness.[104] In sum, unlike neutralizing antibodies, T cell responses to influenza are central to eliciting broader vaccine-induced immunity and are thus an important component of efforts to develop a universal influenza vaccine.

Although less-well characterized, a similar role for Tcell-mediated cross-reactivity has been hypothesized for human papillomavirus (HPV), where available vaccines are effective against viral strains that are not contained in the vaccines.[113–116] Interestingly, post-hoc analyses suggest that the AS04-adjuvanted bivalent HPV vaccine may be more effectively cross-protective than the quadrivalent HPV vaccine which contains an aluminum salt adjuvant.[117,118] The differential cross-protection induced by the two vaccines is hypothesized to relate to the effects of their adjuvants on the T cell responses.[119] AS04 is a toll-like receptor 4 agonist that has been shown to elicit a robust T_H1-biased response, while aluminum salt induces a T_H2-biased response.[120] Although more mechanistic research is needed, it is plausible that the increased cross-protection conferred by the AS04-adjuvanted vaccine is mediated by the T_H1-skewed CD4 T cell response. Because the bivalent vaccine is commonly used in low- and middle-income countries, this T cell-dependent cross-reactivity has major public health implications by contributing to global efforts to eliminate cervical cancer caused by strains of HPV not contained in the vaccines.[116]

T cells improve the durability of vaccine-induced protection

While certain vaccines induce life-long immunity, the efficacy of others wanes over time. It has been reported for several vaccines that long-term humoral and cellular responses are uncoupled, such that T cell responses may continue to provide some degree of protection even in the absence of neutralizing antibodies.[121] The epidemiology of COVID-19 following the wide-spread introduction of vaccines serves as a recent example of this role of T cells. It is now well-documented that mRNA vaccine-induced protection against infection wanes in the months following immunization, but that protection against hospitalization and death is more persistent.[122,123] One hypothesis for this is the relatively rapid waning of vaccine-induced neutralizing antibodies, which prevent infection, compared to the more stable T cell responses, which reduce the severity of established infections.[43, 124–126] An additional consideration is that the mRNA vaccines induce strong systemic immunity but fail to induce persistent immunity at mucosal sites.[127] Once this mucosal immunity wanes, the vaccine no longer prevents infection; however, the circulating T cell recall response can contribute to more rapid elimination of the infection. These trends have led experts to hypothesize that memory T cells play a crucial role in mediating the durable impacts of the COVID-19 mRNA vaccines on morbidity and mortality.[128]

T cells protect special populations

In addition to the more general functions of T cells described above, vaccine-induced T cell responses can play a pivotal role in protecting special populations from VPD. Evidence for increased dependence on T cell-mediated immunity in the context of immunosuppression comes from an in-depth

study of multiple cohorts of hematologic cancer patients hospitalized for COVID-19.[129] These patients had higher mortality and lower anti-SARS-CoV-2 IgG and IgM levels than patients without cancer. Those with the lowest SARS-CoV-2 specific T cells, however, had the highest disease severity and mortality, regardless of IgG levels or the B cell response. In contrast, patients with more robust T cell responses had less severe disease and lower mortality. Further, patients treated with an anti-CD20 monoclonal antibody that depletes circulating B cells were not at increased risk of severe disease or death, despite significantly reduced IgG and IgM responses.[129] Through comparisons with patients with solid cancers and healthy controls, the authors conclude that in the context of impaired humoral immunity, patients with hematological malignancy were more dependent on CD8 T cell responses to reduce disease severity and improve survival.[129]

Another example of the compensatory role of T cell-mediated immunity in special populations is measles vaccination in infants. Measles remains an important cause of morbidity and mortality in young children in low-resource settings and is a growing concern in high-resource settings that have previously eliminated measles.[130–134] Despite a substantial burden of infection in the first year of life, the first dose of measles-containing vaccine is typically given at 9–12 months of age of due to the presence of passive maternal antibodies inhibiting a robust humoral response to vaccination by neutralizing the live attenuated vaccine. In addition, there are concerns about the ability of the immature immune system to respond to the vaccine.[135] This creates a gap in immunity, whereby infants are highly susceptible to measles in outbreak or endemic settings.[136] There is evidence to suggest, however, that vaccination at ≤6 months of age rapidly induces robust and durable T cell responses that are not impacted by passive antibodies[137,138] and these T cells effectively reduce measles-related infection, hospitalization, and mortality.[139,140] Partly due to these robust T cell responses, the WHO recommends a supplementary dose of a measles-containing vaccine delivered to infants beginning at six months in certain high-risk situations, such as during a measles outbreak or for HIV-infected or exposed infants.[141,142]

Discussion

The emergence and reemergence of infectious diseases in the 21st century highlights the ever-present need for continued surveillance of population-level immunity, evaluation of existing vaccine programs, and development of new vaccines candidates. As evidenced by the examples described above, there are clear benefits to considering T cell-mediated immunity in these efforts, yet challenges remain to comprehensively measuring human T cell responses on a large scale. We thus conclude with a discussion of these challenges, along with potential solutions to encourage more wide-spread consideration of cell-mediated immunity in vaccinology.

A first challenge is that relatively large sample volumes are required for assays that seek to identify low-frequency antigen-specific T cells.[46] This volume of blood can be difficult to obtain, particularly from infants and children. As a result, much of what is known about neonatal and infant immunity to infectious diseases and vaccines is derived from animal models or umbilical cord blood samples, which do not always recapitulate human *in vivo* responses.[143] Efforts to address the lack of representative human pediatric and infant immunity data include the use of systems biology approaches to measure a large number of immune cell populations and plasma proteins in small-volume whole blood samples (i.e., 100 μL).[144] Alternatively, various barcoding approaches have been reported, whereby a small number PBMC from individual donors are fluorescently tagged, pooled together for use in standard flow cytometry assays, and then deconvoluted during analysis.[145,146] Barcoding is a reagent-sparing approach that allows for sensitive identification of rare antigen-specific T cell populations in large, pooled samples while maintaining individual-level granularity and is thus an attractive option for comprehensive immunogenicity studies in infants and children.[145]

A second challenge is that measuring T cell-mediated immunity is technically complex. The process of isolating PBMC is labor-intensive and must be completed soon after the sample is collected, further complicating the logistics of large-scale studies, particularly in low-resource settings.[147] Differences in protocols and reagents used to isolate PBMC can introduce variability in downstream analyses,[148] as can cryopreservation protocols.[147,149] The assays themselves also require technical skill and expensive equipment that can impact reproducibility. For example, flow cytometry-based assays require multiple-sample processing steps, various reagents that can vary from lot-to-lot, instruments that must be precisely calibrated, and a multi-step data analysis pipeline.[150] As the complexity of an assay increases, so does the inherent variability between labs, or even between operators within a lab.[151] To address issues associated with the inherent variability of T cell assays, several groups have developed standardized protocols, demonstrating the feasibility of inter-laboratory reproducibility.[151] For example, use of commercial products such as SepMate (StemCell) or Cell Preparation (BD Biosciences) tubes has been shown to increase the quality and reproducibility of the PBMC isolation step.[148] Others have suggested the use centralized labs with expertise in particular assays to facilitate large-scale studies and sharing of standardized standards and reagents.[152] In addition, at the height of the COVID-19 pandemic, certain labs implemented a two-step approach, whereby higher throughput methods like ELISpots were used to survey cellular responses in large cohorts, and then a subset of samples were analyzed with more in-depth techniques.[51] Together, while challenges remain to reproducibly measuring human T cell responses on a large scale, there are several feasible approaches to implementing precise and accurate T cell assays in vaccine research and development.

Third, methods that rely on the stimulation of antigen-specific cells are currently the mainstay of clinical T cell research, but they limit our understanding to T cells that are relatively abundant in peripheral blood and have certain functional properties in response to *in vitro* stimulation (i.e., that either proliferate or produce cytokines when they encounter their antigen).[153] To address the issues of low abundance and selection for functional properties, tetramer staining is

increasingly common, whereby soluble MHC tetramers that are fluorescently labeled and present a peptide of interest are used to identify T cells that bind to that peptide, regardless of that T cell's functional properties.[154–156] Technological advances have allowed for pairing high-throughput sequencing of T cell receptors with phenotypic analysis of these T cells, measured via gene expression.[157,158,159,160] While powerful, the main limitation of tetramer-based approaches is that the HLA type of the study subjects and the epitopes of interest must be identified in advance.[154–156] Moving beyond peripheral blood, there is also increasing data from animal models to support the importance of T_{RM} cells, a subset of T cells that resides in tissues without recirculating, in mediating immunity at barrier sites (i.e., skin and mucosa).[20,161,162] Seminal work on the development and persistence of T_{RM} in humans has been done using organ donors and transplant recipients.[163,164] Strategies for more widespread consideration of human T_{RM} include sampling sites beyond peripheral blood, such as surgical explants, bronchoalveolar lavages, biopsies, and fine-needle aspirates of lymph nodes.[165]

Finally, in the absence of defined cellular correlates of protection, it can be difficult to decide which parameters to measure and to interpret the results of T cell assays in a meaningful way. Analyzing the amount of antibody that is statistically correlated with protection from disease provides a simple binary antibody-based correlate of protection. While this approach is attractive for its simplicity, there is a growing appreciation for the lack of data underpinning some established antibody-based correlates of protection.[166] In addition, the complexity of the mechanisms that mediate protection induced by certain vaccines,[167] and the relative (as opposed to absolute) and synergistic nature of some correlates also highlight the limitations of this approach.[168] While including various measures of T cell-mediated immunity does not replace antibody-mediated correlates of protection, it does allow for capturing a more complete picture of the quality of a vaccine-induced immune response. For example, multiplexed cytokine secretion assays that allow for measurement of ≥15 cytokines in a single sample are now widely accessible.[51] In addition, technological improvements in flow cytometry allow for a larger number of parameters to be measured simultaneously. These advances include spectral flow cytometry, to minimize fluorophore overlap and cytometry by time of flight (CyTOF) to allow for a higher parameter of surface and intracellular markers, including cytokines, to be measured simultaneously.[169] However, as the number of immune parameters measured increases, more advanced statistical methods must be used to analyze and interpret these data beyond a simple threshold of protection based on a single parameter.[170,171] This approach requires inter-disciplinary collaboration between immunologists, biostatisticians, epidemiologists, and bioinformaticians, which will undoubtedly increase the quality and translatability of vaccine research.[172]

Looking forward, there is a pressing need to develop more effective next-generation vaccines for diseases such as influenza, tuberculosis, and SARS-CoV-2, and new vaccine candidates for diseases such as group B streptococcus and HIV.[2] Successful immunization against these targets will require innovative approaches and it will be crucial to incorporate measures of T cell immunogenicity in these efforts. Taking influenza and SARS-CoV-2 as examples, the failure of existing vaccines to elicit durable and cross-protective immunity that prevents transmission has been attributed to the rapid replication of the virus in the respiratory tract.[173] This rapid replication in mucosal tissue, coupled with the absence of viremia, shields the virus from the systemic adaptive immune response while allowing for disease onset and transmission to others.[173] In thinking about next-generation vaccines, we must therefore consider vaccination strategies that elicit mucosal immunity, including alternate routes of vaccine delivery (i.e., intranasal, oral, or intra-dermal).[174,175] To evaluate these novel vaccine candidates, it will be critical tomeasure T cell-mediated immunity early in pre-clinical and clinical development and to harness the protective capacity of T_{RM}.[176]

In conclusion, we use select examples to demonstrate the importance of vaccine-induced T cell responses in mediating protection from VPD and argue that widespread incorporation of cellular immunity assays would allow for more effective use of existing vaccines and development of improved next-generation vaccine candidates.

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Author contributions

JRS, JP, THW, and SB conceptualized the manuscript. JRS, MC, and JP performed the literature search. JRS drafted the manuscript, and all authors provided substantial editorial contributions.

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