


The roles of resident, central and effector memory CD4 T-cells in protective immunity following infection or vaccination

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Introduction

Vaccines are the most cost-effective form of healthcare worldwide. Most current vaccines act by generating protective antibodies that inactivate the pathogen or its toxins.¹ We do not, however, have effective vaccines against pathogens responsible for many millions of deaths each year, such as human immunodeficiency virus (HIV), malaria and tuberculosis. These pathogens present difficult challenges to the immune system either through their complex life cycles and/or via their ability to impair or subvert the host's immune response. Protective immunity to these infections requires a more diverse immune response than antibodies alone. CD4 T-cells are central to all adaptive immune responses, coordinating pathogen control and clearance by both immune and local stromal cells. Harnessing their diverse functions has the potential to provide novel vaccine strategies that generate effective immunity against even complex infections.

CD4 T-cells act in concert with innate and other adaptive immune cells to protect the host from pathogens. During primary immune responses, CD4 T-cells are activated in

Summary

Immunological memory provides rapid protection to pathogens previously encountered through infection or vaccination. CD4 T-cells play a central role in all adaptive immune responses. Vaccines must, therefore, activate CD4 T-cells if they are to generate protective immunity. For many diseases, we do not have effective vaccines. These include human immunodeficiency virus (HIV), tuberculosis and malaria, which are responsible for many millions of deaths each year across the globe. CD4 T-cells play many different roles during the immune response coordinating the actions of many other cells. In order to harness the diverse protective effects of memory CD4 T-cells, we need to understand how memory CD4 T-cells are generated and how they protect the host. Here we review recent findings on the location of different subsets of memory CD4 T-cells that are found in peripheral tissues (tissue resident memory T-cells) and in the circulation (central and effector memory T-cells). We discuss the generation of these cells, and the evidence that demonstrates how they provide immune protection in animal and human challenge models.

Keywords: CD4 T cell; cytokine; infection; memory; vaccine.

secondary lymphoid organs where they amplify the anti-pathogen response by driving B-cell germinal responses and supporting CD8 T-cell activation. Activated CD4 T-cells also migrate from secondary lymphoid organs to inflamed sites where they participate in controlling and/or clearing the pathogen. Following pathogen control or clearance, the vast majority of activated CD4 T-cells undergo apoptosis with the remainder, usually around 10%, forming a population of long-lived memory cells. These memory cells retain knowledge about the initial immune response, enabling them to respond more effectively following a secondary infection. This enhanced response forms the basis for the success of vaccines. Understanding the signals that drive the generation of protective memory CD4 T-cells and the mechanisms by which they act will facilitate the design and development of improved vaccines.

Cytokine production is key to CD4 T-cell protective immunity

Naïve CD4 T-cells can differentiate into a number of distinct functional subsets.^{2,3} This enables them to tailor the

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immune response depending on the type of pathogen, and to perform multiple functions at different sites during a single infection. Memory CD4 T-cells retain characteristics of the activated CD4 T-cells from which they are derived and can, therefore, also be divided based on their functional responses.^{4,5} This cellular memory is thought to be retained by epigenetic changes to the cell's DNA or associated histone proteins that keep genes in an open or closed state depending on their expression during the primary immune response.^{3,6,7} Many genes are thought to be maintained in a poised state in memory T-cells, enabling rapid re-expression of effector molecules following T-cell reactivation by antigen-presenting cells (APCs). However, epigenetic alterations to the genome are not fixed and memory cells can display plasticity.^{8–11} The level of this plasticity is likely dependent on the extent of differentiation during the primary response and on the reactivation environment.

Cytokine responses by memory CD4 T-cells are key to their ability to protect the host from infectious disease. Rapid production of the most appropriate cytokine enables CD4 T-cells to quickly control the pathogen. For example, interferon (IFN)- γ from CD4 T-cells protects against viral infections, while interleukin (IL)-17 aids controls of bacterial and fungal infections, and IL-4 protects against infection by parasitic worms.^{12–20} Most immune protection studies are carried out in animal models where challenge studies are feasible and mechanisms of protection can be identified by loss or gain of function. Human challenge studies are, however, becoming more frequent and have also demonstrated that cytokine-producing memory CD4 T-cells correlate with reduced symptoms following pathogen challenge.^{21–23}

Immune protection by cytokine-producing CD4 T-cells correlates with their production of several related cytokines, with cells producing IFN- γ , tumour necrosis factor (TNF)- α and IL-2 most commonly studied. These multifunctional memory CD4 T-cells are found following vaccination or infection in animal models and in humans.^{24–29} In infection models of *Leishmania major* and *Mycobacterium tuberculosis*, multifunctional CD4 T-cells provide the most effective immune protection, and in humans they correlate with successful recovery from infection with Japanese encephalitis virus.^{25,27,29}

It is currently unclear why multifunctional memory CD4 T-cells offer enhanced protection in comparison to single cytokine-producing cells. The enhanced protective functions of these cells could be because they produce higher levels of the individual cytokines on a per cell basis.^{25,27} Alternatively, or in addition, as multifunctional memory T-cells can simultaneously drive effector responses, for example via IFN- γ , and T-cell survival and proliferation via IL-2 production, they offer a sustained and protective response. Currently we have limited understanding of the priming signals that lead to the generation of multifunctional memory cells. This information will be key to the development

of more effective vaccines capable of producing protective multifunctional CD4 T-cells.

Memory CD4 T-cells are found throughout the body

The consequences of rapid cytokine production by memory CD4 T-cells depend on the location of the cell. Naïve T-cells patrol through lymphoid organs as they have no prior knowledge about where in the body the pathogen they recognize may cause an infection. Memory T-cells have learnt this information during the primary response, and some memory CD4 T-cells, tissue resident memory (Trm) cells, continue to reside at the infection site. Other memory T-cells recirculate through the body. These memory cells can be split into central memory cells (Tcm) and effector memory cells (Tem). Tcm are largely restricted to lymphoid organs and the blood. In contrast, Tem are present in the blood and have the ability to traffic through peripheral organs. All types of memory CD4 T-cells are important. This is because they can provide protection in distinct ways, with recirculating cells acting as reinforcements should resident cells fail to contain the infection (Fig. 1).

Trm T-cells

The anti-pathogen responses coordinated by naïve T-cells are delayed until information about the infection is transferred from the infection site to the draining lymph node. Trm cells can provide much more immediate protection, and various studies have demonstrated that they provide the most effective immune protection to the host.^{14,15,17,30}

Tissue resident memory cells are identified as CD69+ cells that remain within peripheral tissues following pathogen clearance.^{30–32} CD69 is thought to act as a retention signal as it inhibits the surface expression of the sphingosine-1-phosphate (S1P) receptor 1. S1P is a signalling phospholipid that regulates the migration of immune cells out of lymph nodes into efferent lymphatics, and also guides cells out of tissues towards draining lymph nodes.^{33–35} Expression of the integrin CD103 by CD8 Trm cells also contributes to their retention, tethering them to skin or mucosal epithelial cells; it is, however, more rare for CD4 Trm cells to express this molecule.³⁶

There are clear differences between CD4 and CD8 Trm cells first revealed by Gebhardt *et al.* in mice infected with herpes simplex virus (HSV).¹⁴ While CD8 Trm cells become dendritic-like with limited motility in the epithelium of the skin, CD4 Trm cells are mainly found in the dermis and display a more motile behaviour.¹⁴ In humans, Trm cells have been found in multiple organs, with elegant studies from Farber and colleagues tracking memory T-cells in individuals of various ages. Human and mouse Trm cells share many characteristics, including CD69 expression and reduced expression of S1P receptor 1.^{37,38} Similarly,

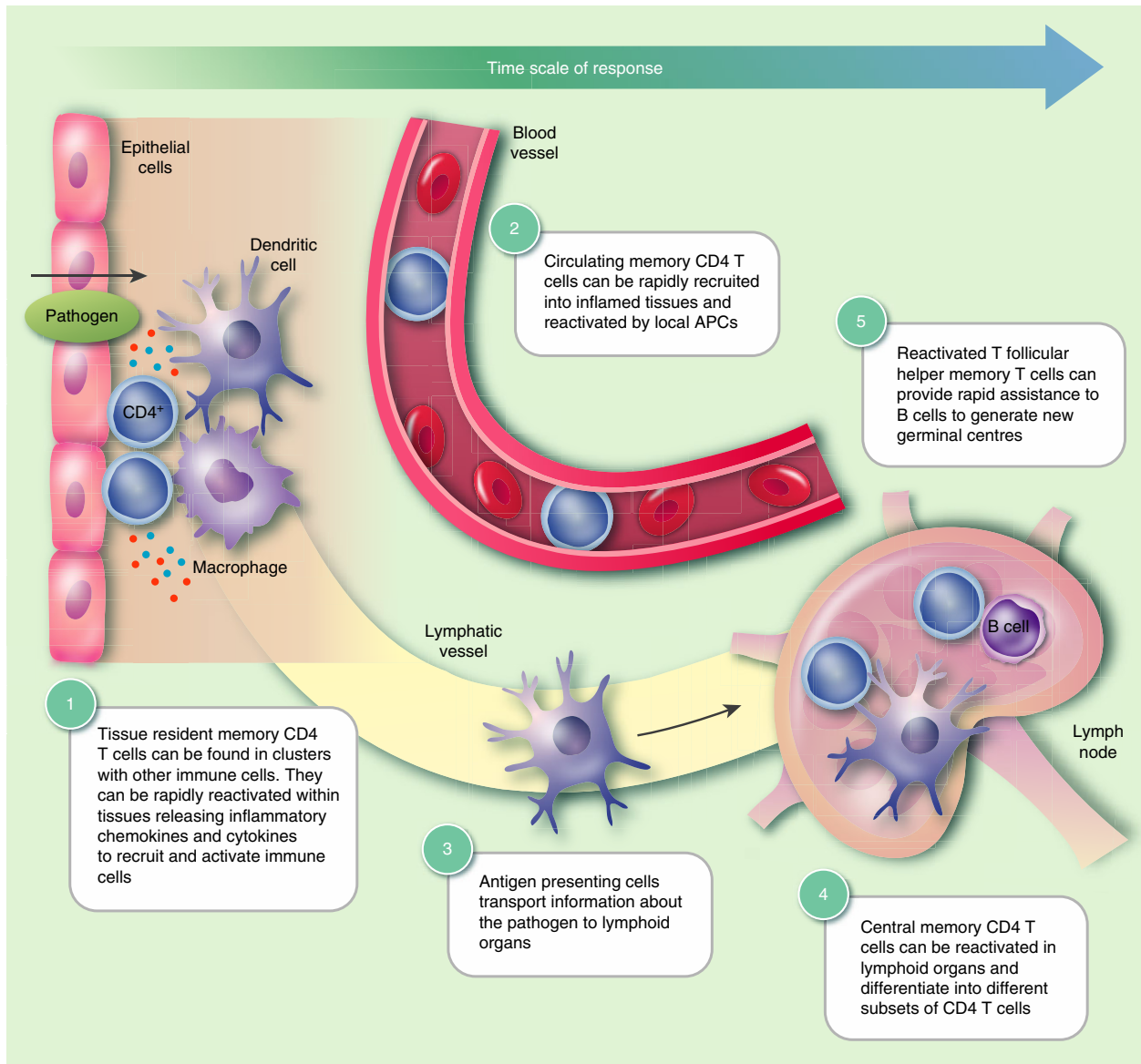


Figure 1. Protective roles of memory CD4 T-cell subsets. The protective response to previously encountered pathogens is dependent on a number of T-cell subsets. Upon pathogen encounter, resident memory CD4 T-cells (Trm) that can be found in clusters with macrophages or dendritic cells respond rapidly by proliferating and releasing inflammatory cytokines and chemokines (1). This stimulates the recruitment of circulating effector memory CD4 T-cells (Tem) to the inflamed tissue to augment the immune response (2). Local antigen-presenting cells (APCs) can subsequently transport antigen to lymphoid organs where they activate central memory CD4 T-cells (Tcm) (3–4). These central memory T-cells then expand and recirculate to confer systemic protection or further amplify the response at inflamed tissues. Memory follicular helper T-cells (Tfh) reactivated by either dendritic cells or B-cells can enter B-cell zones and induce rapid production of class-switched antibodies, which are then released into the circulation (5). These different memory T-cell subsets work in concert to provide long-lasting protection upon re-exposure to the same pathogen.

CD4 Trm cells in skin are, like their mouse equivalent, mainly found in the dermis.³⁹ Transcriptional analyses comparing circulating cells and Trm cells from the same donor indicate that CD69⁺ and CD69[−] CD4 T-cells express overlapping sets of T-cell receptor genes.³⁷ These data indicate that environment, rather than epitope specificity, is the key driver of Trm cell development.

In mice, memory T-cell residency is often identified by their failure to bind fluorescently labelled antibody injected

intravenously shortly before the animal is killed. This demonstrates that these cells are not directly in contact with the vasculature, at least at the time of analysis. Three additional key approaches are used in mice to investigate whether Trm cells are truly resident: *in vivo* treatment with the S1P functional antagonist, FTY720; parabiosis in which the circulatory systems of two animals are surgically connected; and fate mapping using photoconvertible cells. Treatment with FTY720 restricts the migration of

circulating cells, so a stable population of Trm cells in treated mice demonstrates that these cells are neither replenished by, nor lost to, circulating populations.^{15,17,40} However, FTY720 may also inhibit egress of cells from peripheral tissues to draining lymph nodes and/or reduce cell survival.^{35,41–43} Despite these potential caveats, results from FTY720-treated animals reflect those from more elegant parabiosis experiments that demonstrate that Trm cells are a distinct population neither leaving the tissue nor being replenished by circulating cells.^{17,30}

In contrast, data from Collins *et al.* suggest that CD4 Trm cells are a more dynamic population.⁴⁴ CD4 Trm cells in mice that express the photoconvertible molecule, Kaede, were found to migrate from the skin of HSV-infected mice to draining lymph nodes. These cells lost expression of CD69 following migration out of the skin. The migrating cells represented about half of the Trm cell population photoconverted at the start of the study and then examined 3 days later. These findings question the reliability of CD69 as a marker of persistently resident memory T-cells within tissues. They suggest that at least two distinct populations may be present – a more static, true Trm population and a second more dynamic population that alter CD69 expression depending on location. In this study, the specificity of the cells was not determined. These two populations may, therefore, reflect resident T-cells that respond to local antigen and remain in the tissue versus cells that are recruited but are not maintained because they are not re-activated by antigen. Future studies will need to address the functional properties of these two populations and compare the behaviours of T-cells exposed to local antigen versus those recruited by inflammation alone.

To develop vaccines that generate protective CD4 Trm cells, it is imperative that we understand the signals that drive the development and maintenance of these cells. During immune responses, activated T-cells migrate into inflamed tissues under the guidance of chemokines, integrins and adhesion molecules.⁴⁵ T-cells that encounter their antigen at the infection site are likely to receive tissue-specific cues that influence their function and memory potential. The presentation of specific antigen at the infected tissue is required for the formation of CD4 Trm cells, but may not be necessary for CD8 Trm cells.^{31,46–48} These data indicate that vaccines aimed at generating protective Trm CD4 T-cells must drive antigen presentation within the tissue targeted by the pathogen.

Whether persistent antigen presentation following pathogen control is required to maintain CD4 Trm cells is still unclear. CD4 Trm cells in the skin and mucosa are located in clusters of cells with macrophages and/or dendritic cells that express chemokines that maintain the cells at the site. Macrophage-derived CCL5 maintains CD4 T-cells in the vagina following HSV-2 infection.¹⁷ IFN- γ produced by CD4 Trm cells, potentially in response to

low levels of persistent antigen, in turn maintains macrophage CCL5 expression. Similarly, CCL5 produced by CD8 T-cells and macrophages in the skin is responsible for maintaining CD4 T-cells in perifollicular clusters.⁴⁴ In this case, however, antigen presentation was not required for memory T-cells to be incorporated into these immune cell clusters.

Clusters of Trm and APCs may represent nascent versions of ectopic lymphoid structures (ELS), which range from organized clusters of immune cells to lymph node-like structures with designated T- and B-cell zones. ELS are often found in sites of chronic inflammation, and have been associated with the generation of autoreactive antibodies.^{49,50} Whether Trm immune cell clusters represent an early stage in ELS development is unclear. They do provide an excellent location for the rapid reactivation of CD4 Trm cells by local APCs. For example, influenza virus-specific Trm cells, which can be found in clusters with B-cells in the infected lung, provide effective and rapid immune protection at least in part by providing rapid assistance to B-cells to make neutralizing antibodies.¹⁸

In addition, CD4 Trm cells can enhance the actions of cells that are recruited into the infected tissue. Influenza-specific memory CD4 T-cells in the lung drive the production of chemokines that attract innate immune cells that rapidly control viral spread.⁵¹ Similarly, IFN- γ production by *L. major*-specific CD4 Trm cells drives the recruitment of inflammatory monocytes to the infection site.¹⁵ These recruited monocytes produce nitric oxide and reactive oxygen species that are toxic to the parasite.

Recirculating memory T-cells

Circulating antigen-specific CD4 memory T-cells can participate in protective immune responses either acting as reinforcements to Trm cells³² or protecting tissues not challenged in the initial infection or vaccination.^{14,52} Immune protection by circulating memory CD4 T-cells is delayed compared with Trm-mediated protection as they must be first recruited to the site of infection and reactivated by local APCs. Immune protection by circulating cells can require collaboration with other components of the memory immune response. Iijima *et al.* found that while recirculating HSV-2-specific memory CD4 T-cells cannot prevent viral replication at the challenge site, they could prevent virus entry into the dorsal route ganglia.⁵² This protection required the entry of virus-specific antibody into the nervous system, which was dependent on increased vascularization mediated by IFN- γ derived from reactivated memory CD4 T-cells. Similarly, in mice vaccinated with the yellow fever vaccine, transfer of CD4 T-cells and immune serum provide the most optimal immune protection.²⁰

The effector response of recruited antigen-specific memory CD4 T-cells is likely to be influenced by the level

of MHC II and co-stimulatory molecules displayed by the local APC that reactivates the memory cell.^{53–55} Furthermore, this response is likely to be distinct to that from the same cell reactivated in the very different environment of a lymphoid organ. It is key, therefore, to consider not just the type of memory CD4 T-cell that a vaccine should aim to generate but where it is likely to be reactivated, which APCs are involved in this, and the downstream consequences of these interactions.

Memory CD4 T-cells in secondary lymphoid organs

Central memory cells are most likely to be reactivated in secondary lymphoid organs as they lack the chemokine receptors and adhesion molecules necessary to enter peripheral tissues.⁴⁵ Instead they, like naïve T-cells, express high levels of CD62L, which enables entry into lymph nodes from the blood via high endothelial venules and CCR7, the chemokine receptor that is also involved in trafficking to and within lymphoid organs.^{45,56}

While Tcm cells may not rapidly produce protective cytokines, they proliferate upon reactivation, quickly increasing the number of antigen-specific CD4 T-cells. These cells can then either remain in the lymphoid organ to help B-cells or migrate to the site of infection to help tackle the infection directly. As Tcm cells are uncommitted to any particular effector cytokine production, they can differentiate under the influence of the cytokine milieu triggered by the challenge infection.⁵⁷ Vaccines that drive the generation of Tcm cells may provide less immediate protection than those designed to induce Trm cells; however, they offer an adaptable pool of memory CD4 T-cells that can protect via multiple pathways.

In primary immune responses, activated CD4 T-cells drive B-cell germinal centre reactions leading to the production of high-affinity class-switched antibody. The cells that coordinate this response are classed as T follicular helper (Tfh) cells that are initially formed in the T-cell zone of the lymphoid organ before moving to the developing germinal centre.⁷ Depending on the type of infection, Tfh cells can produce T helper type 1 or 2 cytokines to drive appropriate antibody class switching.⁷

Whether Tfh cells can differentiate into specialized memory cells has been an area of recent debate.⁷ Within the pool of Tcm cells, a proportion of cells express higher levels of CXCR5, the chemokine receptor that allows cells to move towards or into B-cell follicles.⁷ Tfh cells can remain for many months in the original draining lymph node where persistent antigen is likely to maintain them in an active state. These cells rapidly expand upon re-challenge and may represent a population of lymph node resident memory cells as they can express CD69.^{58–60} However, this reliance on antigen suggests that these lymph node resident Tfh cells may not represent ‘true resting memory cells’. Moreover, Pepper *et al.* found that the number of antigen-specific

memory cells that expressed Tfh markers declined over time, suggesting that Tfh cells fail to differentiate into long-lived memory cells.⁵⁷ However, we and others have shown that both mouse and human CXCR5+ memory CD4 T-cells provide rapid assistance to B-cells upon reactivation.^{61–69} Importantly, Alexander *et al.* demonstrated that DNA vaccination generated memory CD4 T-cells that, via their rapid assistance to B-cells, protected mice from influenza virus infection.⁷⁰ This rapid assistance to generate high-affinity class-switched antibody is particularly relevant to infections such as influenza virus where regions of the virus targeted by antibody alter much more rapidly than epitopes recognized by CD4 T-cells.⁷¹

CXCR5+ memory cells with an increased ability to help B-cells are commonly referred to as Tfh memory cells,⁷ even though they may be found in circulation rather than contained within a B-cell follicle. How they are distinct from the general CD4 Tcm pool is a complicated question. Indeed, Tfh memory cells do express lower levels of CXCR5 than Tfh cells present during the primary immune response^{62,69} and, as Pepper *et al.* describe, are difficult to distinguish from Tcm cells.⁵⁷ Reactivation of CXCR5+ memory cells can occur in the absence of B-cells,⁶³ but reactivation by B-cells consolidates the T-cell’s expression of Bcl6, the transcription factor associated with Tfh cell function.⁷² These data suggest that if there are Tfh memory cells, their function upon reactivation is likely dependent on the context in which they are reactivated.

A further key question that remains to be addressed is the consequences of reactivation of memory CD4 T-cells by B-cells within either ELS or immune cell clusters at the infection site itself. In lymphoid organs, secondary germinal centres are thought to be formed by reactivated IgM+ memory B-cells, providing a blank canvas for antibody class switching relevant to the pathogen.⁷³ In influenza virus-infected mice, CD4 T-cells and B-cells can be found in clusters within the lung, and many virus-specific B-cells in the lung are class switched.^{74,75} This suggests that germinal centres formed in ELS in peripheral tissues may follow different rules to those in lymphoid organs following re-infection. Careful studies that dissect the contribution of the reactivation of B-cells in the tissue versus those in lymph nodes to immune protection are needed to establish their relative importance in protecting the host from reinfection.

Human vaccines: recent progress and continuing challenges

Dissecting the relative contributions of different populations of memory T-cells to immune protection in mice provides mechanistic understanding of immunological memory. Animal vaccine studies often do not, however, easily translate into protective vaccines for humans. Human challenge studies, therefore, play important roles in evaluating vaccines at early stages of development.

They often cut the costs of large-scale field vaccine trials and reduce tests of non-effective vaccines on large numbers of individuals.⁷⁶ This is especially the case in diseases, including malaria and tuberculosis, in which our understanding of the correlates and mechanisms of immune protection are limited.

Human challenge studies in malaria have demonstrated the relative safety and efficacy of sporozoite vaccines. Experimental sporozoite vaccines have either been delivered as irradiated parasites that can infect host cells but fail to differentiate and cause disease, or as live parasites.^{21,77,78} In this instance, anti-malarial drugs must be given to prevent active disease. Protection in these studies is associated with high levels of multifunctional, antigen-specific memory CD4 T-cells in peripheral blood, although mouse and non-human primate studies point to key roles for liver resident memory CD8 T-cells in preventing parasite growth.^{78,79} The logistical challenges of sporozoite vaccines are significant; current field trials are likely to reveal whether or not these approaches are feasible.⁸⁰

The most advanced vaccine for malaria is GlaxoSmithKline's RTS,S vaccine, which contains the circumsporozoite protein from the pre-erythrocyte stage of the parasite.^{81,82} Antibodies and CD4 T-cells specific to the circumsporozoite protein are correlates of immune protection following RTS,S immunization, which has an efficacy of about 30%.^{22,81,83} While a positive advance, this relatively low efficacy and the short duration of protection means that more robust vaccines are still required.⁸⁴ A key contrast between sporozoite vaccines and the recombinant protein RTS,S vaccine is the need for adjuvants to boost the immune response to the subunit vaccine. RTS,S contains a combined adjuvant with a saponin and toll-like receptor agonist.⁸² This adjuvant combination is one of only a handful approved for use in humans, with few able to induce the size and correctly tailored immune response driven by either natural infection or vaccination with an attenuated or inactivated pathogen.⁸² The live vaccines are, however, more likely to lead to adverse effects or negative side-effects.^{82,85} Improved understanding of the mechanisms of action of current and experimental adjuvants is likely to lead to further improvements allowing us to strike the right balance between immunogenicity and vaccine safety.

Other human challenge models include the use of BCG as a surrogate challenge in tuberculosis vaccine trials. While the BCG vaccine protects against disseminated tuberculosis, especially in young children who are most at risk, its ability to protect from pulmonary disease varies across the world.⁸⁶ Current studies in mice, non-human primates and humans are focussed on increasing the immune protection offered by BCG, for example by using prime-boost strategies.^{86,87} BCG effectiveness can also be achieved by altering the vaccination route, with mucosal and intravenous routes offering enhanced protection

compared with more traditional subcutaneous or intradermal injection in a non-human primate model.⁸⁸ Potentially these injection routes provide enhanced protection as they are better at driving the development of CD4 Trm cells in the lung.

Human immunodeficiency virus presents bigger hurdles still as immune protection does not develop in the vast majority of infected individuals and human challenge studies are not possible. The RV144 HIV vaccine trial has offered a number of key insights into immunity to HIV. The vaccine demonstrated an efficacy of 31.2%.⁸⁹ Protection correlated with high levels of anti-viral IgG, dependent on CD4 T-cell responses, while vaccine-driven IgA was associated with an increased risk of infection.^{90–92} Immune protection to this highly diverse virus is thought to require broadly neutralizing antibodies (bnAbs) that recognize more conserved regions of the virus.⁹³ The development of bnAbs likely requires repeated exposure to the antigen and continued input from Tfh cells.⁹³ Successful HIV vaccines will probably, therefore, require the induction of effective Tfh memory cells that can coordinate the development of protective bnAbs.

Future perspectives

We have appreciated the concept of protective immunological memory for thousands of years and have been manipulating it via vaccination since the 10th century.⁸⁵ The majority of successful vaccines act by generating neutralizing class-switched antibodies produced by long-lived plasma cells.¹ These vaccines must, therefore, drive activation of helper CD4 T-cells that act, at the very least, during the primary response to protect the host. We are now beginning to understand the potential myriad roles memory CD4 T-cells can themselves play in protection from infectious diseases. The recent studies discussed here have revealed the complexity of the memory CD4 T-cell pool. We now need to understand how vaccine formation and delivery can be altered to bias the development of protective memory CD4 T-cells.

As our fundamental understanding of memory CD4 T-cells improves, we must put this into a real-world context. Studies in wild or pet-shop mice have highlighted the major differences between memory compartments and responses to infections in these animals versus their laboratory equivalent.^{94,95} The microbiome plays a major role in these differences, but the exposure to varied and multiple pathogens throughout life is also likely to affect the generation and function of immune memory cells.^{94,95} Human challenge studies offer a valuable extra arm to vaccine studies, but most are carried out on healthy western volunteers. Evidence from the recently introduced rotavirus vaccine, and also polio and cholera vaccine studies, demonstrates reduced vaccine-effectiveness in major at-risk populations in low–middle-income countries.^{96–98}

It is clear, therefore, that protective immunity must be examined and evaluated in multiple experimental settings that can each provide valuable information on both mechanisms of immune protection and real-world effectiveness.

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Disclosures

The authors declare that they have no competing interests.

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