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PERSPECTIVE

Harnessing CD4+ T cell responses in HIV vaccine development

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CD4+ T cells can perform a panoply of tasks to shape an effective response against a pathogen. Limited attention has been paid to the potential importance of functional CD4+ T cell responses in the context of the development of next-generation vaccines, including HIV vaccines. Many CD4+ T cell functions are newly appreciated and only partially understood. A workshop was held as a forum to bring together a small group of experts to exchange ideas on the role of CD4+ T cells in developing durable functional antibody responses, via follicular helper T cells, as well as on the roles of CD4+ T cells in other aspects of protective immunity. Here we discuss whether CD4+ T cell responses may represent a beneficial component of an efficacious HIV vaccine.

Recent findings, including the results of the RV144 Thai trial[1] and the identification of broadly neutralizing HIV antibodies in some HIV-infected individuals[2–11], have provided new hope that humans can make potent anti-HIV antibody responses and that if a candidate HIV vaccine were able to appropriately harness HIV-specific CD4+ T cells together with antibody responses, the vaccine would confer protection. Although there is considerable enthusiasm in the field to pursue these issues, there is uncertainty about how to prioritize each problem and how to formulate appropriate approaches to address them. Hence, a workshop called "Harnessing CD4+ T cell responses in HIV vaccine development," sponsored by the National Institute of Allergy and Infectious Diseases and the Ragon Institute, was held on 30 May 2012. The workshop goal was to bring together leaders with wide expertise to discuss a range of controversial questions and topics to assess where the field stands and, hopefully, to provide guideposts for future research by providing conceptual and technical frameworks to deal with some of the challenges of HIV vaccine development. CD4+ T cells are astonishingly diverse and multifaceted in their capabilities, and they can direct immune responses to maximize antipathogenic processes while suppressing nonessential immune responses[12–14]. The three topics of discussion during the meeting were (i) how to generate broadly neutralizing HIV antibodies in a vaccine, with a focus on follicular helper (T_{FH}) cells and germinal center biology; (ii) what CD4+ T cell effector functions in chronic viral infections are; and (iii) how to initiate potent CD4+ T cell responses. The workshop promoted an intensive idea exchange and, most importantly, an agreement among the participants as to what some of the major questions are in this field.

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How can a vaccine elicit broadly neutralizing antibodies to HIV?

A central problem in HIV vaccine research is how to induce broadly neutralizing antibodies (bnAbs). It is now clear that 5% (refs. 3,5) (or more[6,15,16]) of HIV-infected individuals develop bnAbs—but only multiple years after infection. Importantly, by looking at the sequences of those antibodies, it appears that developing bnAbs to HIV often involves exceptional contortions by the B cell receptor (BCR). The accumulation of amino acid mutations during antibody maturation of most HIV bnAbs is five- to tenfold higher than that of the average human memory BCR. For example, in a study of four HIV+ individuals with HIV bnAbs[4], the heavy chains of the bnAbs are all mutated ~25–33% (compared to a baseline of 0%). Moreover, every one of them had an additional highly unusual feature, either an extremely long CDR3 or an unusual insertion or deletion[4]. The degree of mutation seen in the highly studied HIV bnAb VRC01 is even more extensive, with a 42% amino acid mutation rate in the heavy-chain variable domain gene and a total of more than 70 amino acid mutations in the antibody heavy- and light-chain genes combined[9,10]. BCRs mutated at such extreme levels are very rare in HIV-negative individuals, so although the good news is that it is possible for the human immune system to generate HIV bnAbs, the bad news is that it is an exceptionally difficult accomplishment—or at least it seems to be.

The vast majority of neutralizing antibody responses to pathogens are dependent on CD4+ T cell help. T_{FH} cells are the CD4+ T cells uniquely specialized to provide B cell help[14,17]. Germinal centers are the sites of B cell selection and mutation[18]. T_{FH} cells are required for germinal centers[18–20], as each round of B cell proliferation and selection depends on survival, proliferation and differentiation signals provided by T_{FH} cells in the form of cell surface co-stimulatory molecules (for example, CD40 ligand) and secreted factors (for example, interleukin-21 (IL-21) and IL-4)[17](**Fig. 1**). T_{FH} cells are frequently the limiting factor in determining the magnitude of the

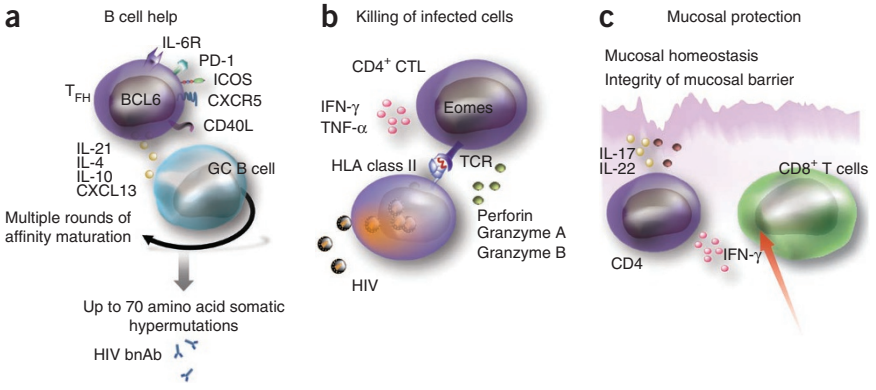

Figure 1 $CD4^+$ T cell functions in protection against HIV. (**a**) T_{FH} cells are defined by their localization in the B cell follicles and expression of the transcription factor BCL6. T_{FH} cells have an essential role in the initiation and maintenance of germinal centers (GCs), the lymphoid tissue sites of B cell proliferation and affinity maturation for the development of high-affinity antibodies. T_{FH} cells select the best germinal center B cells by providing necessary signals for B cell survival, proliferation and differentiation. Moreover, T_{FH} cells are crucial in inducing high levels of somatic hypermutations, which are a common feature of broadly neutralizing antibodies against HIV. (**b**) T_H1 $CD4^+$ T cells can directly recognize infected cells through viral peptides bound to HLA class II and can respond with secretion of the cytokines IFN-γ and tumor necrosis factor-α (TNF-α). $CD4^+$ T cells can also directly kill HIV-infected cells, probably through perforin and granzyme A/B secretion. Eomesodermin (Eomes) is a transcription factor associated with cytolytic $CD4^+$ T cells. T-bet is required for T_H1 cells. (**c**) $CD4^+$ T cells are essential for the maintenance of mucosal integrity and control of gastrointestinal microflora, through IL-17–mediated attraction of neutrophils and IL-22–mediated repair of epithelial cells. $CD4^+$ T cells can also attract $CD8^+$ T cells via IFN-γ–dependent mechanisms and aid their tissue migration.

germinal center response[19,21]. Most HIV bnAbs show high mutation levels, indicating that many rounds of selection must occur in the germinal centers of these individuals before bnAb capacity evolves. Therefore, it is likely that outstanding T_{FH} cell responses must be elicited by an HIV vaccine to meet the overall challenge of having optimal germinal centers for extensive selection events to generate HIV bnAbs.

On the basis of that premise, the workshop discussed the question "What are the most important aspects of T_{FH} cells necessary to understand in order to harness them in HIV vaccine development?" Phenotypically, human T_{FH} cells are best defined by their localization in B cell follicles and expression of the transcription factor Bcl6 (refs. 18–20,22), the chemokine receptor CXCR5 and other migration-related proteins, and the receptor PD-1, among other surface markers[14,22–24]. While the importance of T_{FH} cells has been demonstrated in mice, as well as in the context of human genetic deficiencies[17], knowledge regarding T_{FH} cells in nonhuman primates has until recently been lacking. Richard Koup (US National Institutes of Health, Vaccine Research Center (NIH VRC)) presented new work demonstrating that T_{FH} cells are identifiable in nonhuman primates[25] (though without significant CXCR5 staining by FACS, indicative of a lack of a suitable CXCR5-specific monoclonal antibody), consistent with another report[26]. Strikingly, in simian immunodeficiency virus (SIV)-infected nonhuman primates, T_{FH} cell abundance in lymph nodes correlated well with the magnitude of the SIV-specific IgG response, the magnitude of the germinal center response and the avidity of the SIV-specific IgG[25]. HIV-specific T_{FH} cells have now been identified in lymph nodes of HIV^+ individuals[27]. However, it is not known whether simply the quantity of T_{FH} cells is important for affinity maturation of B cell responses or whether T_{FH} cells with particular functions are a crucial limiting factor. The latter would provide a potential explanation for why only a small minority of HIV^+ humans and SIV^+ macaques develop bnAbs, even though T_{FH} cells are present.

If T_{FH} cells are to be valuable in HIV vaccine development, it is important to be able to track them in the peripheral blood of humans and nonhuman primates. T_{FH} cells may be missed when monitoring immunogenicity in human HIV vaccine trials and nonhuman primate $CD4^+$ T cell assays, as they focus on quantifying antigen-specific $CD4^+$ T cells by their ability to produce common cytokines such as interferon-γ (IFN-γ) and therefore may not detect T_{FH} cells. There were disagreements as to what is necessary to define a T_{FH} cell in peripheral blood and whether peripheral T_{FH} cells are representative of germinal center T_{FH} cells. There is evidence that antigen-specific T_{FH} cells can be detected in human blood[28,29], and some investigators have used CXCR5 or IL-21 as a single marker to identify blood T_{FH} or 'T_{FH}-like' cells. However, although IL-21 is produced by human T_{FH} cells, IL-21 expression is insufficient to identify T_{FH} cells, as a variety of $CD4^+$ T cell types can produce IL-21 (refs. 14,17,30,31). In contrast, CXCR5 is important for migration to B cell follicles. Indeed, blood $CXCR5^+CD4^+$ T cells have a gene expression profile largely distinct from central memory T_H1, T_H2 or T_H17 cells[23,32]. However, the bulk of $CXCR5^+CD4^+$ T cells in peripheral blood are also phenotypically distinct from T_{FH} cells found in lymphoid tissue (lymph nodes or tonsil), including no difference in Bcl6 expression compared to other blood $CD45RO^+CD4^+$ T cells[22,23,32]. Nevertheless, similar arguments can be made about blood central memory T_H1 cells; resting human central memory T_H1 cells have little protein expression of T-bet or other proteins found selectively at high levels in effector T_H1 cells. Many only show full T_H1 phenotypes after multiple days of reactivation. Similarly, blood $CXCR5^+CD4^+$ T cells reacquire more T_{FH} phenotypes upon reactivation[32].

As an alternative to the use of CXCR5 as a phenotypic marker, blood T_{FH} cells can potentially be defined on the basis of function. The function of T_{FH} cells is to help B cells *in vivo*. Co-culture assays can be used to show the capacity of $CD4^+$ T cells to help B cells *in vitro*. Tonsillar T_{FH} cells provide enhanced help to B cells[22,24,29,33], and selective B cell help by blood $CXCR5^+CD4^+$ T cells has also been reported[29]. Yet Koup presented data that no, or modest, differences were detectable in T plus B cell co-culture assays using blood $CXCR5^+CD4^+$ T cells in comparison to $CXCR5^-CD4^+$ T cells, and multiple investigators at the workshop indicated they had similar experiences. Co-culture assays show a modest discriminatory capacity for identifying the function of blood $CXCR5^+$ cells in several published studies, as well[28,32,34]. It was agreed that a robust functional assay or widely agreed upon phenotypic markers for blood T_{FH} cells are required, and this gap poses a considerable impediment for the HIV field and for vaccine studies.

The question "Are there memory T_{FH} cells?" was also raised. Although a preponderance of data indicate that memory T_{FH} cells exist in mice and humans[29,32,35,36], issues do remain, and the topic remains controversial[37,38]. This is at least in part due to the apparent ability of T_{FH} cells to convert to other $CD4^+$ T cell types[39,40].

More studies of antigen-specific human T_{FH} cells are central to resolving this debate[28,29]. It is also crucial to better define which $CXCR5^+CD4^+$ T cell population is comprised of memory cells, as there is evidence that at least some $CXCR5^+CD4^+$ T cells found in the peripheral blood of patients with autoimmune disease have an activated T_{FH} cell phenotype[29,41,42]. Moreover, most activated human $CD4^+$ T cells upregulate CXCR5 (refs. 17,43) (unlike mouse $CD4^+$ T cells[31]), which indicates that at least some $CXCR5^+CD4^+$ T cells in human peripheral blood may be recently activated cells from an ongoing immune response[37]. Koup pointed out that T_{FH} cells in germinal centers are highly prone to apoptosis, making it unlikely that the majority of those cells directly progress to memory cells[25]. S. Crotty noted that germinal center T_{FH} cells have substantial viability *in vitro* when allowed a rest period before re-stimulation and that there is evidence that T_{FH} cells in germinal centers can leave the germinal centers[44,45] and progress to a more resting or memory-like state[36,45]. Further research is required in this area.

As introduced above, T_{FH} cells are directly involved in antibody maturation and selection. Most HIV bnAbs have a high number of mutations, indicating massive selection and affinity maturation in germinal centers before the development of sufficient broadly neutralizing binding capacity[3,4,6,8], as summarized by John Mascola (NIH VRC) at the workshop. The evidence indicates that the majority of those mutations are functional products of the affinity maturation process, not bystander accumulation of irrelevant multations[8]. The primary enzyme required for DNA mutation in germinal center B cells is activation-induced cytidine deaminase (AID). Therefore, the questions of "How does a germinal center work to maximize affinity maturation?" and "How are AID and somatic hypermutation regulated in germinal centers?" were agreed to be of great interest, and much more research is needed to find answers. Several other important issues were also raised about the B cell response. The inferred germline versions of many bnAbs tested have little to no measurable binding affinity to HIV Env in ELISA-based assays. Given that the final affinity-matured $bnAb^+$ B cell has substantially modified specificity and increased affinity for Env spikes from the initial B cell, it is unclear how the initial B cells are primed. It is currently unknown whether intact virions, free Env or antigens of some other form trigger the naïve B cells that eventually become bnAb producers. It is unlikely to be the first, as intact virions have evolved to possess very few spikes[46]. One possibility considered by many is that the initial '$bnAb^+$ potential' B cell is selected by a completely different antigen that happens to cross-react with Env spikes. Deep-sequencing studies and longitudinal analyses of the developing bnAb response are necessary to address this possibility. A different, perhaps more likely scenario is that the initial $bnAb^+$ potential B cell is selected by Env with affinity that is simply too low to measure by conventional assays but is nevertheless biologically relevant and sufficient for activation. Importantly, membrane-bound antigen is much more potent at triggering B cells than soluble antigen[47,48], and interaction of B cells with membrane-bound antigen results in potent BCR-discrimination and antigen-acquisition processes[49–52]. Therefore, are the primary antigens seen by $bnAb^+$ potential B cells actually the HIV Env spikes on the surface of infected cells? Or, are complement- or antibody-bound Env spikes possibly presented by follicular dendritic cells (DCs)? Another process that can occur, perhaps simultaneously, is the increase of the apparent affinity of a B cell for antigen by polyreactivity resulting from engagement of more BCRs[53]. Understanding the relevant antigen form is essential for HIV vaccine Env protein immunogen design, a topic discussed extensively elsewehere[54]. Finally, where does the initial productive B cell–HIV antigen interaction take place? Knowing the answer to this question would aid understanding of the relevant antigen form. Although not all of these questions are posed from a vaccinology perspective, elucidating the biology and mechanism of action of productive B cell recognition of HIV Env spikes is necessary to understand how to elicit $bnAb^+$ B cells via immunization.

One point made during the workshop was that it may be more useful to focus on studying earlier forms of HIV-neutralizing antibodies—pre-bnAbs—before they develop into bnAbs. It may be substantially easier to uncover the developmental steps of pre-bnAbs that may have much less somatic hypermutation than a fully matured bnAb. Importantly, in the context of HIV vaccine development, immunization to develop pre-bnAbs may be much easier than that aimed at developing matured bnAbs because there is far less somatic hypermutation and affinity maturation involved (although it should be noted that somatic hypermutation has also been described for non-neutralizing antibodies against HIV in chronically infected individuals, it is not sufficient for the development of bnAb function, but it does seem to be necessary). Pre-$bnAb^+$ B cells may then be capable of rapidly evolving into bnAb-producing B cells upon HIV exposure and facilitating viral control during the acute phase of HIV infection. In addition, pre-bnAbs may be able to provide some degree of direct protection against HIV if modest levels of neutralization or other antibody functionalities are useful. Multiple recent nonhuman primate studies have found protection to correlate with antibody responses, even though bnAbs were not elicited[55,56]. It has been hypothesized that antibodies targeting the HIV Env V1V2 loops were the source of the modest protection observed in the RV144 human HIV vaccine trial[57,58], even though those antibodies have weak antiviral activity *in vitro*[57,59]. It must be noted that too focused an antibody response can be counterproductive because it can induce rapid escape of the virus, underscoring the importance of the breadth of an induced antibody response in any vaccine strategy. Protective breadth is seen with antiviral memory B cell responses, whereby a population of antigen-specific memory B cells can have sufficient diversity to neutralize viral variants they have never encountered[60]. HIV mutates at a truly exceptional rate, and the significance of breadth of antiviral activity against HIV for preventing escape has been demonstrated for years by the success of multidrug highly active antiretroviral therapy.

Michael Cancro (University of Pennsylvania) proposed a contrarian view that the best way to generate bnAbs via vaccination will not involve mimicking the natural process of massive somatic mutation seen in HIV^+ individuals. Instead, the problem should be viewed as a B cell repertoire problem. Given that B cells can recognize virtually any possible surface, the lack of $bnAb^+$ B cell generation within weeks after HIV infection implies that there is a specific gap in the human B cell repertoire that HIV exploits. To apply this hypothesis to vaccine development, the mature B cell repertoire can be enlarged by experimentally relaxing B cell tolerance checkpoints for a short duration of time around an immunization. Indeed, treatment with BLyS (also known as BAFF) allows B cells to bypass some tolerance checkpoints and create a larger B cell repertoire, and Env immunization of BLyS-treated mice resulted in an improved HIV-neutralizing antibody response[61]. A related concept is to make germinal center B cell selection more inclusive after an immunization to capture and sustain a wider range of Env-binding B cells in the germinal center response, as the selection landscape may be such that B cells that bind Env with low affinity may have BCR sequences amenable to bnAb development over time.

It was pointed out by M.P. D'Souza that the quality of the B cells is largely irrelevant if stable B cell memory is not generated. The cumulative protective efficacy in the RV144 trial was 31% at 42 months after first vaccination, with the highest efficacy observed in the first 12 months[1]. Antibody responses to that vaccine faded rapidly[59]. The lack of durable memory antibody responses is a serious problem for HIV vaccine efforts. Sustained release of antigen may be one solution to this problem[62,63]. Long-term B cell memory and antibody responses can be elicited for decades by many vaccines[64–67], so this should be a solvable problem for HIV vaccine development, but, the cellular biology of what drives long-lasting memory versus short-term B cell and plasma cell memory is still poorly understood[68–70].

CD4+ T cell effector functions in chronic viral infections

Although CD4+ T cell help to B cells is an important component of vaccination strategies, direct CD4+ effector functions may also aid to prevent HIV infection. H. Streeck showed data that a marker of cytotoxic activity by CD4+ T cells (expression of granzyme A) strongly correlated with slower disease progression in HIV+ individuals[71]. Interestingly, only a fraction of HIV-specific CD4+ T cells develop cytolytic activity, and it is not known what signals drive the development of cytotoxic CD4+ T cells. It has been suggested that CD4+ T cells use similar killing mechanisms as CD8+ T cells or natural killer cells, and, indeed, degranulatory activity (measured by surface expression of CD107a[71]) and perforin and granzyme expression have been detected in cytolytic CD4+ T cells during many other viral infections. A recent study of human influenza infection demonstrated that pre-existing influenza-specific cytolytic CD4+ T cell responses—but not CD8+ T cell responses—were associated with reduced severity of flu symptoms and reduced viral shedding[72]. However, the protective role of cytolytic CD4+ T cell responses against HIV acquisition remains to be determined. HIV preferentially infects CD4+ T cells during mucosal transmission, and the levels of human leukocyte antigen (HLA) class II expression on infected CD4+ cells might be too low for cytolytic CD4+ T cells to be able to effectively control viral dissemination. In contrast, HLA class II expression is high on macrophages and DCs, and therefore cytolytic CD4+ T cells might be potent at controlling viral replication in infected macrophages or DCs. It has been suggested that CD8+ T cells are impaired in controlling HIV in macrophages[73], and cytolytic CD4+ T cells may compensate for this by recognition of HIV infection via HLA class II presentation. One potential mechanism of action of granzyme A is the destruction of the SET DNA repair complex, and HIV requires the SET complex for integration[74]. It was noted that cytolytic CD4+ T cell activity was observed in the RV144 trial[75], although no CD4+ T cell function was positively correlated with protection[57].

What other CD4+ T cell types might be involved in the control of HIV infection, directly or indirectly? Jay Kolls (University of Pittsburgh) showed data highlighting the importance of IL-17 and IL-22 in protection against lung bacterial and fungal infections[76]. There was general agreement that T_H17, T_H22 and T_H2 responses have no apparent value in an anti-HIV response, and efforts should be focused on enhancing T_{FH}, T_H1, and cytotoxic mucosal CD4+ T cell responses in HIV vaccine efforts. However, it was also noted that the presence of T_H17 cells and IL-22–producing cells is essential for the maintenance of mucosal integrity. These cells may therefore indirectly contribute to viral control through the reduction of immune activation. In addition, CD4+ T cell help to CD8+ T cells is crucial for memory development and protection from chronic viral infections, but the molecular processes involved remain incompletely understood. Although it has been shown that IL-21 expression by CD4+ T cells maintains effective antiviral CD8+ T cell responses, it remains unknown whether IL-21 made by T_{FH} cells can provide adequate CD8+ T cell help[77,78]. Notably, David Brooks (University of California, Los Angeles) highlighted that CD4+ T cells induced in persistent viral infections in mice are more prone to developing a T_{FH}-like phenotype. Additional unknown CD4+ T cell types and functions with value for protection against HIV may remain undiscovered. One approach to understanding what CD4+ T cell functions are important in protecting against a chronic viral infection is a computational approach to interrogate the heterogeneity in the antigen-specific CD4+ T cell response[79]. High-parameter flow cytometry may also provide a better understanding of how complex the antiviral CD4+ T cell response may be[80].

Initiating potent CD4+ T cell responses

How can potent CD4+ T cell responses best be primed by immunization? CD4+ T cell priming is an intricate and multifaceted biological process, with a wide range of possible inputs and outputs. For example, IL-2 enhances T_H1 responses but potently inhibits T_{FH} responses[81,82]. D.R. Littman highlighted the likely importance of type 1 IFN in priming strong CD4+ T cell responses, particularly antiviral CD4+ T cell responses. SIV and HIV-2 both infect DCs and restrict type 1 IFN production by the DCs[83]. HIV-1, however, avoids substantial infection of DCs, and infected CD4+ T cells are generally very poor producers of type 1 IFN. How does this major difference affect T cell priming and the course of the first week of HIV infection? Whereas type 1 IFN is important for CD4+ T cell priming in some systems, it is dispensable in others[84]. In addition, it was contentious whether type 1 IFN is limiting at the site of infection, as upon SIV infection of the vagina, plasmacytoid DCs are rapidly recruited to make large quantities of type 1 IFN[85]. Another secreted factor produced rapidly by cells of the innate immune system is IL-6. Of note, IL-6 has an important role in T_{FH} cell differentiation and therefore potentially has an important role in CD4+ T cell priming in the context of an HIV vaccine[20,31]. Interestingly, IL-6 produced by nonhematopoietic cells has a crucial role for maintenance of T_{FH} cells at late time points in a chronic mouse lymphocytic choriomeningitis virus infection and is required for control of that chronic infection[86]. Moreover, the expression of the IL-6 receptor strongly correlates with improved T_{FH} cell responses and improved SIV-specific antibody responses[25].

Rama Amara (Emory University) raised the key issue that CD4+ T cells are a double-edged sword in HIV infection and protection. CD4+ T cells are, of course, the primary cell target of HIV-1. HIV-specific CD4+ T cells are preferentially infected[87], and PD-1+CD4+ T cells preferentially serve as an HIV reservoir in chronic infection[88]. In addition, there is evidence that some form of vaccine-specific T cell responses may enhance susceptibility to HIV[89,90]. Nevertheless, the preferential infection of HIV-specific CD4+ T cells is modest compared to massive overall infection of memory CD4+ T cells[91]. The RV144 trial showed modest protection while eliciting CD4+ T cell responses, and the CD4+ T cell responses were not negatively correlated with protection. In addition, Koup presented data showing that T_{FH} cells are not preferentially infected in chronic SIV+ macaques[25]. Amara suggested that, on balance, potent CD4+ T cell responses are highly desired in an HIV vaccine, but they should express low CCR5, if possible, to limit recruitment to sites of infection. As a counterargument, it was pointed out that it is questionable that CD4+ T cell numbers are limiting for the initial HIV or SIV infection[92]. In response, it was noted that the study making that conclusion used an intravenous

route of SIV transmission; as such, although there are a large number of CD4+ T cells available as potential targets in a systemic infection (and in rectal or intestinal mucosa), a noninflamed vaginal mucosa has very few CD4+ T cells. Yet, virtually all of the early infected cells in the vagina are CD4+ T cells[85,93]. Together, these data suggest that CD4+ T cells may be limiting for SIV and HIV infection of the vagina. Moreover, a paucity of CCR5+CD4+ T cells, both systematically and in mucosal tissues, is frequently observed in natural SIV host species that do not succumb to disease[94,95].

The role of mucosal immune responses was discussed extensively. Akiko Iwasaki (Yale University) summarized the importance of CD4+ T cells for recruiting CD8+ T cells to vaginal epithelium[96] and enhancing CD8+ T cell responses in the draining lymph node[97] to provide protective immunity against herpes simplex virus in mice. Even though IFN-γ secreted from CD4+ T cells is required for cytotoxic T lymphocyte (CTL) recruitment in the vaginal mucosa, the mere presence of IFN- γ in the vagina is not sufficient, as natural killer cells, which secrete comparable levels of IFN- γ in the vagina at days 2–3 after infection, are insufficient to enable CTL migration. Thus, the best way to induce CTL migration is to either locally express CTL-attracting chemokines or to first recruit antigen-specific CD4+ T cells into the vaginal mucosa, which prime the vaginal tissue for CTL migration. Iwasaki proposed an intriguing vaccine concept of 'prime and pull', where first the mice are primed with a vaccine that induces systemic T cell responses (prime) and then CD4+ and CD8+ T cells are recruited with localized vaginal application of chemokines to selectively 'pull' activated lymphocytes during the peak of T cell expansion to that mucosal tissue[98]. If successful, this strategy will improve the understanding of the establishment of immunity at mucosal surfaces and lead to new methods to achieve local immunity. A caveat, however, is that mouse vaginal immunology findings might not translate to human vaccine development, as the mouse vagina is a substantially different tissue type than human vagina. Further research will be required to determine whether such a strategy would be applicable to HIV vaccine approaches.

It is expected that the T_{FH} cell responses probably do not need to be mucosal, as IgG can be efficiently passively transcytosed into luminal spaces, and vaccines against other mucosal infections (poliovirus and human papillomavirus) can protect with only IgG responses[99]. However, a mucosal T_{FH} cell response may be necessary for the generation of a strong HIV-specific IgA response, and IgA may provide better protection than IgG at the mucosal surface in a 1:1 comparison. This remains unknown[57].

Future directions

In conclusion, despite the central importance of CD4+ T cells in the immune system, the roles of HIV-specific CD4+ T cell responses in HIV infection are largely unknown, and these cells have mostly been excluded from HIV vaccine design strategies because they can be infected by HIV. Understanding exactly how CD4+ T cells coordinate the immune system, including directing the quality and persistence of protective CD8+ T cell and B cell responses, is likely to be crucial for increasing the effectiveness of candidate vaccines. Understanding whether fine specificity of the CD4+ T cell affects these functions (and CD4+ T cell differentiation itself) is an additional important topic of research that is not covered here. Given the complexity of the different CD4+ T helper responses and their ability to promote different arms of the immune system, a highly refined understanding of CD4+ T cell functions and their inductive signals is needed to engineer the immune system to elicit these particular responses.

Table 1 Open questions and important future areas of investigation

Are there long-lived human memory T_{FH} cells?
What are the best ways to identify and track T_{FH} cells in blood and tissue?
How do T_{FH} cells provide B cell help in germinal centers to regulate germinal center B cell mutation, affinity maturation, survival, proliferation and plasma cell differentiation?
What Env antigen form best elicits bnAbs?
Do vaccine-elicited HIV-specific antibodies need to be bnAbs to be substantially protective against vaginal or rectal HIV infection?
How can B cell memory after immunization against Env be improved? Is this an Env-specific problem?
Do cytotoxic CD4+ T cells contribute to protection against HIV? Might they have a role in candidate HIV vaccines?
What protective functions beyond B cell help, IFN-γ production and cytotoxicity may HIV-specific CD4+ T cells perform in the context of a candidate HIV vaccine?
Do vaccine-elicited HIV-specific CD4+ T cells increase the risk for HIV acquisition? Can potential risk be minimized, for example, by restricting CCR5 expression on these cells?
What immunization conditions best prime human CD4+ T cell responses?
How do CD4+ T cells help CD8+ T cells in vaccine and chronic infection settings?

Future topics highlighted for study include understanding the factors that contribute to the development of T_{FH} cells and how T_{FH} cells regulate the development of neutralizing antibodies, germinal centers and memory B cell responses (**Table 1**). It remains to be determined how T_{FH} cells can be specifically induced by vaccination and how memory T_{FH} cells can be generated. Both of these processes are likely crucial factors for the rational design of an HIV vaccine and the induction of bnAbs. In addition, determining whether the induction of HIV-specific antiviral CD4+ T cell responses through vaccination, both systemically and at viral portals of entry, will contribute to protection from HIV acquisition or rather increase the susceptibility to HIV infection needs to be resolved. These research areas will inform the design of vaccine candidates that can elicit powerful T_{FH} cell responses with the relevant functional profile. Moreover, they will encourage the design of new candidate HIV vaccines capable of inducing potent and broad antibodies and will help define cytolytic and CD8-helper CD4+ T cell functions that could provide an opportunity for an HIV vaccine to control and eliminate HIV at the portal of entry, should vaccine-induced bnAbs fail to fully prevent infection. Much hard work remains, but we are more optimistic now than we have been in many years.

Note: the views expressed herein are those of the authors and should not be construed to represent the positions of the US Army or the Department of Defense.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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