

A Little Help From the Follicles: Understanding the Germinal Center Response to Human Immunodeficiency Virus 1 Infection and Prophylactic Vaccines

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ABSTRACT: Human immunodeficiency virus 1 (HIV-1) is the causative agent of AIDS. There are currently more than 35 million people living with HIV infection worldwide, and more than 2 million new infections occur each year. The global pandemic caused by HIV-1 is the subject of numerous research projects, with the development of a prophylactic vaccine and a therapeutic cure being the ultimate goals. The classic paradigms of vaccinology have proven incapable of producing a viable vaccine due to the complexity of the virus' replication cycle, its genetic diversity, and a lack of understanding of the immune correlates of protection. Here, we briefly discuss recent vaccine approaches and the immune correlates of protection from HIV-1 infection with a focus on the role of the germinal center as a reservoir of replication-competent virus and its role in the development of broadly neutralizing antibodies in response to vaccination.

KEYWORDS: Germinal center, T_{FH}, HIV-1, vaccines, broadly neutralizing antibody

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Introduction

AIDS first surfaced in the human population as an outbreak of *Pneumocystis carinii* pneumonia among homosexual men in the United States.^{1–3} It is thought to have been circulating undiscovered in Africa for many years prior. The causative agent is a Baltimore class VI retrovirus, called human immunodeficiency virus 1 (HIV-1). The virus is spread by direct sexual contact, through intravenous delivery of blood or blood products, and through vertical transmission from mother to child. The primary mode of transmission is sexual and infection is typically initiated at mucosal sites (Figure 1). As the virus replicates within host cells, it inserts a copy of its genome into that of the host—a copy that is reproduced every time that cell divides and is capable of reactivation at any time—a phenomenon called latency. The World Health Organization estimates that 75 million people have been infected since the summer of 1981, and approximately 36 million lives have been lost to the disease. Today, 0.8% of all adults aged between 15 and 50 years are living with HIV.

The alarming statistics of the HIV-1 pandemic have spurred an unprecedented amount of research. Molecular medicine has provided clinicians with an arsenal of drugs that target important aspects of the viral lifecycle in hopes to decrease viral load and transmissibility. However, there is still no vaccine to prevent transmission and no feasible method to cure an HIV-1-infected individual; thus, preventing infection and removing latent virus are major research goals.

Recent discoveries have shed light on the nature of the infection and produced clues that could have major implications for

a cure. In 2009, the transplant of stem cells lacking the HIV-1 co-receptor, chemokine receptor 5 (CCR5), into an HIV-1-positive patient resulted in a decrease in HIV-1 RNA to undetectable levels.⁴ Recently, researchers at Temple University reported the first successful ablation of the HIV-1 proviral genome from latently infected human cells.⁵ Both these represent huge advancements in HIV research and should be pursued, but at present, there are no feasible treatments for HIV-1-infected patients in places such as sub-Saharan Africa where rates of infection are the highest.

Unfortunately, all recent vaccine endeavors have proven unsuccessful. However, these failures have led to clues about the necessary correlates of protection (Table 1). Passive transfer studies have shown that neutralizing antibodies are capable of controlling HIV-1 infection and preventing infection,^{6–8} even though no trials to date have produced neutralizing antibodies.^{9–13} These results are disheartening, and solving the “neutralizing antibody problem” is the subject of much research. Adenovirus vector-delivered vaccines have not protected macaques from autologous challenge but have decreased viral loads and protected T lymphocytes, indicating that cell-mediated control of infection is also possible. The recent failures of AIDSVAX¹¹ and Merck Ad5¹³ vaccines are disappointing but further our understanding of how to effectively prevent infection. It is clear that “traditional” vaccinology is unlikely to yield a successful therapeutic or prophylactic vaccine against HIV-1. Here, we



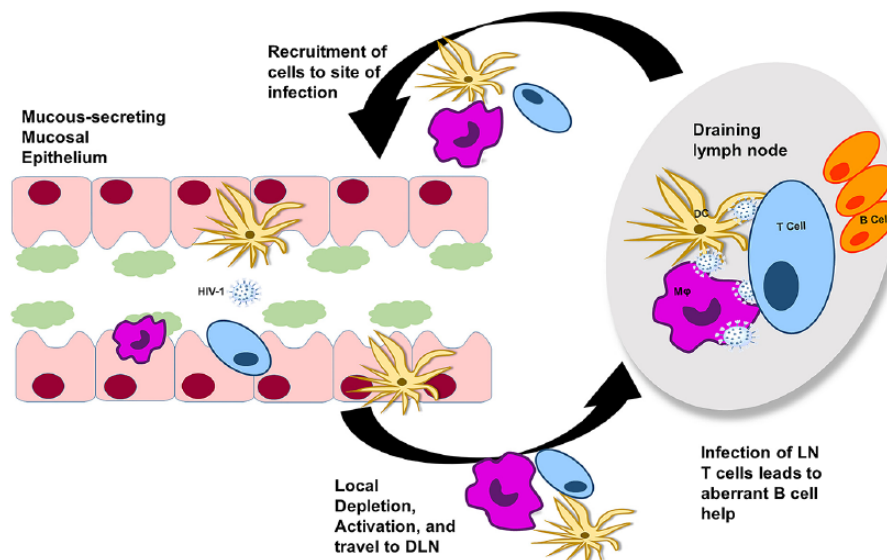


Figure 1. Mucosal pathogenesis of human immunodeficiency virus 1 (HIV-1). Most of the HIV-1 infections occur at mucosal sites via sexual transmission. On gaining access to tissue-resident CD4⁺ T cells or when taken up by tissue-resident dendritic cells (DC), infected cells may be phagocytosed by granulocytes, such as macrophages (Mφ), and the virus is carried to the draining lymph nodes (DLNs) where these antigen-presenting cells may directly infect CD4⁺ T cells. Replication-competent viruses multiply and establish latency. In this way, the DLNs become the largest tissue reservoir during chronic infection.

Table 1. Summary of past HIV-1 vaccine clinical trials.

NAME START DATE (RESULTS PUBLISHED) ^{Ref:}	RV144 SEPTEMBER 2003 (2009) ¹¹	STEP DECEMBER 2004 (2008) ⁹	PHAMBILI/HVTN 503 JULY 2005 (2011) ¹⁰	VRC DNA: AD5HVTN 505 JUNE 2009 (2011) ¹³
	ALVAC-HIV:AIDSVAX B/E (RV144)	HVTN 502/504	HVTN 503	VRC DNA: HVTN 505
Composition	Canarypox-HIV gag/pol/env + recombinant gp120 boost	3 adenovirus serotype 5 vectors encoding HIV-1 gag, pol, and nef	3 adenovirus serotype 5 vectors encoding HIV-1 gag, pol, and nef	HIV clade B gag, pol and nef, and EnvA, EnvB, and EnvC plasmids (6) 4 adenovirus serotype 5 vectors encoding clade B gag-pol fusion and env protein from clades A, B, and C
Regimen	4 ALVAC-HIV injections at 0, 4, 12, and 24 wk plus boosting with AIDSVAX B/E at weeks 12 and 24	3 (0, 4, 26 wk), 1 mL injections of 1.5×10^{10} genome equivalents	3 (0, 4, 26 wk), 1 mL injections of 1.5×10^{10} genome equivalents	3 (0, 4, 8 wk) Biojector injections of 4 mg of each plasmid plus boosting with 10^{10} particle units of rAd5 at week 24
Efficacy	26.4%–31%	–20%	–31%	–25%
Location	Thailand	North and South America, Caribbean, and Australia (clade matched)	South Africa (clade mismatched)	United States
Affiliations	VaxGen (Genentech), Aventis Pasteur	Merck, HVTN, NIAID/NIH	Merck, HVTN, NIAID/NIH	Merck, HVTN, NIAID/NIH
Key findings	V1V2 antibodies contribute to protection, and increased env-specific IgA may inhibit protective antibodies	Preexisting immunity to the adenovirus vector inhibits vaccine efficacy		Natural B-cell clones expressing potential bNAbs are likely deleted during clonal selection due to autoreactivity

Abbreviations: HIV-1, human immunodeficiency virus 1; NIAID, National Institute of Allergy and Infectious Diseases; NIH, National Institutes of Health.

discuss the development of anti-HIV-neutralizing antibodies, the germinal center (GC) response to HIV-1 infection, and the GC response to HIV-1 prophylactic vaccines.

B Cells and Neutralizing Antibodies

B lymphocytes bind antigen via a membrane-bound B-cell receptor (BCR), and they also secrete soluble forms of the

BCR that can bind antigen, called antibodies. The envelope (env) protein of HIV-1 is absolutely critical for viral entry and subsequent replication; thus, antibodies that prevent env-mediated binding and entry are protective. When it was first evidenced that serum samples from some elite HIV-1 controlling patients not undergoing antiretroviral treatment could neutralize a spectrum of HIV-1 viruses,¹⁴⁻¹⁶ researchers grew optimistic that similar broadly neutralizing antibody (bNAb) responses could be elicited by vaccination. In fact, experiments with nonhuman primates (NHPs), as well as analyses from human elite controllers or elite neutralizers, indicate that the presence of neutralizing antibodies correlates with protection from infection.¹⁷⁻¹⁹ Unfortunately, these antibody responses are not typical. In general, these antibodies target conserved but less-immunogenic epitopes of the env protein or the CD4-binding site (CD4bs), whereas most of the natural responses target less well-conserved epitopes. Broadly neutralizing antibodies also tend to share unique features such as polyreactivity, a large variable heavy-chain complementarity-determining region (HCDR3), and evidence of large amounts of somatic hypermutation. In a cross-sectional study of serum samples from more than 200 patients, Hraber et al²⁰ found that most serum displayed some degree of cross-neutralization and that half of the samples were broadly neutralizing, but with titers at or below the thresholds thought to be protective in NHPs.²¹ This indicates that most chronically infected patients develop some form of neutralizing antibody but that bNAbs are less common, and thus, although it may be feasible to induce production of neutralizing antibodies, production of antibodies that are cross-reactive but have a more restricted breadth is likely to be more feasible. Some neutralizing epitopes, such as those in the membrane-proximal external region (MPER), or the variable loops on gp41 remain hidden during infection and are generally poorly immunogenic; thus, finding ways to expose these epitopes is critical to the development of protein antigens that will incite the production of neutralizing antibodies. Novel virucides, such as the peptide triazole-thiols that cause lysis of the HIV-1 virion, present the possibility of creating novel antigens for immunization.²²⁻²⁴ The development of stable, soluble trimeric forms of env with native-like confirmations²⁵ has renewed interest in the generation of neutralizing antibody with protein vaccination.²⁶

When designing an immunogen intended to produce neutralizing antibody, it is important to first consider B-cell ontology. B cells develop in the bone marrow and must pass a tolerance checkpoint intended to prevent self-reactive B cells from entering peripheral circulation. It is estimated that approximately 70% of the B cells produced in the bone marrow die there due to self-reactivity.²⁷ Mature B cells that survive in the periphery are still not completely self-tolerant, and it is estimated that nearly 20% of these are self-reactive.²⁸ What this means for the development of vaccine immunogens is that the repertoire of BCRs that could respond to an immunogen is

much smaller than the total number of BCRs that could be generated from the germ line. If tolerance reduces the affinity of the BCR or the number of available BCRs, this inhibits the ability of the host to mount an adequate humoral response. bNAb 2F5, which binds to the MPER of gp41, is a prime example of an atypical HIV-neutralizing antibody. 2F5 knock-in mice carrying the human heavy-chain variable (V), diversity (D), and joining (J) regions of human 2F5²⁹ have normal pre-B-cell development but are lacking in their ability to produce immature B cells, suggesting tolerance-induced killing of these cells at this stage of B-cell development.³⁰ This was later verified by the discovery that the MPER epitope bound by 2F5 (ELDKWA) is present in both the mouse and the human kynureninase enzyme.^{31,32}

With B-cell ontology in mind, research has shifted to developing immunogens that will induce a particular B-cell lineage. When a naïve B cell encounters an antigen, it recognizes it via the BCR but also undergoes affinity maturation and somatic hypermutation as the humoral response continues to evolve such that the antibodies isolated later in an infection have much higher affinity for their antigen, that is to say, the quality of the B-cell response increases with increasing exposure to the antigen. It is also important to note that HIV rapidly mutates to escape immune pressures encountered in the host such that a constant arms-race occurs inside an infected patient, and usually the B-cell response is outpaced by viral mutagenesis. This is the reason why bNAbs are typically isolated after years of chronic infection.³³ Awareness of this phenomenon has led to the idea of designing a “reverse antigen.” This approach involves isolating a set of clonally related B cells that neutralize HIV-1 and using computational approaches to determine the original or intermediate antibodies that the neutralizing antibodies would have likely developed from during maturation of the humoral response, and designing an antigen that will be recognized by these early antibodies, thus allowing the immune system to mature until neutralizing antibodies are produced.³⁴ Jardine and colleagues³⁵ used this approach to design a gp120 mimetic that was capable of stimulating germ line BCRs and was indeed recognized by the VRC01 class of patient-isolated bNAbs, validating the approach. It has recently been demonstrated that multiple B-cell lineages may cooperate to produce bNAbs,³⁶ indicating that a multipronged approach to this technique is likely necessary. Similarly, in a landmark study, Wu and colleagues demonstrated that over a 15-year period, the mutational rate of antibodies in long-lived plasma cell lineages correlates with the mutational rate of HIV-1 quasispecies and that this high mutational rate leads to the development of the extraordinary antibodies isolated from latently infected patients.³⁷ Finally, McGuire and colleagues developed a modified env immunogen in which certain variable regions of the protein have been removed and demonstrated that this modified env was capable of eliciting germ line-reverted bNAbs as opposed to nonneutralizing antibodies.³⁸ These studies

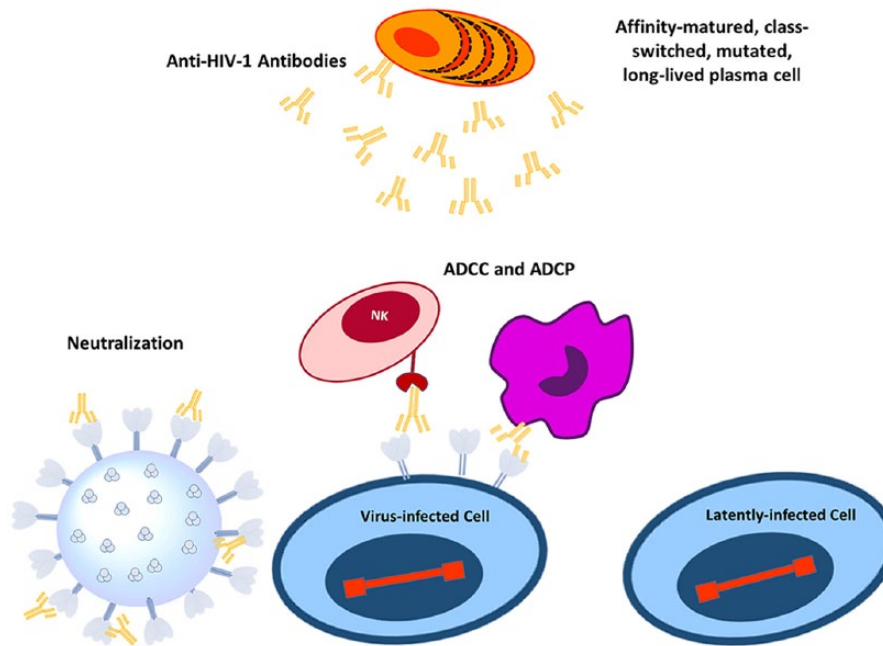


Figure 2. Role of antibodies in HIV-1 infection. Affinity-matured, class-switched, highly-mutated, long-lived plasma cells can secrete anti-HIV-neutralizing antibodies that are capable of neutralizing free virus to prevent infection of target cells (left) as well as mediating ADCC or ADCP (middle) by natural killer cells. Quiescent cells that are latently infected remain immune to anti-HIV responses (right). ADCC indicates antibody-dependent cell-mediated cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; HIV-1, human immunodeficiency virus 1; NK, natural killer.

indicate that rational immunogen design might be used to generate HIV-1 immunogens that specifically result in the production of neutralizing antibodies.

Neutralization is not the only mechanism of antibody-mediated protection (Figure 2). Antibodies also function to induce antibody-dependent cell-mediated cytotoxicity (ADCC). Natural killer (NK) cells mediate classical ADCC by binding to the Fc region of an antibody bound to a cell that expresses viral proteins on its surface. Engagement of the NK-cell Fc receptor leads to release of cytotoxic cytokines that induce apoptosis in the target cell. Studies have shown that early ADCC responses to HIV env proteins can control viremia.³⁹ Interestingly, it has also been shown that the specificity of the antibodies mediating ADCC differs between HIV controllers and noncontrollers, whereas the functionality of their NK cells remains the same with HIV controllers more often producing env-targeted antibodies.⁴⁰ Antibodies can also mediate phagocytosis, complement activation, and mucus entrapment.⁴¹⁻⁴³ In attempts to cure chronic HIV-1 infection, nonclassical HIV immunogens, such as those targeting the transactivator (Tat), have been developed. Indeed, it has been shown that antibodies targeting Tat are capable of preventing HIV reactivation and limiting pathogenesis.⁴⁴

Although production of bNAbs is important to prevent primary HIV-1 infection, it is important to note that the quality of antibody produced as a result of infection or vaccination is greatly enhanced by optimal help from the cell-mediated arm of the immune system. B cells that are activated independently of CD4⁺ helper T cells produce polyclonal antibodies. However, GC formation and the processes of affinity maturation and

isotype switching of antibodies can only occur with the help of T cells. The goal of vaccination would be to prime recipients and induce a memory response that is protective against subsequent HIV-1 challenge, but memory B cells are formed only in GCs. For this reason, it is unlikely that a vaccine platform that solely elicits a B-cell response will provide long-lived immunity. Protein antigens are highly immunogenic and elicit primarily an antibody-mediated response, which may help neutralize infectious viral particles initially but will likely not lead to a robust recall response; thus, a combination of platforms is a better option.

With current data indicating that the traditional approach to vaccine development is likely to be ineffective against HIV, researchers have developed unique antigens and antigen delivery systems. The use of adenovirus and adeno-associated virus (AAV) serotypes to deliver HIV antigens proved a promising way to achieve long-lasting expression of antigens⁴⁵⁻⁴⁷ but can be hindered by the presence of preexisting host immunity against the vector.^{48,49} As the most conserved regions of the envelope protein are the CD4- and co-receptor-binding sites, recombinant fusion proteins targeting these epitopes provide a nontraditional means of controlling or preventing HIV infections.⁵⁰⁻⁵² Interestingly, AAV vectors have been used as a gene therapy vehicle to deliver these molecules.⁵⁰ In the most promising of these approaches, the coding sequence of eCD4-Ig, a recombinant immunoadhesin form of CD4 (CD4-Ig), and CCR5mim1, a CCR5 mimetic peptide derived from bNAbs E51, were delivered to rhesus macaque via intramuscular injection of AAV. Gardner and colleagues demonstrate that eCD4-Ig bound and neutralized most of the HIV-1 isolates better

than either of the components alone and was also capable of neutralizing simian/human immunodeficiency virus (SHIV) isolates. The group also demonstrated stable expression of the construct up to 40 weeks post inoculation in rhesus macaques and protection against challenge with a variety of SHIVs.⁵³ Other promising vectors include modified cytomegalovirus⁵⁴ and adenovirus serotype 26 (Ad26) which may avoid the issue of preexisting immunity that hinders other serotypes.⁵⁵

The GC in HIV-1 Infection

It is important to note that the germinal center reaction (GCR) occurs in the presence of HIV-1 virions. Dendritic cells are known to carry immune complexes containing HIV-1 virions on their processes to the GC, and follicular helper T (T_{FH}) cells are $CD4^+$ and thus targets for HIV-1 infection. Despite this, HIV-1-infected patients have higher numbers of T_{FH} cells and GC B cells,^{56,57} leading to increased numbers of circulating B cells, hypergammaglobulinemia, and, in some cases, B-cell lymphomas.⁵⁸ It is well established that $CD4^+$ T cells in the lymph nodes serve as reservoirs for HIV-1.^{57,59,60} Recently, Kohler et al⁶¹ used spinoculation of human tonsillar cells to demonstrate that GC T_{FH} are highly permissive to HIV infection, more so than extrafollicular or non-GC T_{FH} which express low levels of programmed death 1 (PD-1) and C-X-C chemokine receptor 5 (CXCR5). They further demonstrated, by *in situ* hybridization, the presence of increased HIV-1 RNA in the GCs compared with the extrafollicular areas in sections of inguinal lymph node biopsies from HIV-1-positive, treatment-naïve, non-AIDS patients. It is also well established that T_{FH} from HIV-positive patients provide inadequate help to B cells *in vitro*,⁶² but the underlying cause of this impairment has not been definitively determined. Using a unique cohort of organ transplant patients or patients requiring splenectomy, Colineau et al attempted to elucidate the underlying causes of this impairment. The group determined that there was an increase in GC T_{FH} in HIV-positive patients but that these cells had decreased messenger RNA (mRNA) expression of CD40L, OX40, and inducible costimulator (ICOS)—molecules involved in T_{FH} stimulation.⁶³ Interestingly, although these cells had decreased mRNA levels of STAT3, a master signal transducer required for maintenance of the T_{FH} phenotype, they did not have decreased Bcl6 expression compared with HIV-1-negative cells. Despite this, the number of follicular regulatory T (T_{FR}) cells in HIV-1-positive spleens increased, indicative of continued GC reactions which correlate with the increased numbers of T_{FH} and B cells observed in HIV patients.⁶³ Understanding the source of the defect in T_{FH} help during HIV-1 infection is imperative to the development of an HIV cure.

Although it is evident that lymph node GC T_{FH} cells are major reservoirs of HIV-1 virus in chronically infected patients,⁵⁹ it appears that this location is somehow protected from $CD8^+$ T-cell immune surveillance. There is evidence that the acute increase in $CD8^+$ T cells coincides with a decrease in viremia,⁶⁴ supporting the notion that $CD8^+$ T-cell responses

can mediate clearance of infected cells. Access to lymph node GCs is dependent on the CXCR5/CXCL13 axis. CXCR5⁺ $CD8^+$ T cells have been shown to localize to the B-cell follicle and even exhibit a regulatory function, at least in the context of B-cell lymphoma.⁶⁵ Recently, CXCR5⁺ $CD44^{hi}$ $CD8^+$ T cells with regulatory activity (secretion of interleukin [IL]-10) have been demonstrated to limit HIV replication in T_{FH} , impair IL-21 production, and inhibit IgG secretion during *ex vivo* HIV infection, indicating a unique $CD8^+$ regulatory component of GCs during HIV infection.⁶⁶ Further studies are needed to characterize the access of $CD8^+$ T cells to the GC and to evaluate whether these cells could kill HIV-1-infected T_{FH} cells therein.

The GC and Vaccine-Induced Responses

Germinal centers are dynamic sites within lymphoid organs where mature B cells undergo somatic hypermutation, affinity maturation, and class-switch recombination of the BCR. The outcome of the GCR is that these mature B cells differentiate into long-lived plasma cells and memory B cells, the cells responsible for instituting humoral immunologic memory (Figure 3). Humoral memory is the hallmark of successful vaccination and memory B cells mediate this memory; therefore, optimizing the GC response to HIV-1 vaccine antigens is integral to the development of effective HIV prophylactics.

The selection and differentiation of B cells in the GC are mediated by a special subset of $CD4^+$ T cells called T_{FH} cells. Follicular helper T cells differentiate from naïve $CD4^+$ T cells when antigen is presented to them by antigen-presenting cells that secrete the necessary cytokine milieu, namely, the cytokines IL-21,⁶⁷ IL-6,⁶⁸ and IL-12.⁶⁹ After acquiring the T_{FH} phenotype, these cells migrate to the GC by virtue of their expression of CXCR5 following a gradient of the ligand for CXCR5, C-X-C ligand 13 (CXCL13).^{70,71} In the GC, T_{FH} cells interact both physically and via cytokine signaling with GC B cells. The physical interaction between GC B cells and T_{FH} includes ICOS on T_{FH} and its ligand ICOSL, PD-1⁷² and its ligand PD-L1,⁷³ CD80/CD86,⁷⁴ and CD40/CD40L.⁷⁵ Follicular helper T cells signal GC B-cell differentiation into memory B cells and long-lived plasma cells by secreting IL-4 and IL-21.⁷⁶⁻⁷⁸ Follicular helper T cells are typically identified by the expression of CXCR5, CD4, PD-1, and the transcription factor Bcl-6. It has been shown that PD-1 signaling through PD-L1 is essential for the development of plasma cells and that deficiency in PD-1 signaling leads to a decrease in the number of long-lived plasma cells.⁷⁹

As the GCR is critical to the development of humoral immunity and immune memory, studying this response is critical to the development of vaccines targeting pathogens. Unfortunately, direct examination of the GC response in human patients is rarely feasible. $CD4^+$ cells possessing T_{FH} -like phenotype (CXCR5⁺ PD-1⁺) cells can be isolated from the periphery, and it has been demonstrated that an early preservation of B and T cells with this phenotype predicts a broad

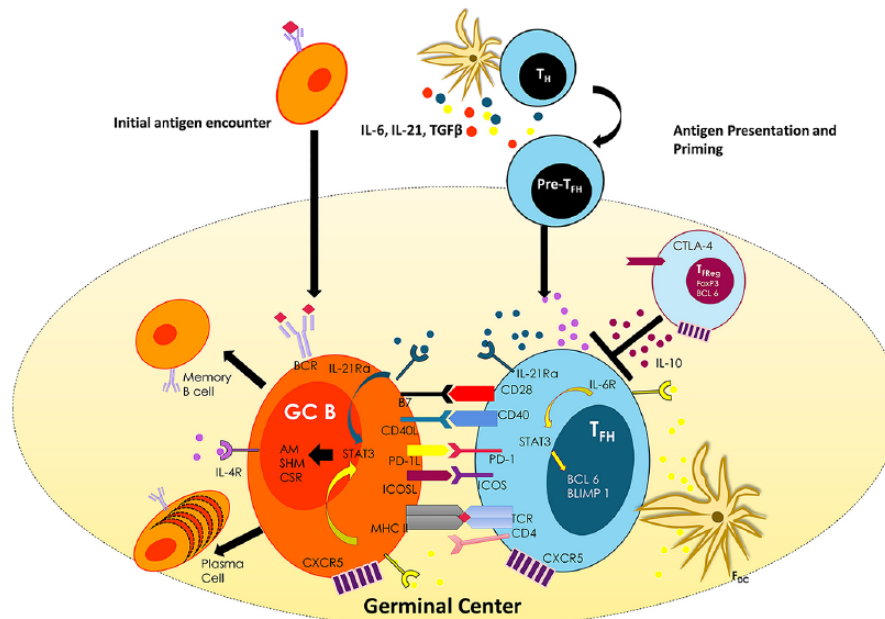


Figure 3. The germinal center reaction. On antigen presentation in the context of the proper cytokine milieu, naïve CD4⁺ T cells differentiate into follicular helper T (T_{FH}) cells and migrate to germinal centers where they select germinal center B cells (GC B) which are responsive to the appropriate antigen via presentation of the antigen on major histocompatibility complex (MHC I). Both T_{FH} and GC B cells traffic to the germinal center by virtue of their expression of the receptor CXCR5, following a chemokine gradient of the CXCR5 ligand CXCL13. T_{FH} cells and GC B cells make physical connection between costimulatory molecules, B7 and CD28, CD40 and CD40L, inducible costimulator (ICOS) and ICOS ligand (ICOSL), as well as PD-1 and PD-1L. This interaction is also cytokine based with the T_{FH} cell secreting interleukin (IL)-21 which is recognized by the IL-12R on GC B cells and mediates STAT3 support of affinity maturation, somatic hypermutation, and class switching of antibodies. T_{FH} cells also secrete IL-4 which is recognized by the IL-4 receptor on GC B cells and supports STAT3 expression. Antigen-specific dendritic cells which also present antigen to T_{FH} secrete IL-6 which promotes Bcl-6 expression in T_{FH} and GC B supporting the GC phenotype. Finally, this reaction is subject to regulation by T follicular regulatory (T_{FR}) cells which secrete IL-10 and inhibit both T_{FH} and GC B function.

neutralizing antibody response in HIV-1 chronically infected patients.^{80,81} These peripheral T_{FH}-like cells have also been connected to the ability to develop an effective humoral response to vaccination.⁸² He et al⁸³ reported that a population of CCR7^{lo}PD-1^{Hi}CXCR⁺ T cells correlated with the formation of GCs in both humans and mice, whereas a CCR7^{Hi}PD-1^{Lo}CXCR5⁺ CD4⁺ T cell possessed a resting, memory-like phenotype and could not be used as a biomarker for GC activity. In another study, conflicting results were observed; when measuring CCR7^{Hi}CXCR5⁺PD-1^{Hi} cells in the periphery T_{FH} (pT_{FH}) cells, Boswell et al⁸⁴ report no relationship between serum-neutralizing capability and pT_{FH} cells, env-specific antibody, or total plasma immunoglobulin levels even though these cells were capable of in vitro B-cell help, concluding that these cells possess a memory-like phenotype as opposed to a classical GC T_{FH} phenotype. These conflicting results indicate that another measure of the GCR is necessary to gauge vaccine-induced responses. Recently, Havenar-Daughton et al⁸⁵ reported the use of soluble CXCL13, the chemokine that attracts T_{FH} and GC B cells to lymph node GCs, in blood as a biomarker for GC activity. The group reported that elevated CXCL13 levels correlated with the development of anti-HIV-1 bNAbs in the well-characterized International AIDS Vaccine Initiative protocol C cohort,^{82,86} as well as in immunized mice and macaques.⁸⁵ Measurement of soluble CXCL13

could provide a feasible means of evaluating HIV-1 vaccine-induced responses as well as a clinical correlate of HIV infection in the lymph node. Recently, this same group correlated the development of tier 2 HIV-neutralizing antibodies post env trimer immunization with GC B-cell and T_{FH}-cell frequencies in a rhesus macaque model using longitudinal fine-needle aspiration.⁸⁷

Finally, inciting a robust GC response to vaccine antigens would mean seeding an antigen-experienced memory cell pool. As T_{FH} select B cells with the appropriate affinity and support their differentiation into long-lived plasma cells and memory B cells, a vaccine that promotes the induction of T_{FH} cells and GCs is likely to induce immunologic memory with fewer immunizations. Although the precise kinetics of memory B-cell formation post antigen exposure are under debate,⁸⁸ it is still widely held that the generation of memory B and plasma cells following exposure to T-dependent antigens depends on the GCR. The T_{FH} phenotype is promoted by signaling through ILs 12, 6, and 21. It has recently been demonstrated that the oil-in-water formulated adjuvant MF59, which has been shown to induce broad, potent, protective humoral responses to influenza vaccination, mediates its adjuvant effect by enhancing T_{FH} cells in early and adult life.^{89,90} Interestingly, it was also reported that this T_{FH} enhancement observed in young adult and adult mice is absent in 1-week-old neonatal mice due to an increased

Table 2. Summary of vaccine adjuvants targeting the germinal center reaction.

	ADJUVANT	FORMULATION/ DELIVERY	ANTIGEN	TARGET	RECEPTOR	MECHANISM	CITATION
Chemical	MF59	Squalene oil, Tween-8, Span-85	Influenza	DCs	N/A	Promotes DC migration, Ag uptake, and Ag presentation in DLNs	Gavillet et al ⁸⁹ and Lofano et al ⁹⁰
Biological	Lipid-based nanoparticles	Lipid bilayers, encapsulating Ag or adjuvant; displaying Ag on surface	Malaria	APCs	N/A	Unknown—increased T _{FH} numbers	Moon et al ¹⁰¹
	RIG-I agonists	5-PPP (dsRNA) or similar RIG-I ligands	Influenza	APCs (hypothesized)	RIG-I	Unknown—enhancement in GC B and T _{FH} numbers; increased plasma and memory B-cell formation	Moon et al ¹⁰¹ and Kulkarni et al ¹⁰²
	Adenosine deaminase	Recombinant protein, plasmid DNA encoded	HIV	DCs, T cells; also B and NK cells	Binds CD26/DPP-4	Enhancement of helper T function and activation via costimulation	Martinez-Navio and colleagues ^{103,104}
	IL-12	Recombinant protein, plasmid DNA encoded	HIV ^a	CD4 T cells, APCs		STAT4-mediated upregulation of Bcl-6	Ma et al, ⁶⁹ Hirao et al, ⁹⁷ Chong et al, ⁹⁸ Kalams et al, ⁹⁹ and Schmitt et al ¹⁰⁰

Abbreviations: APCs, antigen-presenting cells; DCs, dendritic cells; DLNs, draining lymph nodes; dsRNA, double-stranded RNA; GC, germinal center; HIV, human immunodeficiency virus; IL-12, interleukin 12; NK, natural killer; RIG-I, retinoic acid-inducible gene-I; T_{FH}, follicular helper T.

^aUnpublished results.

number of T_{FR} cells which suppress the GCR.⁸⁹ A recent study undertaken to evaluate the benefit of using MF59 instead of alum in the ALVAC vaccine formulation discovered that although MF59 has been shown to enhance GC formation, it did not lead to increased protection from challenge in an SHIV model.⁹¹ The plasticity of CD4⁺ T cells provides another potential avenue for augmenting the GC response. It has been shown that T_H17 cells preferentially differentiate into T_{FH} cells in the mucosa and that this plasticity is causally linked to IgA production in the gut.^{92,93} IgA production has been linked to protection against HIV-1 infection.⁹⁴⁻⁹⁶ This could mean that T_H17 cells are an, as yet, unexplored means of enhancing the GC response and increasing vaccine-induced IgA production in the mucosa, which is the primary site of HIV-1 transmission. DNA vaccines incorporating plasmid-encoded IL-12 have demonstrated the ability to enhance humoral responses to HIV-1 DNA vaccines.⁹⁷⁻⁹⁹ This humoral enhancement is partially linked to increased GC responses induced when IL-12 is included as a molecular cytokine adjuvant (Gary and Kutzler, unpublished results). Interleukin 12 is thought to mediate this effect via STAT4 which supports Bcl-6 expression which promotes and maintains the T_{FH} and GC B phenotype.⁶⁹ Notably, a defect in IL-12 signaling interferes with T_{FH} cell function *in vivo*.¹⁰⁰ The delivery of malaria antigens formulated with nanoparticle-based delivery was recently demonstrated to enhance GC formation and T_{FH} expansion compared with the adjuvant monophosphoryl lipid A.¹⁰¹ The inclusion of ligands that bind

the retinoic acid-inducible gene-I pattern recognition receptor pathway, such as synthetic double-stranded RNA (dsRNA), has also been shown to enhance the GC response to influenza antigens.¹⁰² Adenosine deaminase, a member of the growth factor cytokine family, has been demonstrated to enhance the costimulatory effect of DCs on CD4⁺ T cells¹⁰³ and has been shown to enhance T-cell responses elicited by DCs loaded with inactivated HIV viruses.¹⁰⁴

The discovery of adjuvants or vaccine platforms that enhance the GC response is integral to the development of vaccines with shorter immunization courses (Table 2). Inciting a robust GC response and seeding the memory B-cell pool following a single immunization will mean fewer immunizations, leading to dose sparing and reduced production costs, all important for delivering vaccines to the developing world. The development of reliable biomarkers to gauge the GCR will be critical to evaluate the efficacy of vaccines and adjuvants targeting this reaction.

Perspectives and Future Challenges

The AIDS epidemic has been raging for more than 3 decades. Great advancements have been made in treatment, and the life expectancy of an HIV-1-infected individual approaches that of uninfected individuals. Our ability to drastically decrease the morbidity and mortality associated with chronic infection is impressive; however, 2.5 million people worldwide become infected with HIV-1 each year, that is, more than 6000 people

every day. These intimidating statistics inspire continued research aimed at the development of a prophylactic vaccine.

As immunologic knowledge improves, the ability to create more effective prophylactics increases. In the past 30 years, more has been learned about the types of immune responses elicited by different vaccine platforms since the advent of vaccinology, and this is due in great part to HIV-1 research. Despite this, several obstacles still impede us—preexisting immunity to HIV vaccine vectors may inhibit the efficacy of vaccine candidates, adequate animal models to accurately predict protection in humans do not exist, and the evolution of the virus in response to host selection pressure cannot be accurately predicted. The following question remains: how do we develop a prophylactic vaccine against HIV-1 that is highly immunogenic in humans? The vaccine needs to elicit a neutralizing antibody response, with high titers of plasma antibodies, as well as a large pool of memory B cells, but this response requires T_{FH} cells that are themselves permissive to infection.

The formulation of such a vaccine remains elusive. Protein-based vaccines generally evoke strong antibody responses, and live vectored vaccines can initiate both arms of the immune response, provided they are not hindered by preexisting immunity. DNA vaccines elicit strong cell-mediated responses and appear capable of engendering an effective T_{FH} response, but the recent DNA prime-adenovirus boost human trial did not demonstrate efficacy.¹³ The only approach to demonstrate efficacy to date was the initial phase 2 trial using a canary pox vector bearing HIV-1 gag, pol, and env proteins, boosted with a recombinant gp120 protein, and still the vaccine proved only 25% effective at preventing infection. The plasticity of $CD4^+$ T-cell subsets is of particular interest. Recent studies have demonstrated the ability of mucosal resident T_{H17} cells to differentiate into GC T_{FH} cells and stimulate mucosal antibody secretion. It may be feasible to target these cells prior to differentiation via vaccination, priming an exceptional GCR.

The failure of previous vaccine trial has revealed the complex nature of a successful immune response against HIV-1 as well as the types of immune responses engendered by the different platforms available. This makes better assumptions about the outcomes of a trial possible. The prime-boost regimen will likely prove to be the most effective method for prompting both a humoral and a cell-mediated response. This dearth of knowledge has led to creative approaches to solving the HIV-1 pandemic.

Author Contributions

ENG conceptualized and wrote this article with comments and guidance from MAK.

REFERENCES

- Masur H, Michelis MA, Greene JB, et al. An outbreak of community-acquired *Pneumocystis carinii* pneumonia: initial manifestation of cellular immune dysfunction. *N Engl J Med.* 1981;305:1431–1438.
- Gottlieb MS, Schanker HM, Fan PT, Saxon A, Weisman JD, Pozalski I. *Pneumocystis pneumonia*—Los Angeles. *MMWR Morb Mortal Wkly Rep.* 1981;30:250–252.
- Gottlieb MS, Schroff R, Schanker HM, et al. *Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. *N Engl J Med.* 1981;305:1425–1431.
- Hütter G, Nowak D, Mossner M, et al. Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. *N Engl J Med.* 2009;360:692–698.
- Hu W, Kaminski R, Yang F, et al. RNA-directed gene editing specifically eradicates latent and prevents new HIV-1 infection. *Proc Natl Acad Sci U S A.* 2014;111:11461–11466.
- Baba TW, Liska V, Hofmann-Lehmann R, et al. Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal simian-human immunodeficiency virus infection. *Nat Med.* 2000;6:200–206.
- Mascola JR. Passive transfer studies to elucidate the role of antibody-mediated protection against HIV-1. *Vaccine.* 2002;20:1922–1925.
- Mascola JR, Lewis MG, Stiegler G, et al. Protection of macaques against pathogenic simian/human immunodeficiency virus 89.6 PD by passive transfer of neutralizing antibodies. *J Virol.* 1999;73:4009–4018.
- Buchbinder SP, Mehrotra DV, Duerr A, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. *Lancet.* 2008;372:1881–1893.
- Gray GE, Allen M, Moodie Z, et al. Safety and efficacy of the HVTN 503/Phambili study of a clade-B-based HIV-1 vaccine in South Africa: a double-blind, randomised, placebo-controlled test-of-concept phase 2b study. *Lancet Infect Dis.* 2011;11:507–515.
- Reks-Ngarm S, Pitisuttithum P, Nitayaphan S, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N Engl J Med.* 2009;361:2209–2220.
- Nicholson O, DiCandilo F, Kublin J, et al. Safety and immunogenicity of the MRKAd5 gag HIV type 1 vaccine in a worldwide phase 1 study of healthy adults. *AIDS Res Hum Retroviruses.* 2011;27:557–567.
- Hammer SM, Sobieszczyk ME, Janes H, et al. Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. *N Engl J Med.* 2013;369:2083–2092.
- Binley JM, Lybarger EA, Crooks ET, et al. Profiling the specificity of neutralizing antibodies in a large panel of plasmas from patients chronically infected with human immunodeficiency virus type 1 subtypes B and C. *J Virol.* 2008;82:11651–11668.
- Li Y, Svehla K, Louder MK, et al. Analysis of neutralization specificities in polyclonal sera derived from human immunodeficiency virus type 1-infected individuals. *J Virol.* 2009;83:1045–1059.
- Sather DN, Armann J, Ching LK, et al. Factors associated with the development of cross-reactive neutralizing antibodies during human immunodeficiency virus type 1 infection. *J Virol.* 2009;83:757–769.
- Bruck C, Thiriart C, Fabry L, et al. HIV-1 envelope-elicited neutralizing antibody titres correlate with protection and virus load in chimpanzees. *Vaccine.* 1994;12:1141–1148.
- Mascola JR, Stiegler G, VanCott TC, et al. Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. *Nat Med.* 2000;6:207–210.
- Trkola A, Kuster H, Rusert P, et al. Delay of HIV-1 rebound after cessation of antiretroviral therapy through passive transfer of human neutralizing antibodies. *Nat Med.* 2005;11:615–622.
- Hraber P, Seaman MS, Bailor RT, Mascola JR, Montefiori DC, Korber BT. Prevalence of broadly neutralizing antibody responses during chronic HIV-1 infection. *AIDS.* 2014;28:163–169.
- Hessell AJ, Poignard P, Hunter M, et al. Effective, low-titer antibody protection against low-dose repeated mucosal SHIV challenge in macaques. *Nat Med.* 2009;15:951–954.
- Bastian AR. Microbicides that can Pop HIV-1. *J Antivir Antiretrovir.* 2014;6:054–056.
- Bastian AR, Contarino M, Bailey LD, et al. Interactions of peptide triazole thiols with Env gp120 induce irreversible breakdown and inactivation of HIV-1 virions. *Retrovirology.* 2013;10:153.
- Bastian AR, McFadden K, Duffy C, et al. Cell-free HIV-1 virucidal action by modified peptide triazole inhibitors of Env gp120. *ChemMedChem.* 2011;6:1335–1339.
- Sanders RW, Vesanen M, Schuelke N, et al. Stabilization of the soluble, cleaved, trimeric form of the envelope glycoprotein complex of human immunodeficiency virus type 1. *J Virol.* 2002;76:8875–8889.
- Khattar SK, DeVico AL, LaBranche CC, Panda A, Montefiori DC, Samal SK. Enhanced immune responses to HIV-1 envelope elicited by a vaccine regimen consisting of priming with Newcastle disease virus expressing HIV gp160 and boosting with gp120 and SOSIP gp140 proteins. *J Virol.* 2016;90:1682–1686.
- Wardemann H, Yurasov S, Schaefer A, Young JW, Meffre E, Nussenzweig MC. Predominant autoantibody production by early human B cell precursors. *Science.* 2003;301:1374–1377.

28. Wardemann H, Nussenzweig MC. B-cell self-tolerance in humans. *Adv Immunol.* 2007;95:83–110.
29. Verkoczy L, Diaz M, Holl TM, et al. Autoreactivity in an HIV-1 broadly reactive neutralizing antibody variable region heavy chain induces immunologic tolerance. *Proc Natl Acad Sci U S A.* 2010;107:181–186.
30. Verkoczy L, Chen Y, Bouton-Verville H, et al. Rescue of HIV-1 broad neutralizing antibody-expressing B cells in 2F5 VH × VL knockin mice reveals multiple tolerance controls. *J Immunol.* 2011;187:3785–3797.
31. Yang G, Holl TM, Liu Y, et al. Identification of autoantigens recognized by the 2F5 and 4E10 broadly neutralizing HIV-1 antibodies. *J Exp Med.* 2013;210:241–256.
32. Yang G, Holl T, Liu Y, et al. Kynureninase is the conserved self-antigen mimicked by the 2F5 neutralizing epitope of HIV-1 gp41. *J Immunol.* 2012;188:105–135.
33. Gray ES, Madiga MC, Hermanus T, et al. The neutralization breadth of HIV-1 develops incrementally over four years and is associated with CD4+ T cell decline and high viral load during acute infection. *J Virol.* 2011;85:4828–4840.
34. Haynes BF, Kelsoe G, Harrison SC, Kepler TB. B-cell-lineage immunogen design in vaccine development with HIV-1 as a case study. *Nat Biotechnol.* 2012;30:423–433.
35. Jardine J, Julien J-P, Menis S, et al. Rational HIV immunogen design to target specific germline B cell receptors. *Science.* 2013;340:711–716.
36. Gao F, Bonsignori M, Liao H-X, et al. Cooperation of B cell lineages in induction of HIV-1-broadly neutralizing antibodies. *Cell.* 2014;158:481–491.
37. Wu X, Zhang Z, Schramm CA, et al. Maturation and diversity of the VRC01-antibody lineage over 15 years of chronic HIV-1 infection. *Cell.* 2015;161:470–485.
38. McGuire AT, Dreyer AM, Carbonetti S, et al. Antigen modification regulates competition of broad and narrow neutralizing HIV antibodies. *Science.* 2014;346:1380–1383.
39. Chung AW, Navis M, Isitman G, et al. Activation of NK cells by ADCC responses during early HIV infection. *Viral Immunol.* 2011;24:171–175.
40. Johansson SE, Rollman E, Chung AW, et al. NK cell function and antibodies mediating ADCC in HIV-1-infected viremic and controller patients. *Viral Immunol.* 2011;24:359–368.
41. Chung AW, Schoen MK, Robinson H, et al. Polyfunctional non-neutralizing Fc-antibody responses in acute HIV infection predict spontaneous viral control. *AIDS Res Hum Retrovir.* 2014;30:A89.
42. Ackerman ME, Mikhailova A, Brown EP, et al. Polyfunctional HIV-specific antibody responses are associated with spontaneous HIV control. *PLoS Pathog.* 2016;12:e1005315.
43. Gunn B, Schneider J, Shansab M, et al. Enhanced binding of antibodies generated during chronic HIV infection to mucus component MUC16. *Mucosal Immunol.* 2016;9:1549–1558.
44. Loret EP, Darque A, Jouve E, et al. Intradermal injection of a Tat Oyi-based therapeutic HIV vaccine reduces of 1.5 log copies/mL the HIV RNA rebound median and no HIV DNA rebound following cART interruption in a phase I/II randomized controlled clinical trial. *Retrovirology.* 2016;13:21.
45. Toromanoff A, Chérel Y, Guilbaud M, et al. Safety and efficacy of regional intravenous (RI) versus intramuscular (IM) delivery of rAAV1 and rAAV8 to nonhuman primate skeletal muscle. *Mol Ther.* 2008;16:1291–1299.
46. Schnepf BC, Clark KR, Klemanski DL, Pacak CA, Johnson PR. Genetic fate of recombinant adeno-associated virus vector genomes in muscle. *J Virol.* 2003;77:3495–3504.
47. Schnepf BC, Jensen RL, Clark KR, Johnson PR. Infectious molecular clones of adeno-associated virus isolated directly from human tissues. *J Virol.* 2009;83:1456–1464.
48. Calcedo R, Franco J, Qin Q, et al. Preexisting neutralizing antibodies to adeno-associated virus capsids in large animals other than monkeys may confound in vivo gene therapy studies. *Hum Gene Ther Methods.* 2015;26:103–105.
49. Calcedo R, Wilson JM. AAV natural infection induces broad cross-neutralizing antibody responses to multiple AAV serotypes in chimpanzees [published online ahead of print June 1, 2016]. *Hum Gene Ther Clin Dev.* doi:10.1089/hum.2016.048.
50. Johnson PR, Schnepf BC, Zhang J, et al. Vector-mediated gene transfer engenders long-lived neutralizing activity and protection against SIV infection in monkeys. *Nat Med.* 2009;15:901–906.
51. Jacobson JM, Lowy I, Fletcher CV, et al. Single-dose safety, pharmacology, and antiviral activity of the human immunodeficiency virus (HIV) type 1 entry inhibitor PRO 542 in HIV-infected adults. *J Infect Dis.* 2000;182:326–329.
52. Fletcher CV, DeVille JG, Samson PM, et al. Nonlinear pharmacokinetics of high-dose recombinant fusion protein CD4-IgG2 (PRO 542) observed in HIV-1-infected children. *J Allergy Clin Immunol.* 2007;119:747–750.
53. Gardner MR, Kattenhorn LM, Kondur HR, et al. AAV-expressed eCD4-Ig provides durable protection from multiple SHIV challenges. *Nature.* 2015;519:87–91.
54. Deere JD, Chang WW, Castillo LD, et al. Utilizing a TLR5-adjuvanted cytomegalovirus as a lentiviral vaccine in the nonhuman primate model for AIDS. *PLoS ONE.* 2016;11:e0155629.
55. Baden LR, Walsh SR, Seaman MS, et al. First-in-human evaluation of the safety and immunogenicity of a recombinant adenovirus serotype 26 HIV-1 Env vaccine (IPCAVD 001). *J Infect Dis.* 2012;207:240–247.
56. Chahroudi A, Silvestri G. HIV and Tfh cells: circulating new ideas to identify and protect. *Immunity.* 2016;44:16–18.
57. Lindqvist M, van Lunzen J, Soghoian DZ, et al. Expansion of HIV-specific T follicular helper cells in chronic HIV infection. *J Clin Invest.* 2012;122:3271–3280.
58. Moir S, Fauci AS. B cells in HIV infection and disease. *Nat Rev Immunol.* 2009;9:235–245.
59. Perreau M, Savoye A-L, De Crignis E, et al. Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production. *J Exp Med.* 2013;210:143–156.
60. Miles B, Connick E. T FH in HIV latency and as sources of replication-competent virus. *Trends Microbiol.* 2016;24:338–344.
61. Kohler SL, Pham MN, Folkvord JM, et al. Germinal center T follicular helper cells are highly permissive to HIV-1 and alter their phenotype during virus replication. *J Immunol.* 2016;196:2711–2722.
62. Cubas RA, Mudd JC, Savoye A-L, et al. Inadequate T follicular cell help impairs B cell immunity during HIV infection. *Nat Med.* 2013;19:494–499.
63. Colineau L, Rouers A, Yamamoto T, et al. HIV-infected spleens present altered follicular helper T cell (Tfh) subsets and skewed B cell maturation. *PLoS ONE.* 2015;10:e0140978.
64. Pereyra F, Heckerman D, Carlson JM, et al. HIV control is mediated in part by CD8+ T-cell targeting of specific epitopes. *J Virol.* 2014;88:12937–12948.
65. Chu F, Neelapu SS. CXCR5+CD8+ T cells are localized in B cell follicles and germinal centers and exhibit regulatory and anti-tumor function. *J Immunother Cancer.* 2015;3:P321.
66. Miles B, Miller S, Folkvord J, Levy D, Rakasz E, Skinner P. Follicular regulatory CD8 T cells impair the germinal center response in SIV and ex vivo HIV infection. *PLoS Pathog.* 2016;12:e1005924.
67. Nurieva RI, Chung Y, Hwang D, et al. Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. *Immunity.* 2008;29:138–149.
68. Eto D, Lao C, DiToro D, et al. IL-21 and IL-6 are critical for different aspects of B cell immunity and redundantly induce optimal follicular helper CD4 T cell (Tfh) differentiation. *PLoS ONE.* 2011;6:e17739.
69. Ma CS, Suryani S, Avery DT, et al. Early commitment of naïve human CD4(+) T cells to the T follicular helper (T(FH)) cell lineage is induced by IL-12. *Immunol Cell Biol.* 2009;87:590–600.
70. Hardtke S, Ohl L, Förster R. Balanced expression of CXCR5 and CCR7 on follicular T helper cells determines their transient positioning to lymph node follicles and is essential for efficient B-cell help. *Blood.* 2005;106:1924–1931.
71. De Guinoa JS, Barrio L, Mellado M, Carrasco YR. CXCL13/CXCR5 signaling enhances BCR-triggered B-cell activation by shaping cell dynamics. *Blood.* 2011;118:1560–1569.
72. Pedros C, Zhang Y, Hu JK, et al. A TRAF-like motif of the inducible costimulator ICOS controls development of germinal center TFH cells via the kinase TBK1. *Nat Immunol.* 2016;17:825–833.
73. Sage PT, Francisco LM, Carman CV, Sharpe AH. PD-1 controls lymph node and blood T follicular regulatory cells. *Nat Immunol.* 2013;14:152–161.
74. Good-Jacobson KL, Song E, Anderson S, Sharpe AH, Shlomchik MJ. CD80 expression on B cells regulates murine T follicular helper development, germinal center B cell survival, and plasma cell generation. *J Immunol.* 2012;188:4217–4225.
75. Adem J, Hämäläinen A, Ropponen A, et al. ERK1/2 has an essential role in B cell receptor- and CD40-induced signaling in an in vitro model of germinal center B cell selection. *Mol Immunol.* 2015;67:240–247.
76. Linterman MA, Beaton L, Yu D, et al. IL-21 acts directly on B cells to regulate Bcl-6 expression and germinal center responses. *J Exp Med.* 2010;207:353–363.
77. Zotos D, Coquet JM, Zhang Y, et al. IL-21 regulates germinal center B cell differentiation and proliferation through a B cell-intrinsic mechanism. *J Exp Med.* 2010;207:365–378.
78. Ozaki K, Spolski R, Feng CG, et al. A critical role for IL-21 in regulating immunoglobulin production. *Science.* 2002;298:1630–1634.
79. Good-Jacobson KL, Szumilas CG, Chen L, Sharpe AH, Tomayko MM, Shlomchik MJ. PD-1 regulates germinal center B cell survival and the formation and affinity of long-lived plasma cells. *Nat Immunol.* 2010;11:535–542.
80. Cohen K, Altfeld M, Alter G, Stamatatos L. Early preservation of CXCR5+ PD-1+ helper T cells and B cell activation predict the breadth of neutralizing antibody responses in chronic HIV-1 infection. *J Virol.* 2014;88:13310–13321.
81. Locci M, Havenar-Daughton C, Landais E, et al. Human Circulating PD-1+CXCR3-CXCR5+ memory Tfh cells are highly functional and correlate

- with broadly neutralizing HIV antibody responses. *Immunity*. 2013;39:758–769.
82. Pallikkuth S, Parmigiani A, Silva SY, et al. Impaired peripheral blood T-follicular helper cell function in HIV-infected nonresponders to the 2009 H1N1/09 vaccine. *Blood*. 2012;120:985–993.
 83. He J, Tsai LM, Leong YA, et al. Circulating precursor CCR7(lo)PD-1(hi) CXCR5+ CD4+ T cells indicate Tfh cell activity and promote antibody responses upon antigen reexposure. *Immunity*. 2013;39:770–781.
 84. Boswell KL, Paris R, Boritz E, et al. Loss of circulating CD4 T cells with B cell helper function during chronic HIV infection. *PLoS Pathog*. 2014;10:e1003853.
 85. Havenar-Daughton C, Lindqvist M, Heit A, et al. CXCL13 is a plasma biomarker of germinal center activity. *Proc Natl Acad Sci U S A*. 2016;113:2702–2707.
 86. Landais E, Huang X, Havenar-Daughton C, et al. Broadly neutralizing antibody responses in a large longitudinal sub-Saharan HIV primary infection cohort. *PLoS Pathog*. 2016;12:e1005369.
 87. Havenar-Daughton C, Carnathan DG, De la, Peña AT, et al. Direct probing of germinal center responses reveals immunological features and bottlenecks for neutralizing antibody responses to HIV Env trimer. *Cell Rep*. 2016;17:2195–2209.
 88. Weisel FJ, Zuccarino-Catania GV, Chikina M, Shlomchik MJ. A temporal switch in the germinal center determines differential output of memory B and plasma cells. *Immunity*. 2016;44:116–130.
 89. Gavillet BM, Eberhardt CS, Auderset F, et al. MF59 mediates its B cell adjuvanticity by promoting T follicular helper cells and thus germinal center responses in adult and early life. *J Immunol*. 2015;194:4836–4845.
 90. Lofano G, Mancini F, Salvatore G, et al. Oil-in-water emulsion MF59 increases germinal center B cell differentiation and persistence in response to vaccination. *J Immunol*. 2015;195:1617–1627.
 91. Vaccari M, Gordon SN, Fourati S, et al. Adjuvant-dependent innate and adaptive immune signatures of risk of SIVmac251 acquisition. *Nat Med*. 2016;22:762–770.
 92. Hirota K, Turner J-E, Villa M, et al. Plasticity of Th17 cells in Peyer's patches is responsible for the induction of T cell-dependent IgA responses. *Nat Immunol*. 2013;14:372–379.
 93. Milpied PJ, McHeyzer-Williams MG. High-affinity IgA needs Th17 cell functional plasticity. *Nat Immunol*. 2013;14:313–315.
 94. Kaul R, Trabattini D, Bwayo JJ, et al. HIV-1-specific mucosal IgA in a cohort of HIV-1-resistant Kenyan sex workers. *AIDS*. 1999;13:23–29.
 95. Devito C, Broliden K, Kaul R, et al. Mucosal and plasma IgA from HIV-1-exposed uninfected individuals inhibit HIV-1 transcytosis across human epithelial cells. *J Immunol*. 2000;165:5170–5176.
 96. Kaul R, Plummer F, Clerici M, Bomsel M, Lopalco L, Broliden K. Mucosal IgA in exposed, uninfected subjects: evidence for a role in protection against HIV infection. *AIDS*. 2001;15:431–432.
 97. Hirao LA, Wu L, Khan AS, et al. Combined effects of IL-12 and electroporation enhances the potency of DNA vaccination in macaques. *Vaccine*. 2008;26:3112–3120.
 98. Chong S-Y, Egan MA, Kutzler MA, et al. Comparative ability of plasmid IL-12 and IL-15 to enhance cellular and humoral immune responses elicited by a SIVgag plasmid DNA vaccine and alter disease progression following SHIV(89.6P) challenge in rhesus macaques. *Vaccine*. 2007;25:4967–4982.
 99. Kalams SA, Parker S, Jin X, et al. Safety and immunogenicity of an HIV-1 gag DNA vaccine with or without IL-12 and/or IL-15 plasmid cytokine adjuvant in healthy, HIV-1 uninfected adults. *PLoS ONE*. 2012;7:e29231.
 100. Schmitt N, Bustamante J, Bourdery L, et al. IL-12 receptor β 1 deficiency alters in vivo T follicular helper cell response in humans. *Blood*. 2013;121:3375–3385.
 101. Moon JJ, Suh H, Li AV, Ockenhouse CF, Yadava A, Irvine DJ. Enhancing humoral responses to a malaria antigen with RIG-I particle vaccines that expand Tfh cells and promote germinal center induction. *Proc Natl Acad Sci U S A*. 2012;109:1080–1085.
 102. Kulkarni RR, Rasheed MAU, Bhaumik SK, et al. Activation of the RIG-I pathway during influenza vaccination enhances the germinal center reaction, promotes T follicular helper cell induction, and provides a dose-sparing effect and protective immunity. *J Virol*. 2014;88:13990–14001.
 103. Martinez-Navio JM, Casanova V, Pacheco R, et al. Adenosine deaminase potentiates the generation of effector, memory, and regulatory CD4+ T cells. *J Leukoc Biol*. 2011;89:127–136.
 104. Climent N, Martinez-Navio JM, Gil C, et al. Adenosine deaminase enhances T-cell response elicited by dendritic cells loaded with inactivated HIV. *Immunol Cell Biol*. 2009;87:634–639.