T cell immune responses to HIV-1

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1. ABSTRACT

The recent use of multiparametric flow cytometry to monitor T cell immune responses complements traditional assays, such as IFN-gamma ELISPOT, to provide more information on the functional complexity of $CD4^+$ and $CD8^+$ T cell immune responses induced either by natural infection, or by immunization. In this review, we provide a general background on T cell subsets, and describe the cellular immune response during natural HIV-1 infection. We then review T cell responses to current candidate HIV-1 vaccines. Taken together, this helps to formulate our understanding of the immune correlates of protection required for an effective prophylactic HIV-1 vaccine. Finally, we emphasize current dendritic cell based vaccine strategies designed to modulate immunity to establish immune protection against HIV-1.

2. INTRODUCTION

Recent advances in the development of multiparametric flow cytometry (1) have provided more information on the functional complexity of both $CD4^+$ and $CD8^+$ T cell immune responses induced either during natural infection, or by immunization. In this review, we will first provide general background on T cell subsets, and then describe the cellular immune response during natural HIV-1 infection. We will then review T cell responses to current candidate HIV-1 vaccines, and how this knowledge, taken together, helps inform our understanding of the immune correlates of protection required for an effective vaccine against HIV-1. Finally, we will emphasize some current vaccination strategies designed to direct the immune system towards this protective response.

3. GENERAL T CELL IMMUNE RESPONSES

After infection or vaccination, naïve T cells that traffic through lymphoid organs encounter specific antigens presented at the surface of antigen presenting cells (APCs), specifically dendritic cells (DCs). Following this interaction, the antigen-specific T cells undergo a program of extensive division and differentiation, and become activated effector T cells (2) that migrate to tissues and defend against infection. This massive expansion is followed by a rapid and well-regulated contraction phase, which might or might not coincide with clearance of the antigen, in which approximately 90-95% of the effector cells are eliminated (3). These successive events give rise to a pool of antigen experienced memory T cells which are maintained, in some cases, for life (4). The establishment of this population of memory T-cells allows the individual to respond quickly and efficiently to subsequent encounters with the same pathogen.

Although both $CD4^+$ and $CD8^+$ T cells initiate their program of differentiation simultaneously, $CD8^+$ T cells divide sooner and more rapidly, and therefore more readily develop into effector cells after short-term primary stimulation than do $CD4^+$ T cells (5). Meanwhile, evidence indicates that $CD4^+$ T cells can regulate the quality of the memory $CD8^+$ T cells generated. These cells are likely important for optimal generation of memory $CD8^+$ T cells following acute infections (6-8), and for sustained $CD8^+$ T cell responses during chronic infections (9).

In both humans and mice, memory $CD4^+$ and $CD8^+$ T cell populations exhibit considerable heterogeneity. The first distinction between memory and effector T cells, early after *in vivo* priming, can be demonstrated by the expression of IL-7R (CD127) at the cell surface (10). The $CD127^+$ memory T cells then further divide, based on homing characteristics and effector functions, into central memory (Tcm) and effector memory (Tem) subsets (11). This clear method of differentiation is particularly true after resolution of infection, in which the exposure to antigen is transient due to the clearance of the pathogen by effectors. However, during chronic /persistent infections, with pathogens such as HIV-1, the strength and the duration of antigenic stimulation affect both the differentiation process, and the functional competency of the resulting effector and memory cells (12, 13) . Indeed, several studies have validated that memory T cells constitute intermediates arrested at different stages of differentiation (14, 15). Thus, the functional properties of a given antigen specific T cell response are determined by the relative proportions of memory T cell subsets generated (16).

3.1. Effector T cells

Effector T cells constitute the first line of defense against pathogens, and circulate during acute infections. Primed T cells are highly activated and dividing cells that initially express CC-chemokine receptor 7 (CCR7), the lymph-node homing receptor CD62 ligand (CD62L) and the co-stimulatory receptors CD28 and CD27. These markers are progressively down regulated, as these primed T cells become effector T cells. $CD8^+$ T cell effectors can secrete IFN-gamma, and TNF-alpha in an antigen-specific manner to induce cell death. They also express perforin which directly mediates cytotoxicity of target cells. Their ability to produce IL-2 is low, but increases gradually during memory $CD8^+$ T cell differentiation (12). Accordingly, analysis of $CD4^+$ T cell responses in patients with primary HIV-1 and primary CMV infection have shown that the large majority of antigen-specific $CD4^+$ T cells are single IFN-gamma-secreting cells, while IFN-gamma/IL-2- secreting cells and single IL-2-secreting cells are poorly represented (17).

3.2. Memory T cells: Tem and Tcm

Memory T cells persist for extended periods of time due to antigen-independent homeostatic turnover. They constitute a potent line of immediate defense, because they are present at higher numbers than naïve cells, and respond rapidly upon reencounter with a pathogen. Memory T cells can be divided into two subsets: central memory T cells (Tcm) and effector memory T cells (Tem). Both are present in the spleen and the blood, and are thought to play complementary roles in this defense process.

Effector memory T cells mainly reside within, or recirculate through, peripheral non-lymphoid tissues, and provide an immediate/rapid effector defense. These cells do not express CCR7 or CD62L ($CCR7^-CD62L^-$), and rapidly acquire effector functions such as cytokine production (e.g. IFN-gamma) upon antigen restimulation. $CD8^+$ Tem cells also acquire the ability to directly kill target cells through perforin and granzyme secretion. Tem cells are characterized by a limited proliferative capacity (11). Through their expansion and differentiation, these cells constitute a pool of secondary effector cells (as compared to the effector cells during the acute response) which ultimately lead to a subset of $CD45\ RA^+\ CD27^-\ CCR7^-$ T cells expressing CD95 ligand, high levels of perforin and granzyme B, and are directly cytotoxic *ex vivo*. These T cells have been designated as late differentiated effector T cells (14) .

Central memory T cells (Tcm) have the potential to generate a functional secondary (recall) response and provide a reserve of defense. They express CCR7 and CD62L molecules ($CCR7^+\ CD62L^+$) that permit trafficking into the lymph nodes. In steady state, these cells are capable of self-renewal by homeostatic proliferation (18). Upon antigenic restimulation, Tcm also convert to secondary effector cells, but their ability to expand is more vigorous than that of Tem. Their full proliferative capacity correlates with their ability to maintain interleukin-2 (IL-2) production. This ability to produce IL-2 is a hallmark of Tcm cells. For example, in the tetanus model of Ag clearance, it has been shown that the dominant population of antigen-specific T cells was represented by $CD4^+$ T cells with a typical Tcm phenotype, that secreted IL-2, but not IFN-gamma (17). Most importantly, due to their ability to generate a larger population of highly activated secondary effector cells (e.g, generate a potent recall response), Tcm are more effective mediators of protective

immunity than Tem, and play a crucial role in long term protection (19-23).

The relationship between Tem and Tcm, and whether they represent interconnected or distinct lineages, is still subject to debate. Three different models of differentiation have been proposed. The detection of human CD4$^+$ and CD8$^+$ Tem by flow cytometry analysis, several years after priming suggests that they are intrinsically stable or continuously replenished through differentiation of Tcm. In line with this hypothesis, *in vitro* culture systems using human T cells have shown that Tcm cells differentiate into Tem cells (11). However, in contrast with this study, analyses of the TCR repertoire of human blood CD8$^+$ memory T cells indicate that Tcm and Tem represent mostly separate lineages (24). Moreover, another study has shown that human CD4$^+$ and CD8$^+$ Tem cells exhibit dynamic differentiation, involving transient and stable changes to the Tcm phenotype and its properties (25). Lastly, investigation of the molecular profile of human CD8$^+$ Tcm and Tem using gene expression microarrays distinguish Tcm from Tem cells based on their ability to express genes involved in self renewal. This study reinforces the concept that Tcm represent memory stem cells (26).

Understanding the relationship between these populations is particularly important for the design of interventional therapeutics as well as prophylactic vaccines. It will be important to determine whether naïve T cells can be educated to become Tcm, or whether Tcm can be expanded from existing Tem during chronic infection.

4. T CELL IMMUNE RESPONSES DURING HIV-1 INFECTION

Although untreated HIV-1 infection follows a progressive course in virtually all infected individuals, there is strong evidence that anti-HIV-1 immune T cell responses are essential in limiting HIV-1 replication.

Cytotoxic CD8$^+$ T cells (CTLs) have been strongly implicated in the control of virus replication in HIV-1-infected humans and SIV-infected monkeys. For example, rhesus macaques fail to contain the initial peak SIV viremia if their CD8$^+$ T cells are depleted at the time of infection. In humans, evidence for the protective role of CD8$^+$ T cells comes from the temporal association of CTL responses with the decline in plasma viremia following acute infection and peak viremia (27) and also, from the presence of a vigorous proliferative response in long-term non-progressors (LTNPs).

4.1. CD4$^+$ T cell response

During primary infection, a large majority of anti-HIV-1 specific CD4$^+$ T cells with an effector phenotype (single IFN-gamma secreting cells) develop in response to the high antigen load (17). Later in the course of untreated infection, despite severe depletion of CD4$^+$ T cells, the functional CD4$^+$ T cell response to different HIV-1 antigens (Gag, Nef, Pol, Env) persists in the peripheral blood of infected patients, as demonstrated by detection of

IFN-gamma and class II tetramer staining (28). In general, in chronically infected patients, Gag-specific responses dominate the CD4$^+$ T cell response to HIV-1 (29). Despite the presence of HIV-1 specific IFN-gamma secreting cells CD4$^+$ T cells, many groups have documented that these cells have lost their ability to produce IL-2 and to proliferate in response to antigen stimulation (30-32). Thus, upon chronic exposure, there is a skewed representation of IFN-gamma only producers that are not associated with control of HIV-1 replication. Phenotypic analysis of these cells have shown that high viremia skews the Gag-specific CD4$^+$ T cells away from an IL-2 producing Tcm phenotype (CCR7$^+$ CD45RA$^-$) and toward poorly proliferative IFN-gamma producing Tem phenotype (CCR7$^-$ CD45RA$^-$) (33). When this CCR7$^-$ T cell population was further subdivided based on CD57 expression, CD57$^+$ CD4$^+$ T cells were found to be proliferation incompetent cells associated with increased apoptosis (34). In contrast, the presence of HIV-1 specific CD4$^+$ T cells that are able to strongly proliferate in response to HIV-1 antigens has been associated with the control of HIV-1 replication in the naturally protected, LTNPs (35). Whether these highly proliferating cells fall into the subset of Tcm versus Tem remains to be elucidated. In direct contrast with progressors, PBMC from clinical non-progressors (LTNPs) exhibit strong and broad responses to many HIV-1 antigens associated with secretion of both type 1 and type 2 cytokines (IL-2 and IL-4, respectively) expressing a normal memory phenotype (36). Finally, during infection with HIV-2, which is associated with a better clinical outcome, it has been shown that the frequency of CD4$^+$ T cells able to produce IL-2 is better preserved than in HIV-1 infection (37, 38). Thus, the ability of HIV-1 specific CD4$^+$ T cells to secrete IL-2 constitutes a correlate with protective immunity. Recently, a comprehensive analysis of T cell phenotype and function was performed within a group of 45 antiretroviral naive controllers with low level viremia. These patients exhibited higher frequencies of HIV-1 specific IL-2$^+$ IFN-gamma $^+$ CD4$^+$ T cells, as previously described, with a low level of proliferating cells within the less differentiated T cell subpopulation (CD45RA$^+$, CD27$^+$, CD28$^+$ CCR7$^+$). Thus the apparent T cell control of HIV-1 replication is associated with an immunological state in which the host responds to HIV-1 by expanding, but not exhausting HIV-1 specific T cells, while maintaining a relatively quiescent immune system (39). This low level of immune activation has also been associated with low susceptibility to HIV-1 infection in high risk exposed seronegative individuals(40).

4.2. CD8$^+$ T cell response

During acute HIV-1 infection, the induction of HIV-1 specific CD8$^+$ effector T cells with the capacity to kill HIV-1 infected cells and secrete IFN-gamma is associated with a rapid and dramatic decline in viremia (41-43) probably reflecting the strong antiviral activity of these cells. In this early phase of infection, the HIV-1 specific CD8$^+$ T cell response is typically low in magnitude and narrowly directed against some viral epitopes, such as Nef, Tat and Env (44-46). In the absence of HAART treatment, these initial CD8$^+$ T cells responses tend to disappear. This is likely due in part to escape variants within the viral

epitopes which lead to the emergence of further responses which are less efficient in reducing viral load (46).

In striking contrast, during chronic HIV-1 infection, high levels of viral replication occur in the presence of large numbers of HIV-1 specific IFN-gamma producing CD8$^+$ T cells. These cells comprise high avidity, antigen-specific CD8$^+$ T cells reactive to several viral proteins, including Gag, Pol and Env (47-51). These data suggest that the HIV-1 specific CD8$^+$ T cell responses become progressively less effective, and these defects are not detected by assays that quantify antigen specific interferon gamma production alone.

In fact, several studies of chronically HIV-1 infected patients have shown that the HIV-1 specific cell pool is predominantly composed of pre-terminally or intermediately differentiated effector memory T cells having a CD45RA$^-$ CCR7$^-$ CD62L$^-$ CD8$^+$ phenotype and relative low levels of perforin (52-54). Papagno *et al.* have also demonstrated that HIV-1 replication in chronically infected individuals leads to activation of the early differentiated (CD27$^+$ CD28$^+$) antigen experienced CD8$^+$ T cells. This activation occurs both in the HIV-1 specific and unrestricted cells, and results in further differentiation of these cells into a state of replicative senescence, characterized by a CD27$^-$CD57$^+$ phenotype (55). Altogether, these results, plus the absence of HIV-1 specific central memory T cells support the idea that CD8$^+$ T cell differentiation is incomplete or arrested in HIV-1 infected individuals, and evidence an exhaustion of T cell competence.

Beside phenotypic alterations, functional defects have also been detected in HIV-1 progressors: several studies using MHC class I tetramer binding or IFN-gamma detection to identify antigen-specific CD8$^+$ T cells have clearly established that HIV-1-specific CD8$^+$ T cells can not always be propagated after *in vitro* culture of PBMCs in response to HIV-1 peptides (56-58), polyclonal stimulation (54) or HIV-1-infected autologous CD4$^+$ T cells (59). We have also reported this HIV-1 -specific CD8$^+$ T cells defect in proliferation, even when these cells have been challenged with potent mature dendritic cells (60).
In contrast to progressor patients, CD8$^+$ T cells in acute HIV-1 infection have strong proliferative capacities, which are rapidly lost in the presence of continuing viral replication. This proliferation is critically dependant on the presence of IL-2 secreting HIV-1 specific CD4$^+$ T cells. These data suggest that the proliferative impairment of HIV-1 specific CD8$^+$ T cells during chronic infection is not primarily due to an intrinsic functional defect of these cells, but rather represents a direct consequence of the progressive loss of IL-2 secreting, HIV-1 specific CD4$^+$ T cells (61).

Also, HIV-1 specific CD8$^+$ T cells from LTNPs have a greater capacity to proliferate than T cells from progressors, and this proliferation is tightly coupled to increase in perforin expression (59). In LTNPs, the strong proliferative capacity of HIV-1 specific CD8$^+$ T cells, assessed by 3H thymidine incorporation, has also been associated with an increase in IL-2 in the supernatant upon *in vitro* expansion with specific peptides (36). In these unique patients, we also reported the association of HIV-1 specific CD8$^+$ effector T cell expansion with the presence of a small subset of Gag-specific, IL-2 producing CD8$^+$ T cells which might represent functional central memory, part of a complete maturation process in the CD8$^+$ T cell compartment (60). Recently, Zimmerli and colleges have demonstrated that the HIV-1 specific IFN-gamma/IL-2 secreting CD8$^+$ T cells support the CD4 independent proliferation of HIV-1 specific CD8$^+$ T cells (62). Phenotypically, it has also been shown through tetramer staining that up to 50% of HIV-1 specific CD8$^+$ T cells in non-progressors are characterized by a fully differentiated phenotype (CD45RA$^+$ CCR7$^-$), suggesting that full maturation can take place in HIV-1 infected individuals in the appropriate immunological setting (63). Thus, superior proliferative and effector functions distinguish LTNPs patients from typical HIV-1 infected progressors, suggesting that the capacity to make perforin and IL-2, and to vigorously expand in culture, represent essential functions in HIV-1 immunological control.

Overall, polyfunctional IFN-gamma$^+$/IL-2$^+$ HIV-1 specific CD4$^+$ and CD8$^+$ T cell responses define the best correlates of protective immune response during HIV-1 infection known to date (64, 65).

However, we are still far from a precise definition of a T cell mediated immune correlate of protection in HIV-1 infection. New studies that challenge our understanding of these correlates continue to emerge. Thus, very recently, Betts *et al.* focused on the quality of the T cell response in 79 HIV-1 infected progressors and 9 non-progressors by using multicolor flow cytometry technology. The measurement of five CD8$^+$ T cell functions (degranulation(CD107a), IFN-gamma, MIP-1-beta, TNF-alpha and Il-2) directed towards multiple antigens (Gag, Pol, Env , Nef , Tat, Rev) in each patient allowed them to define a functional profile of HIV-1 specific CD8$^+$ T cells. Based on their ability to detect anywhere from two to five different functions in the same cell, they found that progressors had limited functional profile compared to non-progressors. The response in progressors is characterized by antigen specific cells with three or less simultaneous functions (IFN-gamma, MIP-1-beta, TNF-alpha, CD107a), an absence of cells expressing all five measured functions, and a paucity in IL-2 production. In contrast, non-progressors had a response notably shifted to cells positive for all five functions, a larger proportion of cells positive for four functions, and a higher percentage of responding cells producing IL-2. They also found that individual HIV proteins can stimulate qualitative diverse response profiles in both populations. Altogether their results indicate that measuring responses by five functions provides a better differentiation between progressors and non-progressors than measuring only IFN-gamma and Il-2 (66). Of interest, their results also indicate that memory phenotype is not necessarily predictive of functionality. Thus the presence of five positive function cells in the non-progressors was not simply due to an over representation of cells with central memory phenotype.

5. T CELL IMMUNE RESPONSES TO HIV-1 VACCINES

5.1. Challenges in HIV-1 Vaccine Development

Despite vigorous efforts for more than two decades, an effective vaccine to prevent HIV-1 eludes us. Current licensed vaccines for other pathogens, both bacterial and viral, rely primarily on the antibody response to eradicate circulating virus. Furthermore, the majority of these vaccines were developed empirically, based on whole killed or live attenuated pathogens. These approaches fail with regard to HIV for a number of reasons. First, an effective vaccine against a retrovirus likely requires both efficient humoral and cell mediated immunity to eradicate free and cell-associated virus. Second, administration of whole killed or live attenuated HIV has raised safety concerns (67) that prevent their use. Lastly, natural host clearance of HIV-1 has not been documented to date, so we have no clear indication of the type of immunologic response required for protection from infection. These are some of the factors that have hindered development of an effective vaccine against HIV-1.

5.1. Types of HIV-1 vaccines, and known responses to date

5.2.1. Recombinant Proteins

Initial vaccine attempts to induce neutralizing antibodies against HIV-1 included recombinant envelope proteins, gp120 and rgp160. In early studies, these vaccines generated neutralizing antibodies in chimpanzees (68). Exogenous proteins are presented by MHC Class II to elicit a predominant CD4$^+$ T cell response, characterized by antigen-specific CD4$^+$ T cells capable of lysing HIV-1 infected CD4$^+$ target cells in humans (69, 70). Despite this, rgp120 failed to protect against HIV-1 infection in the only Phase III efficacy trial of a candidate HIV-1 vaccine to date (71).

5.2.2. DNA

The goal of a successful T-cell based vaccine is to expand the magnitude and breadth of T cell epitopes recognized after natural infection (72). Approaches to elicit a strong cell mediated immune response include DNA-based vaccinations, viral vectors, and adjuvants. In contrast to proteins, recombinant DNA plasmids expressing one or more genes from HIV, simian immunodeficiency virus (SIV), or chimeric SHIV elicit CD8+ T cell responses in nonhuman primates (73) and humans (74). These responses are characterized by cytotoxic T cell lysis of antigen expressing cells, cellular proliferation to antigen, antigen-specific tetramer staining, and IFN-gamma secretion in response to peptide antigen (75-77). Despite the fact that DNA based vaccines have shown protection against HIV-1 challenge in chimps (78), DNA vaccines alone are relatively weak immunogens in comparison to viral vectors, so several strategies are being developed to improve the immunogenicity of DNA vaccines in humans (79).

5.2.3. Poxviridae

Several researchers have used recombinant viral vectors encoding one or more HIV or SIV genes to improve gene delivery to cells, in order to drive endogenous expression and MHC Class I presentation. Canarypox was one of the earliest viral vectors to move forward in clinical development. Intramuscular administration leads to antigen-specific CD4$^+$ and CD8$^+$ cytotoxic T lymphocytes in humans (80, 81), including a memory component, defined as CD3$^+$CD8$^+$ (or CD4$^+$) CD45RO$^+$ (82). Viral vectors have the additional advantage of stimulating the innate immune response. Canarypox has been shown to expand natural killer cells elicit gamma delta cells, as well as lead to IFN-gamma secretion in response to vector, but not HIV-1, antigens (83). Intramuscular administration has also been shown to elicit mucosal CD8$^+$ MHC-Class I restricted antigen specific CTL in the rectal mucosa, which may be important in preventing a sexually transmitted pathogen. Despite these responses, vaccination of recombinant canarypox expressing gp120, Gag and protease did not afford protection from heterologous HIV-1 challenge in chimpanzees (84). A second viral vector in the poxvirus family is modified vaccinia ankara (MVA), an attenuated, non-replicating form of vaccinia virus. It elicits similar antigen-specific CTL against expressed genes in macaques (85).

5.2.4. Adenovirus

Replication-defective adenovirus serotype 5 (Ad5) is the third vector that has progressed the farthest in clinical development. Adenoviral vectors are advantageous because they have a high insert capacity, are highly immunogenic, and are easily manipulated for large scale production (86). In rhesus monkeys, intramuscular immunization of Ad5 expressing SIV Gag led to high levels of antigen-specific CD3$^+$ CD8$^+$ T cells by tetramer assay and IFN-gamma ELISPOT. This led to attenuation, but not protection, from subsequent SIV challenge (87). In humans, a replication defective adenoviral vector expressing Gag, Pol and Env elicited primarily a CD8$^+$ response by IFN-gamma ELISPOT and intracellular cytokine staining, with 20-30% of responders also developing a CD4$^+$ T cell response (Casimiro DR and Merck Research Group, 2005, unpublished data).

5.2.5. Additional Viral and non-viral Vectors

Measles virus expressing HIV-1 antigens elicit effective CTL in mice, and may be a good candidate for pediatric vaccines (88). Additional viral vectors that elicit HIV-antigen specific CTL in macaques include, but are not limited to, recombinant forms of poliovirus (89), venezuelan equine encephalitis virus (VEE) (90), vesicular stomatitis virus (VSV) (91), and semliki forest virus (SFV) (92). Non-viral recombinant vectors include recombinant BCG vector (93), which is capable of eliciting HIV-1 specific CD8$^+$ effector (CD44^{hi}, CD127$^-$, CD62L^{lo}) and central (CD44^{hi}, CD127$^+$ CD62L^{hi}) memory in mice. Salmonella (94) and Shigella (95) induce systemic CTL against HIV-1 in mice. Salmonella, shigella, and adenovirus also induce mucosal immunity, which may be important in preventing sexual transmission of HIV-1.

5.3. Prime-Boost Strategies

Those viral vectors previously seen by the human immune system have the disadvantage of pre-existing viral immunity against the virus, which can diminish delivery of the genes of interest to target cells, subsequent antigen expression, and immunity. This is particularly true of adenovirus serotype 5, vaccinia virus in smallpox vaccinees, poliovirus, and measles virus. In addition, repeated boosting of any viral vector can create anti-vector immunity, diminishing the effect of subsequent boosts.

To overcome these limitations, vaccine strategies can be combined to elicit a broader synergistic response. Different methods of antigen delivery (protein, DNA, viral vectors) can lead to qualitatively different T cell responses. For example, DNA and MVA control virus similarly with differing mechanisms (96). Priming with DNA before a viral boost may also help overcome pre-existing anti-vector immunity (97). Such combination vaccine approaches are known collectively as heterologous prime-boost vaccination strategies.

As one example, the heterologous DNA-prime, MVA-boost vaccine strategy has proven immunogenic against a variety of pathogens in humans (98, 99), and has been shown to elicit cellular immunogenicity and control viremia after challenge in non-human primates (100-106). Sadagopal, *et al.* nicely demonstrated that 22/23 rhesus macaques vaccinated with DNA-prime, MVA boost regimen expressing Gag-Pol-Env, controlled viremia for 200 weeks after challenge with SHIV 89.6P. These controllers developed high levels of neutralizing antibodies, in combination with high levels of antigen-specific CD4$^+$ and CD8$^+$ IFN-gamma producing cells 2 weeks after viral challenge. Over time, however, the frequency of Gag-specific CD8$^+$ cells contracted (0.04-0.16%), while Gag-specific CD4$^+$ cells expanded (0.02%-0.27%). A significant fraction of both populations produced both IFN-gamma and IL-2. Overall, successful control was characterized by low-frequency, low-breadth CD4$^+$ and CD8$^+$ T cells co-producing IFN-gamma and IL-2 (22). Subsequent depletion of CD8$^+$ cells resulted in rebound viremia despite high neutralizing antibody levels and antigen specific CD4$^+$ cells, confirming that CD8$^+$ cells play a central role in long-term viral control (107). Despite this, DNA priming did not significantly augment the IFN-gamma ELISPOT response to a Clade-A based MVA vaccine in humans (108).

DNA is efficient at priming other viral vectors as well, including adenovirus (109), fowlpox (110) and Sendai virus (111) However, despite the fact that a DNA prime, Ad5 boost elicited a strong Gag-specific CD8$^+$ T cell response in baboons (109), the same DNA prime did not significantly boost immunogenicity of Ad5Gag in humans (E. Emini and Merck Research Team, 2002 – unpublished data).

Finally, heterologous viral prime-boost approaches appear better than homologous prime-boost approaches in generating CTL, likely due to the differing mechanisms of presentation, as well as avoidance of anti-vector immunity. Casimiro *et al.* found higher frequencies of antigen-specific

IFN-gamma-producing PBMCs Ad5 prime, canarypox boost regimen that either vector in homologous prime boost (112). Other heterologous prime-boost viral regimens include high levels of antigen-specific CTL, including SFV/MVA (113, 114), VSV/Vaccinia (115) and VSV/MVA (116).

5.4. Assay Limitations

The majority of analysis of T cell response to HIV-1 vaccines to date has relied on functional CTL killing assays, antigen-specific tetramer staining, or IFN-gamma ELISPOT (117-119). However, the advent of multicolor flow cytometry (1) allows for finer characterization of these responses. A detailed study of responses to tetanus and hepatitis B vaccines using multicolor flow cytometry by De Rosa *et al.* indicated that many CD4$^+$ T cells produced interleukin-2 (IL-2) without IFN-gamma. This study also describes a detailed immunologic characterization of four individuals who had been vaccinated with a clade A candidate DNA based vaccine. In this very limited sample, responses were heterogeneous and included CD4$^+$ T cells that secreted IL-2 and/or tumor necrosis factor alpha (TNF-alpha) without IFN-gamma (120). Therefore, use of the IFN-gamma ELISPOT assay alone may be insufficient to detect critical memory responses to candidate vaccines (64, 121).

5.5. Breakthrough Infections

Our lack of understanding of the true correlates of protection from HIV-1 is best evidenced by reports of breakthrough HIV-1 infections in subjects previously vaccinated in clinical trials. Three separate trials of recombinant gp120 or gp160 antigens, expressed either as recombinant proteins or in recombinant vaccinia virus reported breakthrough infections in one or more individuals, despite development of CTL and antibody responses to vaccine (122-124). The only Phase III efficacy study of a candidate HIV-1 vaccine to date, consisting of rgp120, showed no protection from HIV-1 (71). Furthermore, in 28 canarypox vaccinees who later acquired HIV-1 infection despite CTL responses, the course of infection was not attenuated compared to placebo recipients (125).

Betts, *et al.* recently described the immune response in a healthy vaccine volunteer receiving recombinant canarypox expressing gp120, gp41, Gag and protease. Detailed flow characterization revealed the vaccine induced both Gag-specific central and effector/effector memory CD8$^+$ T cells, defined as CD28$^+$ CD27$^+$CCR7$^-$ CD45RO$^+$CD57$^-$ and CD28$^-$ CD27$^-$CCR7$^-$ CD45RO$^-$CD57$^+$, respectively. Of the antigen-specific CD8$^+$ cells, more than 25% produced IL-2 in response to Gag peptides. A significant fraction of CD4$^+$ T cells produced IL-2 as well. Despite these varied T cell responses, the subject subsequently became infected with HIV-1. Over time, the virus escaped the dominant epitope sequences, and the T cell response took on the phenotype of a chronically infected subject (126). Despite our more recent understanding of components of protective central memory, it is clear we still do not have a full grasp of what is required for protective immunity.

6. CONCLUSIONS AND PERSPECTIVES

As described above, in the chronic progressive stage of disease, HIV-1 specific CD4^{+} and CD8^{+} T cells become progressively more dysfunctional, and CTLs against new and previously targeted epitopes do not fully mature, resulting in increasing viral load, and clinical immunodeficiency. In large scale (phase III) completed vaccination trials, vaccinees acquired HIV-1 infection despite documented CTL or neutralizing antibodies responses. Indeed, this lack of efficacy prompted the development of alternative or complementary strategies to attempt to restore antigen specific T cell responses in chronically infected patients and to improve the quality of HIV-1 vaccine candidates. Multiple strategies to improve the quality, quantity, and duration of the T cell response to vaccines, including the use of adjuvant therapies (79) are beyond the scope of this review.

We will conclude by focusing on one potentially promising strategy, involving the dendritic cell (DCs) to restore or stimulate the HIV-1 immune response. DCs are professional antigen presenting and capturing cells that are able to stimulate effective immune responses both *in vitro* and *in vivo* (127). Exploiting the full immunostimulatory potential of DCs may be key to achieving an effective immune response to prevent or control HIV-1 infection.

Several techniques have been studied to allow DCs to present specific antigens, including pulsing with peptides, transducing with recombinant viral vectors, loading with apoptotic infected cells, or electroporating with autologous mRNA. Using such methods, many groups have successfully used DCs expressing HIV-1 antigens, to stimulate memory or even primary HIV-1 specific CD8^{+} T cell responses *in vitro* (128-133). In those studies, the stimulatory effect of the DCs was mostly represented by an increase in the frequency of IFN-gamma ELISPOT responses and in increased perforin expression of the effector T cells. However, as evidenced by the chronic infection state, higher quantities of IFN-gamma alone are not sufficient to control the viremia. Thus, in addition to quantity, the quality of the immune response, in terms of differentiation and function, must now be investigated more deeply to try to better define correlates of protection. In our hands, the use of potent mature DCs to restimulate HIV-1-specific CD8^{+} T cells in chronically infected patients with high viremia *in vitro*, does not help to restore the deficit in proliferation of those cells (60). This underlines the need to determine under which circumstances DC-based interventions may be appropriate to help establish a good immune response and not to exhaust an already exhausted one.

Interestingly, in the murine model of *Listeria monocytogenes* immunization, it has been shown that using peptide-pulsed DCs as an adjuvant accelerates the generation of memory T cells. In contrast, the administration of CpG oligodeoxynucleotides, a potent inflammatory agent that allows the action of IFN-gamma on the responding T cells, prevents memory T cells from

developing (134). This reinforces the concept that it is important to maintain a relatively quiescent immune system while establishing a memory T cell response.

Importantly however, Lu and coworkers have published promising results regarding therapeutic DC vaccination in chronically HIV-infected individuals. These subjects, untreated but with a stable viral load for at least 6 months, were immunized with autologous monocyte derived DCs loaded with autologous aldrithiol-2 inactivated HIV-1. In the majority of subjects, viral load was suppressed for at least one year. Control of viremia was associated with a robust HIV-1-specific CD4^{+} T helper type 1 response, comprised of IFN-gamma and IL-2 producing CD4^{+} T cells, and perforin expressing CD8^{+} effector cells (135). Again, this demonstrates that HIV-1 specific CD4^{+} T cells can sustain and restore HIV-1 specific CD8^{+} T cell function, as also demonstrated by Litcherfeld and colleges (61) .

It is interesting to note that in a mouse model, one single vaccination with HIV-1 Gag fused to anti DEC-205, a DC-targeting antibody, leads to a high frequency of IFN-gamma and Il-2 Gag-specific CD4^{+} T cells which persist long-term, and protect from virus challenge in a vaccinia-Gag challenge model (136).

Finally, alternative strategies using a combination of co-stimulatory molecules expressed at the surface of APC demonstrate that expansion and acquisition of effector function by antigen experienced CD8^{+} T cells can be achieved. Thus, Bukczynski and colleges have shown that the dual co-stimulation with CD80 and CD137L of HIV-1-specific CD8^{+} T cells *in vitro* can lead to better expansion and accumulation of effector molecules such as perforin (137).

Recently, a new approach based on inhibition of antigen presentation attenuators (SOCS1) in murine DCs have demonstrated that SOCS1 silenced DCs broadly induced enhanced HIV-1- specific CTLs and CD4^{+} helper T cells as well as antibody responses. Furthermore, the co-immunization with SOCS1 siRNA expressor DNA significantly enhanced the potency of HIV-1 DNA vaccination (138).

Globally, this review reminds us that the requirements for controlling HIV-1 infection are complex, and not completely defined. In addition to a strong neutralizing antibody response, and a polyfunctional CD4^{+} and CD8^{+} T cell response, several other factors may influence the ability of a host to control HIV-1 infection. A more detailed dissection of the quality of T cell response must be systematically addressed in humans and animals models capable of controlling HIV-1 viral replication to try to better define correlates of immune protection. Determinants of the quality of T cell response include the breadth of the response to various HIV-1 antigens, multiple cytokine secretion, memory phenotype, proliferation in response to antigen re-exposure, cytotoxicity, regulatory functions, anatomic location, and the kinetics of response. Thus ,in addition to an adequate memory response, CTL

expansion must occur rapidly enough to control initial infection (139). Localization of the response to the mucosal compartment may therefore be important in containing initial infection.

Attempts to improve the method of HIV-1 vaccine delivery are also critical, as the route, dosage, and vector used for vaccination influence the quality of the T cell response (140). Improved understanding of the quality of immune responses induced by both natural HIV-1 infection, as well as by various vaccine regimens, will allow us to design better strategies to direct the initial vaccine response towards a more protective response against HIV-1.

7. ACKNOWLEDGMENTS

8. REFERENCES

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Abbreviations: APC: Antigen Presenting Cell, LTNPs: Long Term Non-Progressor, DC: Dendritic cell, Tem – effector memory T cell, Tcm – central memory T cell

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