CD4+ T-cell subsets: what really counts in preventing HIV disease?

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Despite the urgent need, no HIV vaccine currently exists. While binding antibody responses to HIV are readily generated *in vivo*, eliciting broadly neutralizing antibody responses to HIV has proven extraordinarily difficult [1]. Studies demonstrating the importance of HIV- and SIV-specific CD8+ T cells (cytotoxic T lymphocytes [CTLs]) in the initial control of viremia during acute infection led to a glut of vaccines designed to induce specific CTL responses [2]. The recent failure of adenovirus-vector HIV vaccines (which typically induce robust HIV-specific CTL responses) in proof-of-efficacy Phase IIb trials, highlights the enormity of the challenge [3].

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Compared with HIV-specific CD8+ CTL and antibody responses, less attention has been paid to CD4+ T-cell responses in controlling or preventing HIV. The contribution of HIV-specific CD4+ T cells to control of viremia or prevention of infection remains controversial. The importance of CD4+ T cells in HIV infection is, however, firmly established: CD4+ T-cell decline is a hallmark of HIV infection.

However, CD4+ T cells are not all created equal. Several recent important studies have begun to suggest a role for various subsets of CD4 T cells in HIV disease progression. Compelling evidence now exists that CD4+ T-central memory (T_{CM}) and T-regulatory (Treg) cell subsets play a significant role in mediating the control of HIV viremia and survival.

Loss of CD4+ T_{CM} cells

Depletion of CD4+ T cells during acute HIV infection occurs primarily within the CC chemokine receptor (CCR)5+ memory population, which comprise the majority of CD4+ T cells in the GI tract [4]. Selective infection and depletion of CD4+ memory T cells is also observed in SIV-infected nonhuman primates [5–7]. During late HIV infection, as the proportion of CCR5+ memory CD4+ T cells declines, there is frequently a shift to CX chemokine receptor (CXCR)4-tropic virus targeting naive CD4+ T cells [8,9]. Moreover, chimeric SHIV, which use CXCR4 (expressed on virtually all naive and a large proportion of memory CD4+ T cells), leads to a rapid and almost complete loss of naive and CD4+ T_{CM} in macaques [10]. Further compounding loss within the CD4+ memory T-cell compartment is the observation that HIV preferentially infects HIV-specific memory CD4+ T cells *in vivo* during all stages of HIV infection [11]. Taken together, these studies highlight a crucial problem: if the majority of CD4+ T cells, including a large number of CD4+ T_{CM} cells, are rapidly infected during primary infection, then therapeutic intervention needs to occur before this initial rapid

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depletion of $CD4^+$ T cells [12]. On the other hand, there is evidence to suggest that although a large number of $CD4^+$ T cells are lost to direct infection, the majority of $CD4^+$ T cells remain uninfected during HIV/SIV infection; thus, loss of $CD4^+$ T_{CM} cells during chronic infection via other mechanisms may still be managed by HIV immunotherapy throughout the later stages of infection.

Fas-mediated apoptosis has also been proposed as a primary cause of $CD4^+$ T-cell loss during HIV/SIV infection. In SIV-infected macaques, $CD4^+$ but not $CD8^+$ T cells undergo apoptosis, which parallels $CD4^+$ T-cell decline [7]. The apoptosis-related FasR (CD95), which is expressed on activated/memory but not naive T cells, is increased on $CD4^+$ T cells during HIV infection and correlates with $CD4^+$ T-cell decline and disease progression. Likewise, apoptosis of CD95 upregulated $CD4^+$ T cells also occurs during acute and chronic SHIV infection. More importantly, since $CD4^+$ T_{CM} and T-effector memory (T_{EM}) cells express high and intermediate levels of CD95, respectively, it appears that the former are more susceptible to Fas-mediated apoptosis than either T_{EM} or naive $CD4^+$ T cells. Preferential apoptosis of HIV-specific $CD4^+$ T cells has also been reported [13]. Control of CD95-mediated apoptosis would be a useful adjunct to HIV immunotherapy of HIV-infected individuals [14].

In addition to the death of infected cells and apoptosis of uninfected bystander cells, chronic virus-induced immune activation also drives $CD4^+$ T-cell depletion and the development of opportunistic infections [15,16]. Translocation of microbes and microbial products across the GI tract following massive depletion of mucosal $CD4^+$ T cells is one cause of chronic immune activation. Plasma levels of lipopolysaccharide (LPS), a marker of microbial translocation, correlate with immune activation and are higher in chronically HIV-infected humans and SIV-infected rhesus macaques [17]. The intensity of apoptosis correlates with degree of immune activation in HIV-infected individuals. If chronic immune activation, driven by circulating plasma LPS or other bacterial products, is causing $CD4^+$ T-cell loss, it may be possible to target plasma LPS in HIV-infected patients in order to limit the extent of $CD4^+$ T-cell loss, which ultimately compromises effective control of HIV.

HIV/SIV-infected primates that are naturally resistant to disease progression provide clear evidence for the importance of limiting chronic immune activation. HIV-infected chimpanzees and SIV-infected sooty mangabeys that are relatively resistant to AIDS exhibit a lack of chronic immune activation. In fact, sooty mangabeys that are naturally infected with SIV maintain normal $CD4^+$ T-cell counts and remain disease free despite high-level viremia and attenuated SIV-specific T-cell responses [18]. Furthermore, $CD4^+$ T cells in SIV-infected sooty mangabeys are relatively resistant to anergy and this anergy resistance resides predominantly within the $CD4^+$ T_{CM} population, which may be crucial in conferring disease resistance in this species [19].

Preservation of $CD4^+$ T_{CM} subset

The targeting of $CD4^+$ T_{CM} cells via direct infection, apoptosis and chronic immune activation during HIV/SIV infection suggests that this cell population may be crucial to effective immune control of HIV/SIV. Additional evidence for the importance of $CD4^+$ T_{CM} cells in HIV/SIV infection comes from studies demonstrating preservation of $CD4^+$ T_{CM} cells is associated with survival in vaccinated and SIV-challenged rhesus macaques [20] and maintenance of Gag-specific T_{CM} $CD4^+$ T cells in macaques that control viremia compared with animals that have progressive disease [21]. However, although the association between preservation of $CD4^+$ T_{CM} cells and control of viremia or survival is evident, the cause and effect relationship remains unclear. Notably, highly active antiretroviral therapy (HAART) of HIV-infected individuals increases total as well as $CD4^+$ T_{CM} cell counts, yet antigen-specific $CD4^+$ T_{CM} cell responses remain functionally impaired [22] indicating that merely increasing total $CD4^+$ T_{CM} cell counts may be insufficient to effect improvement in the immune control of HIV.

Regulatory T cells in HIV/SIV infection

Another important subset of $CD4^+$ T cells are $CD4^+CD25^+$ forkhead box transcription factor (Foxp)3^+ Treg cells. Given the lack of chronic immune activation observed in AIDS-resistant HIV-infected chimpanzees and SIV-infected sooty mangabeys, there is growing interest in the role of natural (nonantigen specific) Treg cells in HIV/SIV infection. Although it is plausible to hypothesize that Treg cells play a beneficial role in HIV/SIV infection by moderating immune activation, there is considerable debate as to whether Treg cells facilitate or hinder control of HIV/SIV infection. Treg cells could contribute to HIV/SIV pathogenesis by suppressing HIV-specific immune responses, as shown *in vitro* [23,24]. Premature induction of Treg cells during acute SIV infection in rhesus macaques appears to facilitate establishment of chronic infection by blunting SIV-specific T-cell responses before the virus can be cleared [25]. This is supported by studies in humans showing proportions of circulating and lymphoid Treg cells correlate with viral load [26,27]. A direct interaction between HIV and Treg cells, probably via glycoprotein 120, may drive accumulation of Treg cells in lymphoid tissues and facilitate disease progression [28]. On the other hand, significantly lower viral loads and higher $CD4^+$ T-cell counts have also been reported for HIV-infected individuals with strong Treg cell function *in vitro* [29]. Furthermore, high levels of Treg cells are present in the cord blood of HIV-exposed, uninfected infants, potentially limiting T-cell activation and reducing vertical transmission [30].

Some of these apparently conflicting results may reflect differences in phenotypic analyses used to identify Treg cells, compartments monitored, measurement of Treg cells based on

absolute numbers versus total CD4+ T-cell frequency and various animal models (e.g., humans, rhesus macaques, sooty mangabeys and pigtail macaques). However, whatever the role of Treg cells in HIV/SIV, they probably play a crucial role in modulating not only virus-specific immunity, but the overall immune milieu during the course of infection.

Defining a role for Treg cells in HIV/SIV infection

If Treg cells dampen HIV-specific immune responses, would HIV vaccines that target and disable Treg cells generate better immunity to HIV? Therapeutic treatment of HIV may benefit from the coupled use of agents to selectively target Treg cells to boost HIV-specific T-cell immune responses. Transient depletion of Treg cells in macaques with (or prior to) SIV infection could be illuminating.

T regulatory cells express T-cell receptors with broad specificity and recognize a number of diverse pathogens, including tumor, bacterial and viral antigens. HIV-specific Treg cell activity has also been reported, although not yet mapped to specific epitopes [29]. Unfortunately, screening for antigen-specific Treg cell responses by measuring Treg cell-associated cytokines, such as IL-10 and TGF-β, has proven difficult. Methodologies that can be used to identify and fine-map putative Treg epitopes are needed, particularly in view of the high failure rate of HIV vaccine trials to date. Recent studies highlight the use of surface markers $CD25^+127^{lo}$ as accurately identifying Treg cells [31]: sorting or gating on this population should help identify live HIV-specific Treg cells without the requirement to fix cells to intracellularly stain for Foxp3. Vaccines designed to elicit HIV-specific T-cell immunity may also inadvertently stimulate and expand HIV-specific Treg cells that prevent optimal production of T-cell responses. Selectively inducing or avoiding HIV/SIV-specific Treg responses (once the relevant epitopes are known), for example using peptide-pulsed blood cells [32], would help sort out the utility of Treg cells *in vivo*.

Conclusion

Much remains to be done in determining how to generate protective immunity against or long-term control of HIV. Lumping all CD4 T-cell responses together belies the extraordinary complexity of this cell population. $CD4^+$ T_{CM} and Treg subsets and their potential contribution to the development of HIV vaccines and immunotherapies warrant further examination, and the following questions need answers:

- What influences preservation of $CD4^+$ T_{CM} cells during HIV/SIV infection?
- Is there a critical threshold for $CD4^+$ T_{CM} cell counts for disease progression?
- Could lack of immune escape in $CD4^+$ T-cell epitopes be consequence of loss of $CD4^+$ T_{CM} cells?
- Can different HIV/SIV antigens preferentially induce HIV/SIV-specific $CD4^+$ memory responses that are skewed towards expansion of either T_{CM} or T_{EM} cells? Could this account for different associations between T-cell responses to different HIV antigens and control of viremia?
- Do $CD4^+$ Treg cells facilitate or suppress HIV infection?
- Do HIV-specific Treg cells exist and can HIV Treg epitopes be targeted or avoided with vaccines?

There is an imperative to explore all avenues that might lead to generating more effective immunity to HIV.

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References

1 Burton DR, Desrosiers RC, Doms RW *et al.* HIV vaccine design and the neutralizing antibody problem. *Nat. Immunol.* 5(3), 233–236 (2004).

2 Johnston MI, Fauci AS. An HIV vaccine – evolving concepts. *N. Engl. J. Med.* 356(20), 2073–2081 (2007).

3 Steinbrook R. One step forward, two steps back – will there ever be an AIDS vaccine? *N. Engl. J. Med.* 357(26), 2653–2655 (2007).

4 Brenchley JM, Schacker TW, Ruff LE *et al.* $CD4^+$ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *J. Exp. Med.* 200(6), 749–759 (2004).

5 Mattapallil JJ, Douek DC, Hill B, Nishimura Y, Martin M, Roederer M. Massive infection and loss of memory $CD4^+$ T cells in multiple tissues during acute SIV infection. *Nature* 434(7037), 1093–1097 (2005).

6 Wang X, Rasmussen T, Pahar B *et al.* Massive infection and loss of $CD4^+$ T cells occurs in the intestinal tract of neonatal rhesus macaques in acute SIV infection. *Blood* 109(3), 1174–1181 (2007).

7 Li Q, Duan L, Estes JD *et al.* Peak SIV replication in resting memory $CD4^+$ T cells depletes gut lamina propria $CD4^+$ T cells. *Nature* 434(7037), 1148–1152 (2005).

8 Davenport MP, Zaunders JJ, Hazenberg MD, Schuitemaker H, van Rij RP. Cell turnover and cell tropism in HIV-1 infection. *Trends Microbiol.* 10(6), 275–278 (2002).

9 Ribeiro RM, Hazenberg MD, Perelson AS, Davenport MP. Naive and memory cell turnover as drivers of CCR5-to-CXCR4 tropism switch in human immunodeficiency virus type 1: implications for therapy. *J. Virol.* 80(2), 802–809 (2006).

10 Nishimura Y, Igarashi T, Donau OK *et al.* Highly pathogenic SHIVs and SIVs target different $CD4^+$ T cell subsets in rhesus monkeys, explaining their divergent clinical courses. *Proc. Natl Acad. Sci. USA* 101(33), 12324–12329 (2004).

11 Douek DC, Brenchley JM, Betts MR *et al.* HIV preferentially infects HIV-specific CD4$^+$ T cells. *Nature* 417(6884), 95–98 (2002).

12 Picker LJ, Watkins DI. HIV pathogenesis: the first cut is the deepest. *Nat. Immunol.* 6(5), 430–432 (2005).

13 Yue FY, Kovacs CM, Dimayuga RC *et al.* Preferential apoptosis of HIV-1-specific CD4$^+$ T cells. *J. Immunol.* 174(4), 2196–2204 (2005).

14 Li-Weber M, Weigand MA, Giaisi M *et al.* Vitamin E inhibits CD95 ligand expression and protects T cells from activation-induced cell death. *J. Clin. Invest.* 110(5), 681–690 (2002).

15 Sousa AE, Carneiro J, Meier-Schellersheim M, Grossman Z, Victorino RM. CD4 T cell depletion is linked directly to immune activation in the pathogenesis of HIV-1 and HIV-2 but only indirectly to the viral load. *J. Immunol.* 169(6), 3400–3406 (2002).

16 Hazenberg MD, Otto SA, van Benthem BH *et al.* Persistent immune activation in HIV-1 infection is associated with progression to AIDS. *AIDS* 17(13), 1881–1888 (2003).

17 Brenchley JM, Price DA, Schacker TW *et al.* Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat. Med.* 12(12), 1365–1371 (2006).

18 Dunham R, Pagliardini P, Gordon S *et al.* The AIDS resistance of naturally SIV-infected sooty mangabeys is independent of cellular immunity to the virus. *Blood* 108(1), 209–217 (2006).

19 Bostik P, Noble ES, Mayne AE, Gargano L, Villinger F, Ansari AA. Central memory CD4 T cells are the predominant cell subset resistant to anergy in SIV disease resistant sooty mangabeys. *AIDS* 20(2), 181–188 (2006).

20 Letvin NL, Mascola JR, Sun Y *et al.* Preserved CD4$^+$ central memory T cells and survival in vaccinated SIV-challenged monkeys. *Science* 312(5779), 1530–1533 (2006).

21 Felber BK, von Gegerfelt A, Valentin A *et al.* Preservation of a subset of SIV-specific central memory CD4$^+$ T cells correlates with control of viremia in SIVmac251 infected macaques. *Retrovirology* 3(Suppl. 1), S93 (2006).

22 Elrefaei M, McElroy MD, Preas CP *et al.* Central memory CD4$^+$ T cell responses in chronic HIV infection are not restored by antiretroviral therapy. *J. Immunol.* 173(3), 2184–2189 (2004).

23 Kinter A, McNally J, Riggin L, Jackson R, Roby G, Fauci AS. Suppression of HIV-specific T cell activity by lymph node CD25$^+$ regulatory T cells from HIV-infected individuals. *Proc. Natl Acad. Sci. USA* 104(9), 3390–3395 (2007).

24 Kinter AL, Horak R, Sion M *et al.* CD25$^+$ regulatory T cells isolated from HIV-infected individuals suppress the cytolytic and nonlytic antiviral activity of HIV-specific CD8$^+$ T cells *in vitro*. *AIDS Res. Hum. Retroviruses* 23(3), 438–450 (2007).

25 Estes JD, Li Q, Reynolds MR *et al.* Premature induction of an immunosuppressive regulatory T cell response during acute simian immunodeficiency virus infection. *J. Infect. Dis.* 193(5), 703–712 (2006).

26 Andersson J, Boasso A, Nilsson J *et al.* The prevalence of regulatory T cells in lymphoid tissue is correlated with viral load in HIV-infected patients. *J. Immunol.* 174(6), 3143–3147 (2005).

27 Lim A, Tan D, Price P *et al.* Proportions of circulating T cells with a regulatory cell phenotype increase with HIV-associated immune activation and remain high on antiretroviral therapy. *AIDS* 21(12), 1525–1534 (2007).

28 Nilsson J, Boasso A, Velilla PA *et al.* HIV-1-driven regulatory T-cell accumulation in lymphoid tissues is associated with disease progression in HIV/AIDS. *Blood* 108(12), 3808–3817 (2006).

29 Kinter AL, Hennessey M, Bell A *et al.* CD25(+)CD4(+) regulatory T cells from the peripheral blood of asymptomatic HIV-infected individuals regulate CD4(+) and CD8(+) HIV-specific T cell immune responses *in vitro* and are associated with favorable clinical markers of disease status. *J. Exp. Med.* 200(3), 331–343 (2004).

30 Legrand FA, Nixon DF, Loo CP *et al.* Strong HIV-1-specific T cell responses in HIV-1-exposed uninfected infants and neonates revealed after regulatory T cell removal. *PLoS ONE* 1, e102 (2006).

31 Seddiki N, Santner-Nanan B, Martinson J *et al.* Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. *J. Exp. Med.* 203(7), 1693–1700 (2006).

32 Chea S, Dale CJ, De Rose R, Ramshaw IA, Kent SJ. Enhanced cellular immunity in macaques following a novel peptide immunotherapy. *J. Virol.* 79, 3748–3757 (2005).