T-cell Exhaustion in HIV Infection

Mohamed El-Far, PhD, Rabih Halwani, PhD, Elias Said, PhD, Lydie Trautmann, PhD, Mehrnoosh Doroudchi, PhD, Loury Janbazian, MSc, Simone Fonseca, PhD, Julien van Grevenynghe, PhD, Bader Yassine-Diab, PhD, Rafick-Pierre Sékaly, PhD, and Elias K. Haddad, PhD

Corresponding author
Rafick-Pierre Sékaly, PhD
Centre de Recherche du CHUM, Hôpital Saint-Luc, 264, René-Lévesque est, Bureau 1317, Montréal, Québec, H2X 1P1, Canada.
E-mail: rafick-pierre.sekaly@umontreal.ca

Current HIV/AIDS Reports 2008, **5:**13–19
Current Medicine Group LLC ISSN 1548-3568

Generation of memory T cells, which mediate immunity against microbes and cancers, relies, for optimal activity, on the interactions of multiple cell types that are highly regulated through the expression of soluble factors and negative and positive receptors. Their disruption will lead to aberrant immune responses, which can result in the invasion of the host by foreign pathogens. In chronic viral infections including HIV and hepatitis C virus, persistence of antigen and lack of CD4 help (HIV) disrupt memory T-cell function and induce defects in memory T-cell responses, which have been defined as T-cell exhaustion. In this review, we examine the molecular mechanisms involved in such T-cell dysfunction. Better understanding of these mechanisms will assist in the development of novel therapies to prevent the immune damage mediated by HIV infection.

Introduction

A hallmark of the immune system is protection of the host against microbial pathogens through the coordination of its two major arms, namely the innate and the adaptive immune systems. Proper recognition, processing, and presentation of antigens will lead to the activation of a specific adaptive immune response, which plays a major role in controlling the infection. T lymphocytes, upon recognition of cognate pathogenic antigen (Ag), enter an activation phase during which they undergo clonal expansion, secrete cytokines, and enhance their cytolytic activity. Once Ag has been cleared, most effector cells die via activation-induced cell death and apoptosis. However, a small proportion of Ag-specific T cells survive the contraction phase and mature to become memory T cells with the capacity to react to previously encountered Ags with enhanced strength, efficiency, and speed [1]. Memory T cells are able to self renew by undergoing homeostatic proliferation and can persist for many years after the initial antigenic exposure and even, in some cases, in the absence of re-exposure to Ag [2,3]. However, T cells lose this characteristic control under chronic exposure to Ags. This functional impairment has been defined as exhaustion [4••]. Herein, much focus will be paid to two major negative regulators of T-cell activation: programmed cell death (PD-1) and cytotoxic T-lymphocyte antigen-4 (CTLA-4). These factors play a central role in driving T cells into dysfunction so far in HIV, simian immunodeficiency virus (SIV), lymphocytic choriomeningitis virus (LCMV) and in HCV infections. Such mechanisms that interfere with the function of memory T cells or induce their dysfunction constitute a major impediment to control viral infections, thereby allowing viral dissemination and persistence.

T-cell Exhaustion

T-cell exhaustion was initially described in the murine LCMV model [5]. Barber et al. [4••] showed that in the context of chronic LCMV infection, LCMV-specific CD8 T cells were unable to kill virally infected target cells. They further showed that CD4 T cells played a major role in augmenting CD8 T-cell function [4••]. It was later shown that antigen persistence is directly associated with CD8 T-cell exhaustion and that CD4 T cells can partially rescue its exhaustion [6]. A gradient of exhaustion of CD8 function was demonstrated with interleukin (IL)-2 production, cytotoxicity, and proliferation being the first functions to disappear during chronic LCMV infection, whereas interferon (IFN)-γ production is the last function to be lost [7]. Exhausted T cells finally die by apoptosis. As CD4 T cells play a critical role in preventing the establishment of CD8 T-cell dysfunction, this study strongly suggested that under conditions of lack of CD4 T-cell help, as in HIV infection, CD8

T-cell dysfunction would be more pronounced. In fact, during HIV infection, HIV-specific CD8 T cells display an overall dysfunctional phenotype characterized by loss of IL-2 production, proliferation, and effector functions [8]. This dysfunction can be significantly, although not completely, rescued by blocking the PD-1 pathway, suggesting that CD4 T-cell–associated factors can contribute in the rescue of CD8 T-cell dysfunction.

Interestingly CD4 T cells are exhausted during chronic viral infections, show impairment of cytokine production, and decrease in their proliferative potential [9••]. CD4 T-cell exhaustion paralyzes the immune system and renders it unable to control viral replication, thereby leading to disease progression. In that overall context, efforts should be directed at investigating the molecular mechanisms involved in this exhaustion with the aim of restoring both CD4 and CD8 functions in infected patients.

Exhausted CD8 and CD4 T cells in HIV Infection

HIV-specific T-cell impairment has been extensively studied over the past few years in the chronic phase of infection. These studies did not provide answers for whether the described defects are a consequence of chronic infection and chronicity of Ag presentation or if they were the result of molecular interaction of the negative regulators of T-cell activation that took place during acute infection. Studies using Ki67 expression as a marker of T-cell proliferation have indicated that CD4 and CD8 T-cell turnover increases in both naïve and memory subsets during HIV infection. This hyperimmune activation, which compromises the homeostatic process, has been suggested to be responsible for the slow depletion of CD4 T cells and the clonal senescence of CD8 T cells [10,11]. The critical question that remains to be answered is how early in acute infection T-cell exhaustion starts. For CD4 T cells, studies have been conducted in acute HIV infection and seem to propose that HIV-specific CD4 T cells expand at high frequency during the early phase of HIV infection but are lost subsequently, either physically through direct or indirect cytopathic effects of the virus or functionally through mechanisms, which will be described in this review [12,13]. A recent SIV model suggests that loss of functional CD4 T cells during primary infection is associated with both selective depletion of memory CD4 T cells and a loss of the functional capacity of the memory T lymphocytes that escape virus-associated destruction [14].

For CD8 T cells, several studies have documented an impaired immune response in primary HIV infection; these cells become non-cytolytic [15], exhibit a lower breadth of the functional response as they produce mostly IFN-γ and express lower levels of perforin in lymphoid tissues [16]. The phenotypic analysis of HIV-specific and total CD8 T-cell responses in acute infection showed highly activated T cells susceptible to apoptosis ($CD38^{hi}$, $Bcl\text{-}2^{lo}$, $CD95^{hi}$). Another study showed the loss of HIV-1–specific CD8 T-cell proliferation after acute HIV-1 infection and the restoration of this proliferation in the presence of vaccine-induced HIV-1–specific CD4 T cells [17]. More recently, SIV-specific CD8 T cells were shown to present a survival defect and a skewed phenotype—both established early within the first few weeks of infection [18]. Altogether, these studies do not give a complete picture of the events that take place in the acute phase of infection and do not define the mechanisms that lead to the establishment of HIV-specific T-cell dysfunction. In that context, more work should be performed at the beginning of the infection to provide a more thorough understanding of the fate of HIV-specific T cells from the onset of infection and identify the mechanisms leading to T-cell exhaustion which has been described in chronic infection.

Several groups have characterized distinct memory subsets based on different surface marker expression. Sallusto et al. [19] reported two subsets of memory T cells based on CCR7 expression: the $CCR7^-$ subset, effector memory T cells (T_{EM} cells), and the $CCR7^+$ subset, central memory T cells (T_{CM} cells) [19]. Whereas T_{EM} cells respond rapidly to Ag by differentiating into effector cells, T_{CM} cells produce a small number of cytokines, show weak effector function, and serve as precursors for the generation of T_{EM} subset. We and others [4••,9••,20•,21] have examined whether chronic viral infections, such as HIV, impact the maturation of memory T cells. We have found that HIV infection induces HIV-specific CD8 T cells to arrest at a late differentiated memory phenotype (RA^-, $CCR7^-$) which is not seen in cytomegalovirus (CMV)-specific CD8 T cells. These terminally differentiated memory T cells express low levels of perforin and are unable to mount a cytolytic response [22].

Another memory phenotype that has also been shown to accumulate during HIV infection has been described by Appay et al. [23]. Based on the expression of CD27 and CD28, they proposed a second model of Ag-specific memory CD8 T-cell differentiation [23]. In this model, Ag-experienced cells could be $CD28^+CD27^+$ (early), $CD28^-CD27^+$ (intermediate), or $CD28^-CD27^-$ (late). Although no phenotypic changes were observed among different viruses in acute infection, striking differences were obvious in the chronic phase for different infections. Mainly, HIV-specific CD8 T cells had an intermediate phenotype ($CD28^-CD27^+$) whereas CMV- and Epstein-Barr virus–specific CD8 T cells populated in the late and early phases of differentiation, respectively. Moreover, intermediate-differentiated HIV-specific T cells exhibited limited cytotoxic and proliferative ability in contrast to the late differentiated CMV-specific CD8 T cells. However, there was no correlation between the phenotype of HIV-specific CD8 T cells in these two models. Hence, future studies should characterize memory responses based on functional and surface markers.

The dysfunction of HIV-specific CD4 T cells during disease progression is a multifactorial process that leads to the impairment of the immune response. HIV disease state and dysfunction have been associated with several markers on HIV-specific CD4 T cells, including CTLA-4, CD57, and PD-1. Several studies have focused on some of these molecules in order to understand their role in disease progression. However, most studies focused on chronic infection, and few studies exist on acute infection. Therefore, it seems conceivable that profound phenotyping characterization of CD4 and CD8 in the acute infection would help understanding the early impairment of T-cell functions. Consequently, early therapeutic intervention may prevent the depletion of Ag-specific T cells or their dysfunction, thereby allowing a better control of viremia.

Factors and Mechanisms Mediating T-cell Dysfunction

The T-cell response to Ag is largely dependent on the balance between positive and negative costimulatory signals. The net outcome following T-cell receptor (TCR) engagement depends on the relative expressions of positive and negative costimulatory receptors on T cells and their ligands on Ag-presenting cells (APCs). The heightened expression of positive costimulatory receptors would enhance TCR-induced proliferation, cytokine production, and cell migration. On the other hand, heightened expression of negative receptors would dampen T-cell function. Several cell-surface molecules are well known for their negative regulation on T-cell function, such as Fas, tumor necrosis factor (TNF)-α receptor, CTLA-4, and PD-1. Although these negative regulators are critical for controlling immune responses quantitatively and qualitatively, their persistent expression, as is the case in chronic viral infections, would lead to aberrant immune responses, T-cell dysfunction, and exhaustion.

Recently, we and others [4••,9••,20•,21] have shown that, during chronic viral infections, CD8 T-cell exhaustion is directly associated with the heightened expression of the negative regulator of T-cell activation, namely PD-1. Signaling pathways initiated upon the interaction of PD-1 with its ligands (PDL-1/PDL-2) negatively regulate signals downstream of TCR and dampen TCR-induced cytokine production and proliferation. As Ag persistence is a hallmark of chronic infections, we have shown that PD-1 expression levels in HIV-1 infection were correlated with both viral load and the reduced capacity of HIV-specific CD8 T cells to produce cytokines and to proliferate. CTLA-4 is another inhibitory molecule belonging to the B7-CD28 family that correlated with disease progression during HIV infection. The increase of CTLA-4 on the surface of HIV-specific CD4 T cells has also been shown in acute and chronic HIV infection. In chronic infection, the blockade of CTLA-4 in vitro increased the HIV-specific T-cell production of IFN-γ and IL-2 [24•].

What Is Known About the Signaling Pathways of These Negative Regulators?

CTLA-4 interacts with the same ligands of the co-stimulatory receptor, namely CD28 B7.1 and B7.2 [25]. Its expression on the cell surface is tightly regulated by the phosphorylation of its tyrosine motifs within the cytoplasmic tail. The function of this receptor is primarily regulated by its rapid endocytosis and by the phosphorylation of its cytoplasmic tail. In fact, phosphorylation of CTLA-4 cytoplasmic tail by the Src kinases Lck and Fyn stabilizes and increases its expression on the cell surface [26].

Although PD-1 and CTLA-4 are similar in function, they differ with respect to their ligands and in the signaling pathways involved. PD-1 lacks the dimerization motif in the extracellular part of the receptor whereas it is conserved in CTLA-4, which is known to be expressed as a homodimer. The cytoplasmic tails of these two proteins are also different. PD-1 contains two conserved motifs, immunoreceptor tyrosine-based inhibitory motif and immunoreceptor tyrosine-based switch motif, both of which are important to the interaction with Src phosphatases SHP-1 and SHP-2 [27]. These motifs are absent from the cytoplasmic tail of CTLA-4, which contains two tyrosine motifs which regulate its surface expression, YVKM (Y201) and YFIP (Y218).

CTLA-4 also impedes TCR downstream signals by interfering with the extracellular signal-regulated kinase and Jun N-terminal kinase activation as well as with CD28-induced nuclear factor κB activity. The third mechanism involves proximal TCR signaling where it has been shown that CTLA-4 coligation with the TCRζ chain prevents its phosphorylation leading to abortion of the signal transduction [28]. Moreover, recent evidence also points to the role of CTLA-4 in modulating the threshold of T-cell activation by preventing the stop signal of TCR and increasing cell motility. Consequently, the reduction of the contact time between T cells and APCs leads to the decrease in cytokine production and cell proliferation [29].

As in the case of CTLA-4, PD-1–deficient mice display multiple autoimmune defects and a breakdown in peripheral tolerance [30]. Upon ligation of PD-1 to PDL-1 and PDL-2 [31], cells show reduced profiles of the breadth of cytokine production as well as proliferation [32]. These negative effects of PD-1 are mediated, in part, through the inhibition of downstream TCR-positive signals by abolishing TCR-mediated phosphorylation of ZAP70 and association with CD3ζ. In addition, and similarly to CTLA-4, PD-1 ligation inhibits CD3/CD28-induced glucose metabolism and Akt activity. However, PD-1 achieves this effect by preventing CD28-mediated activation of phosphatidylinositol 3-kinase.

T-cell dysfunction can also be regulated by cytokines such as IL-10. The natural role of IL-10 is to balance the cytokine network, particularly during the differentiation of helper T cell (Th)-1 and Th2. IL-10 knockout mice showed polarized Th1 responses and developed severe

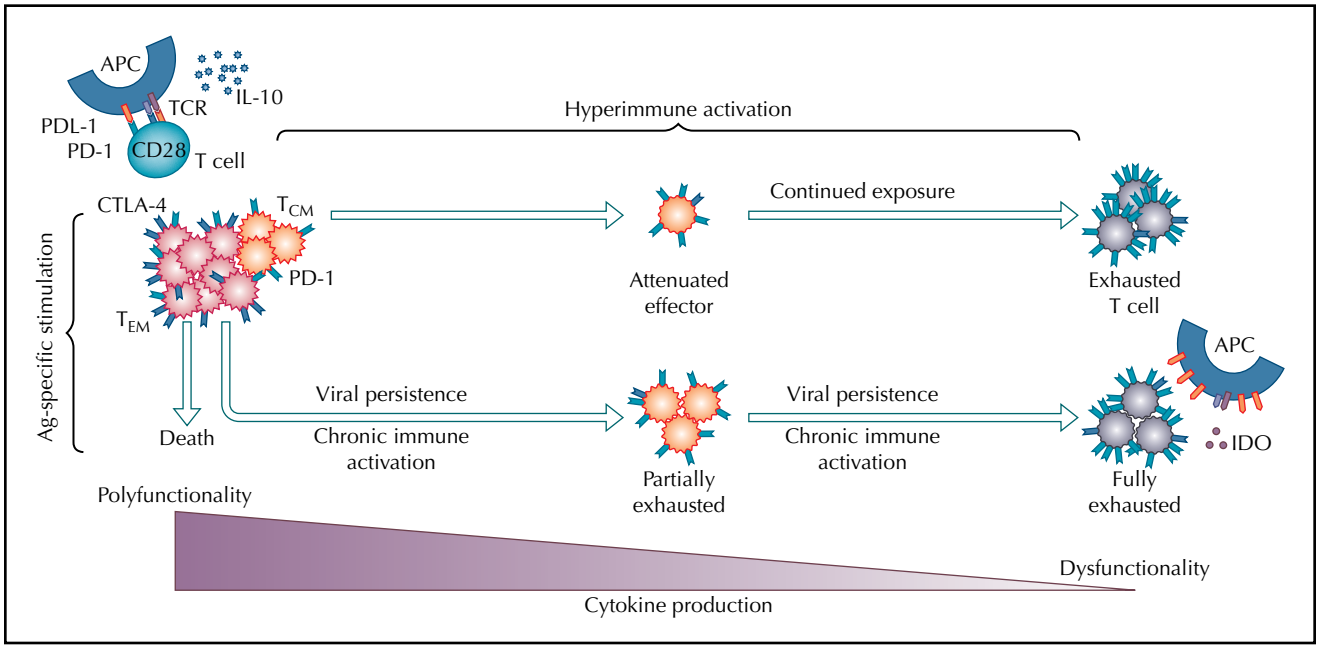

Figure 1. T-cell dysfunction is controlled by cytokines and negative regulatory receptors. During initial exposure to antigen-presenting cells (APCs), T cells differentiate into effector and memory subsets. However, persistence and continuous exposure to antigen (Ag) leads to hyperimmune activation. The engagement of cytotoxic T-lymphocyte antigen-4 (CTLA-4) and programmed cell death (PD-1) in the presence of interleukin (IL)-10 and/or indoleamine 2,3-dioxygenase (IDO) gradually drives T cells into exhaustion. PDL-1—programmed cell death ligand; T_{CM}—central memory T cells; TCR—T-cell receptor; T_{EM}—effector memory T cells.

colitis. Therefore, IL-10 is known to inhibit a wide array of immune factors in vitro. These include proinflammatory cytokine production by macrophages, Ag presentation, Ag-specific T-cell proliferation, and type 1 cytokine production by T cells [33,34]. The impact of IL-10 on Ag presentation involves the inhibition of dendritic cell (DC) maturation. McBride et al. [35] have shown that IL-10 desensitizes DCs to lipopolysaccharide-induced expression of costimulatory molecules, major histocompatibility complex class II, and the secretion of IL-12, TNF-α, IL-6, and IL-1β. Moreover, it has also been shown in vitro and in vivo that IL-10 can induce anergy [36]. Interestingly, using a LCMV model in mice, Brooks et al. [36] showed that viral persistence drives higher levels of IL-10 by APCs leading to impaired T-cell function. These effects raise IL-10 as a potent inhibitor of T-cell activation and an important inducer of tolerance, a mechanism exploited by viral infections.

Another important player in the dysfunction of T cells is indoleamine 2,3-dioxygenase (IDO). This enzyme plays a regulatory role in the mechanisms that limit T-cell proliferation through its catabolism of tryptophan, an essential amino acid for T-cell proliferation. In HIV/AIDS, it has recently been shown that HIV-1 infection induces the expression of IDO in plasmacytoid dendritic cells (pDCs), which impair T-cell function [37].

Therefore, during chronic viral infections, T cells are exposed to continuous Ag presentation that leads to hyperimmune activation (Fig. 1). This activation upregulates the expression of the negative receptors CTLA-4 and PD-1, which drive T-cell dysfunction. IL-10 and IDO, both secreted by APCs, can highly participate in this impairment of T-cell function. The blockade of all these factors can readily rescue T cells from dysfunction.

Role of the Innate Immune System in T-cell Exhaustion During HIV Infection

The presence of a competent innate immune response is essential for the induction and regulation of an efficient adaptive immune response. It is very likely that T-cell exhaustion may result from defects in the innate immune system in HIV infection. Monocytes/macrophages and DCs, two major components of the innate immune system, impact T-cell responses during HIV infection.

Impairment in the Ag presentation and stimulation capacity of DCs and monocytes/macrophages

The capacity of both DCs and monocytes/macrophages to present APCs is damaged during HIV infection. On one hand, several studies have shown that myeloid DCs (mDCs) and pDCs absolute numbers in the blood are reduced during HIV infection and that this loss of DCs correlates with the viral load [38]. Similarly, loss of DCs has also been observed in lymph nodes of individuals with HIV infection [39]. In addition to the significant loss in the number of DCs, the functions of DCs obtained from individuals with HIV infection are also impaired [40]. For example, both mDCs and pDCs of individuals with HIV infection have reduced capacity to stimulate allogeneic T-cell proliferation [38]. Other phenotypic defects have also been observed in

DCs obtained from the lymph nodes of patients with HIV infection as these cells express low levels of CD80 and CD86 indicating a costimulatory defect [39]. In addition, HIV-associated DC dysfunction has been demonstrated in DC matured with HIV peptides. It was reported that pDCs isolated from healthy donors displayed reduced capacity to produce IFN-α production when matured with HIV peptides and not with peptides obtained from herpes simplex virus [41]. The reasons for the DC loss and DC dysfunction during HIV infection are poorly understood. Some could be explained by the fact that mDCs and pDCs from individuals with HIV infection were found to contain proviral DNA. Accordingly, it has been shown that following the infection of monocyte-derived DCs by HIV, their maturation can be blocked reducing the efficacy of Ag presentation, and their capacity to prime functional T cells. Moreover, HIV envelope glycoprotein, gp120, induced abnormal maturation of DCs which might lead to profound suppression of their activities. Also, HIV Nef protein was shown to inhibit the capacity of DCs to prime alloreactive CD8 T-cell responses downregulating their proliferation and functional competence.

During HIV infection monocyte/macrophage functions are also impaired, as they are unable to present Ags, phagocytosis, intracellular killing, chemotaxis, and cytokine production, such as the production of IL10. Actually, Polyak et al. [42] described an impaired class II expression and Ag uptake in monocytes after HIV-1 infection. Furthermore, the altered capacity of HIV-infected monocytes to stimulate and present Ag to CD4 T cells was related to downmodulation of CD4 expression on T cells and appeared to occur via membrane-associated cellular factors on HIV-infected monocytes [42,43].

Deregulation of cytokine production by DCs and monocytes/macrophages

The impairment in the regulation of cytokine production by monocytes and DCs contributes to immunologic deficiencies occurring in patients with HIV infection [44]. IL-10, a regulatory cytokine that inhibits T-cell proliferation and activation, is increased during HIV infection and was reported to be associated with impaired CD4 and CD8 T-cell responses [45,46]. Moreover, monocytes were identified as the major source of IL-10 during HIV infection. Also, an in vitro study has shown that HIV-infected DCs interact with T cells from patients with HIV-1 infection to stimulate IL-10 production and immune suppression. However, whereas DCs have the potential to produce IL-10, but HIV-1–infected DCs produce less of this cytokine.

Upregulation of Immune-suppressor Molecule Expression

The upregulation of immune-suppressor molecules, such as PDL-1 and the immunoglobulin-like transcript 4 (ILT4), on APCs in HIV infection was documented. The PD-1/PDL-1 pathway appears to be involved in downregulating T-cell functionality. Trabattoni et al. [47] has shown that PDL-1 expression was upregulated on monocytes during HIV infection. Increased levels of PDL-1 were found in aviremic chronically infected patients who were HIV-1 positive. This possibly contributed to an incomplete immune reconstitution after highly active antiretroviral therapy. Moreover, ILT4, a tolerogenic molecule expressed on APCs, could also impair regulation of the adaptive immune system. In fact, it has been shown that monocytes from individuals with HIV infection have a tolerogenic phenotype ($ILT4^{hi}$), which is induced by elevated levels of serum IL-10 and may account, in part, for their impaired capacity to present Ags and stimulate T cells [48].

In Vitro Restoration of T-cell functions by Blockade of Negative Regulators of T-cell Function

Although negative signals are required to downregulate the activity of T cells following acute infection, blockading these pathways in mice chronically infected with LCMV or in peripheral blood mononuclear cells from patients with chronic HIV infection has been shown to restore the capacity of these cells to proliferate, secrete cytokines and to kill viral-infected cells [4••,9••,20•,21]. Interestingly, in HIV-1 infection, blocking PD-1 signal by soluble anti–PDL-1 leads to a potent restoration of HIV-specific CD8 T cells and secretion of TNF-α and IFN-γ as well as increasing the proliferation in response to HIV peptides. Similar results were also reported for hepatitis C virus as well as SIV-specific T cells upon in vitro blocking of PD-1/PDL-1 interactions. The implication of IL-10 triggering in T-cell exhaustion during HIV infection is also demonstrated by some in vitro studies, which show that the defective Ag-specific CD4 T cells in patients who are HIV positive can be reversed by anti–IL-10 antibody, including the response to HIV envelope synthetic peptides. In addition, defective functions of T cells in individuals who are HIV positive can be restored in vitro after negative modulation of the CTLA-4–mediated pathway. Indeed, in vitro blockade of CTLA-4 was associated with an increase in the effector function of CD4 and CD8 T cells in both SIV macaque and HIV human model [24•,49]. Finally, in vitro inhibition of IDO with the competitive blocker 1-methyl tryptophan results in increased CD4 T-cell proliferative responses in peripheral blood mononuclear cells from patients with HIV infection [37].

Conclusions

Converging evidence suggests that T-cell exhaustion plays a primary role in the pathogenesis of multiple diseases, such as chronic viral infections, tumors, and autoimmune diseases. Understanding the mechanisms by which

these immune factors influence the competence of T cells may pave the way for therapeutic intervention capable of blocking or neutralizing their effect.

Disclosures

Dr. Rabih Halwani is a fellow through the Canadian Institute of Health Research.

Dr. Elias Said is a fellow through the Canadian Institute of Health Research.

No further potential conflict of interest information relevant to this article was reported.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Dutton RW, Bradley LM, Swain SL: **T cell memory.** *Annu Rev Immunol* 1998, **16:**201–223.
2. Mahnke YD, Schwendemann J, Beckhove P, Schirrmacher V: **Maintenance of long-term tumour-specific T-cell memory by residual dormant tumour cells.** *Immunology* 2005, **115:**325–336.
3. Swain SL: **CD4 T-cell memory can persist in the absence of class II.** *Philos Trans R Soc Lond B Biol Sci* 2000, **355:**407–411.
4.•• Barber DL, Wherry EJ, Masopust D, et al.: **Restoring function in exhausted CD8 T cells during chronic viral infection.** *Nature* 2006, **439:**682–687.

This article reported the first evidence regarding the implication of PD-1 in T-cell exhaustion in a murine model.

5. Matloubian M, Concepcion RJ, Ahmed R: **CD4+ T cells are required to sustain CD8+ cytotoxic T-cell responses during chronic viral infection.** *J Virol* 1994, **68:**8056–8063.
6. Zajac AJ, Blattman JN, Murali-Krishna K, et al.: **Viral immune evasion due to persistence of activated T cells without effector function.** *J Exp Med* 1998, **188:**2205–2213.
7. Wherry EJ, Blattman JN, Murali-Krishna K, et al.: **Viral persistence alters CD8 T-cell immunodominance and tissue distribution and results in distinct stages of functional impairment.** *J Virol* 2003, **77:**4911–4927.
8. Wodarz D, Klenerman P, Nowak MA: **Dynamics of cytotoxic T-lymphocyte exhaustion.** *Proc Biol Sci* 1998, **265:**191–203.
9.•• Day CL, Kaufmann DE, Kiepiela P, et al.: **PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression.** *Nature* 2006, **443:**350–354.

PD-1 expression controls T-cell exhaustion on Ag-specific T cells and is related to disease progression.

10. Douek DC, Brenchley JM, Betts MR, et al.: **HIV preferentially infects HIV-specific CD4+ T cells.** *Nature* 2002, **417:**95–98.
11. Papagno L, Spina CA, Marchant A, et al.: **Immune activation and CD8+ T-cell differentiation towards senescence in HIV-1 infection.** *PLoS Biol* 2004, **2:**E20.
12. Seth N, Kaufmann D, Lahey T, et al.: **Expansion and contraction of HIV-specific CD4 T cells with short bursts of viremia, but physical loss of the majority of these cells with sustained viral replication.** *J Immunol* 2005, **175:**6948–6958.
13. Younes SA, Trautmann L, Yassine-Diab B, et al.: **The duration of exposure to HIV modulates the breadth and the magnitude of HIV-specific memory CD4+ T cells.** *J Immunol* 2007, **178:**788–797.
14. Sun Y, Permar SR, Buzby AP, Letvin NL: **Memory CD4+ T-lymphocyte loss and dysfunction during primary simian immunodeficiency virus infection.** *J Virol* 2007, **81:**8009–8015.
15. Mackewicz CE, Yang LC, Lifson JD, Levy JA: **Non-cytolytic CD8 T-cell anti-HIV responses in primary HIV-1 infection.** *Lancet* 1994, **344:**1671–1673.
16. Andersson J, Kinloch S, Sonnerborg A, et al.: **Low levels of perforin expression in CD8+ T lymphocyte granules in lymphoid tissue during acute human immunodeficiency virus type 1 infection.** *J Infect Dis* 2002, **185:**1355–1358.
17. Lichterfeld M, Kaufmann DE, Yu XG, et al.: **Loss of HIV-1–specific CD8+ T cell proliferation after acute HIV-1 infection and restoration by vaccine-induced HIV-1-specific CD4+ T cells.** *J Exp Med* 2004, **200:**701–712.
18. Petrovas C, Price DA, Mattapallil J, et al.: **SIV-specific CD8+ T cells express high levels of PD1 and cytokines but have impaired proliferative capacity in acute and chronic SIVmac251 infection.** *Blood* 2007, **110:**928–936.
19. Sallusto F, Lenig D, Forster R, et al.: **Two subsets of memory T lymphocytes with distinct homing potentials and effector functions.** *Nature* 1999, **401:**708–712.
20.•• Trautmann L, Janbazian L, Chomont N, et al.: **Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction.** *Nat Med* 2006, **12:**1198–1202.

This work demonstrates for the first time that PD-1 is implicated in the exhaustion of Ags CD8 T cells in HIV infection. This exhaustion could be restored by blocking the PD-1 pathway leading to recovery of cell proliferation and cytokine secretion.

21. Petrovas C, Casazza JP, Brenchley JM, et al.: **PD-1 is a regulator of virus-specific CD8+ T cell survival in HIV infection.** *J Exp Med* 2006, **203:**2281–2292.
22. Champagne P, Ogg GS, King AS, et al.: **Skewed maturation of memory HIV-specific CD8 T lymphocytes.** *Nature* 2001, **410:**106–111.
23. Appay V, Dunbar PR, Callan M, et al.: **Memory CD8+ T cells vary in differentiation phenotype in different persistent virus infections.** *Nat Med* 2002, **8:**379–385.
24.• Kaufmann DE, Kavanagh DG, Pereyra F, et al.: **Upregulation of CTLA-4 by HIV-specific CD4(+) T cells correlates with disease progression and defines a reversible immune dysfunction.** *Nat Immunol* 2007, **8:**1246–1254.

CTLA-4 expression on Ag-specific CD4 T cells in HIV infection is highly involved in T-cell exhaustion and is related to disease progression. Antibody blockade of CTLA-4 restores CD4 functions.

25. Greenwald RJ, Latchman YE, Sharpe AH: **Negative co-receptors on lymphocytes.** *Curr Opin Immunol* 2002, **14:**391–396.
26. Shiratori T, Miyatake S, Ohno H, et al.: **Tyrosine phosphorylation controls internalization of CTLA-4 by regulating its interaction with clathrin-associated adaptor complex AP-2.** *Immunity* 1997, **6:**583–589.
27. Chemnitz JM, Parry RV, Nichols KE, et al.: **SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of programmed death 1 upon primary human T cell stimulation, but only receptor ligation prevents T cell activation.** *J Immunol* 2004, **173:**945–954.
28. Lee KM, Chuang E, Griffin M, et al.: **Molecular basis of T cell inactivation by CTLA-4.** *Science* 1998, **282:**2263–2266.
29. Schneider H, Downey J, Smith A, et al.: **Reversal of the TCR stop signal by CTLA-4.** *Science* 2006, **313:**1972–1975.
30. Nishimura H, Honjo T, Minato N: **Facilitation of beta selection and modification of positive selection in the thymus of PD-1–deficient mice.** *J Exp Med* 2000, **191:**891–898.
31. Carreno BM, Collins M: **The B7 family of ligands and its receptors: new pathways for costimulation and inhibition of immune responses.** *Annu Rev Immunol* 2002, **20:**29–53.
32. Freeman GJ, Long AJ, Iwai Y, et al.: **Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation.** *J Exp Med* 2000, **192:**1027–1034.

33. Fiorentino DF, Zlotnik A, Mosmann TR, et al.: **IL-10 inhibits cytokine production by activated macrophages.** *J Immunol* 1991, **147:**3815–3822.
34. Mosmann TR, Moore KW: **The role of IL-10 in cross-regulation of TH1 and TH2 responses.** *Immunol Today* 1991, **12:**A49–53.
35. McBride JM, Jung T, de Vries JE, Aversa G: **IL-10 alters DC function via modulation of cell surface molecules resulting in impaired T-cell responses.** *Cell Immunol* 2002, **215:**162–172.
36. Brooks DG, Trifilo MJ, Edelmann KH, et al.: **Interleukin-10 determines viral clearance or persistence in vivo.** *Nat Med* 2006, **12:**1301–1309.
37. Boasso A, Herbeuval JP, Hardy AW, et al.: **HIV inhibits CD4+ T-cell proliferation by inducing indoleamine 2,3-dioxygenase in plasmacytoid dendritic cells.** *Blood* 2007, **109:**3351–3359.
38. Donaghy H, Gazzard B, Gotch F, Patterson S: **Dysfunction and infection of freshly isolated blood myeloid and plasmacytoid dendritic cells in patients infected with HIV-1.** *Blood* 2003, **101:**4505–4511.
39. Lore K, Sonnerborg A, Brostrom C, et al.: **Accumulation of DC-SIGN+CD40+ dendritic cells with reduced CD80 and CD86 expression in lymphoid tissue during acute HIV-1 infection.** *AIDS* 2002, **16:**683–692.
40. Macatonia SE, Lau R, Patterson S, et al.: **Dendritic cell infection, depletion and dysfunction in HIV-infected individuals.** *Immunology* 1990, **71:**38–45.
41. Yonezawa A, Morita R, Takaori-Kondo A, et al.: **Natural alpha interferon-producing cells respond to human immunodeficiency virus type 1 with alpha interferon production and maturation into dendritic cells.** *J Virol* 2003, **77:**3777–3784.
42. Polyak S, Chen H, Hirsch D, et al.: **Impaired class II expression and antigen uptake in monocytic cells after HIV-1 infection.** *J Immunol* 1997, **159:**2177–2188.
43. Louie AT, Wahl LM, Hewlett IK, et al.: **Impaired antigen presentation to CD4+ T-cells by HIV-infected monocytes is related to down-modulation of CD4 expression on helper T-cells: possible involvement of HIV-induced cellular factors.** *FEBS Lett* 1996, **398:**1–6.
44. Kedzierska K, Crowe SM, Turville S, Cunningham AL: **The influence of cytokines, chemokines and their receptors on HIV-1 replication in monocytes and macrophages.** *Rev Med Virol* 2003, **13:**39–56.
45. Clerici M, Wynn TA, Berzofsky JA, et al.: **Role of interleukin-10 in T helper cell dysfunction in asymptomatic individuals infected with the human immunodeficiency virus.** *J Clin Invest* 1994, **93:**768–775.
46. Kumar A, Angel JB, Daftarian MP, et al.: **Differential production of IL-10 by T cells and monocytes of HIV-infected individuals: association of IL-10 production with CD28-mediated immune responsiveness.** *Clin Exp Immunol* 1998, **114:**78–86.
47. Trabattoni D, Saresella M, Biasin M, et al.: **B7-H1 is up-regulated in HIV infection and is a novel surrogate marker of disease progression.** *Blood* 2003, **101:**2514–2520.
48. Vlad G, Piazza F, Colovai A, et al.: **Interleukin-10 induces the upregulation of the inhibitory receptor ILT4 in monocytes from HIV positive individuals.** *Hum Immunol* 2003, **64:**483–489.
49. Hryniewicz A, Boasso A, Edghill-Smith Y, et al.: **CTLA-4 blockade decreases TGF-beta, IDO, and viral RNA expression in tissues of SIVmac251-infected macaques.** *Blood* 2006, **108:**3834–3842.