Role of CD40 Ligand dysregulation in HIV-associated dysfunction of antigen-presenting cells

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Abstract: Cellular interactions between antigen-presenting cells and activated CD4+ T cells are central to the regulation of adaptive immunity. Among the many receptor–ligand pairs involved, the critical importance of CD40–CD40 Ligand (CD40L) interactions has been demonstrated in many experimental systems. Dysregulation of antigen-presenting cell function is a hallmark of HIV-associated defects in cell-mediated immunity. Much evidence suggests a mechanistic role for defective CD40–CD40L interactions in such a defect. Consistent with this hypothesis, the capacity to upregulate CD40L on purified CD4+ T cells becomes progressively impaired in HIV infection, in parallel with the progression of clinical immunosuppression. The mechanisms underlying CD40L dysregulation in HIV infection remain unknown. Because CD40L expression is tightly regulated (transcriptionally, post-transcriptionally and post-translationally), HIV may interfere at several levels. However, a transcriptional defect in CD40L expression, mediated by the engagement of CD4 by HIV gp120, appears to play a primary role. Clear elucidation of mechanism may well lead to the development of novel immunotherapeutic approaches to HIV infection. J. Leukoc. Biol. 74: 000–000; 2003.

Key Words: gp120 · interleukin-12 · immune suppression · CD4

INTRODUCTION

HIV-1 infection is associated with a gradual loss of immune competence, leading to an increased susceptibility to infections and cancers. Although HIV infection is associated with abnormalities in most compartments of the immune system, defects in cell-mediated immunity appear to be of greatest clinical import. Such defects include (1) aberrant or absent CD4+ T cell responses; (2) inefficient CD8+ T cell activity; and (3) dysregulation of antigen-presenting cell (APC) function, which will be the focus of this review. Functional consequences include poor control of HIV replication, as well as of other pathogens for which cell-mediated immunity is essential for clearance.

Defective APC function in HIV

HIV infection is associated with decreased numbers of both plasmacytoid and myeloid dendritic cells (DC) [1–6]. Both populations are targets for HIV infection [7–9], as indicated by the presence of provirus in the vast majority of DC from these two subsets in HIV-infected individuals [9]. In addition to depletion and infection, both myeloid and plasmacytoid DC exhibit impaired function, as evidenced by poor stimulation of allogeneic T lymphocytes [9], a functional defect also demonstrated in Langerhans cells from HIV-infected donors [10] and recapitulated in enriched populations of in vitro infected monocyte-derived DC [11]. The mechanisms underlying impaired DC functions are not fully elucidated but do not appear to involve infection of the allogeneic T cells [11].

In the (SIV)/macaque model, other DC functional defects have been demonstrated. During acute SIV infection, Langerhans cell density is reduced in the skin, but the proportion of activated DC is increased in lymph nodes. In contrast, during AIDS, both DC migration from skin and DC activation within lymph nodes are suppressed [12]. Altered expression of the chemokines and chemokine receptors that drive DC trafficking has also been shown during SIV infection, with increases in lymph node expression of CCL19/MIP-3B, CCL20/MIP-3α, CCR6, and CCR7 during acute infection and progressive decreased expression of CCL21/SLC through progression to AIDS [13]. These findings suggest that disruption of homeostatic chemokine expression may be responsible for alterations in APC trafficking to lymphoid tissues, ultimately contributing to systemic immunodeficiency.

Studies of monocyte/macrophage (MΦ) function in HIV infection also indicated a broad range of defects, including abnormalities in phagocytosis [14, 15] and intracellular killing [15]. Antigen presentation by MΦ is abnormal [16], which may be linked to reduced expression of MHC class II and B7 costimulatory molecules [17–20]. Increased expression of Fas and the de novo appearance of Fas Ligand have been reported [21, 22]. Importantly, monocytes and macrophages show dysregulation in their production of cytokines (rev. in [23]). In particular, peripheral blood mononuclear cells (PBMC) from HIV-infected patients and MΦ infected in vitro are markedly

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impaired in their ability to produce interleukin-12 (IL-12), a cytokine that is critical for the generation and regulation of cell-mediated immunity (rev. in [24]).

IL-12 is a key link between innate and adaptive immunity. It is a potent inducer of IFN-γ production in T cells and natural killer (NK) cells, and enhances NK cytotoxicity, as well as the generation of cytolytic CD8+ T lymphocytes. IL-12 is also comitogenic for T and NK cells and is necessary for in vivo delayed-type hypersensitivity reactions in most models. Interestingly, all of these functions are known to be dysfunctional in HIV-infected patients. As might be expected, IL-12 has been shown to play a critical role in resistance in murine models of infection with a variety of intracellular microbes, including Mycobacteria, Cryptosporidia, Toxoplasma, and Histoplasma—all pathogens that cause disease of greater frequency and/or severity in HIV-infected individuals. In agreement with these murine data, IL-12 appears to have an important role in resistance to human tuberculosis [25].

IL-12, which consists of two disulfide-linked subunits (p40 and p35) that form functionally active p70 heterodimers, is produced mainly by APC (MΦ and DC). Production of IL-12 is induced by a variety of bacterial stimuli (likely acting through toll-like receptors), as well as by T cell-derived stimuli (rev. in [24]). Importantly, bacterial and T cell-derived stimuli synergize to induce production of high levels of IL-12 [26]. One of the most potent inducers of IL-12 by human APC is Staphylococcus aureus Cowan strain (SAC). Although SAC can induce IL-12 production in purified MΦ and DC, its activity in PBMC is strongly dependent on APC interactions with activated CD4+ T cells in order to induce high levels of IL-12 [27]. Specifically, interactions between CD40 (on APC) and CD40 Ligand [CD40L] (on activated CD4+ T cells) are crucial for maximum production of IL-12 by PBMC [28].

Other colleagues and we have demonstrated severe defects in the production of IL-12 by PBMC from HIV-infected patients after stimulation with a broad panel of microbial stimuli [28–38]. This defect in IL-12 production does not reflect a global deficiency in proinflammatory cytokine secretion by APC, since TNF-α and IL-1β production remains intact [29, 32]. The in vitro treatment of PBMC from HIV-infected donors with IL-12 ameliorates defective T cell responses [36, 39–42]. Furthermore, recombinant IL-12 protects macaques in vivo from SIV-induced disease; long-term survival correlates with the sustained presence of high levels of SIV-specific cytotoxic T lymphocytes [43]. These encouraging results suggest a critical role for IL-12 in protecting against HIV disease, although similar results have not been obtained in HIV-infected individuals [44]. Discrepant results have been reported about the effect of highly active antiretroviral therapy (HAART) on IL-12 production. In some studies, prolonged suppression of viral replication in HAART-treated patients resulted in improved production of IL-12 [45–47]. Our own results, following a cohort of children with chronic HIV infection, did not show any increases in IL-12 production even after more than two years of therapy [34, 37]. However, in our study, few subjects actually achieved therapy-mediated viral suppression. Of note, none of the above-mentioned studies assessed CD40L expression and its link to IL-12 production.

As for possible mechanism(s) underlying IL-12 suppression in HIV infection, abnormal CD4+ T cell signaling, and CD40L-mediated signaling in particular, may be critical.

**CD40–CD40L interactions**

Among the various receptor–ligand interactions important for CD4+ T cell-APC communication, CD40–CD40L interactions appear crucial for APC activation. CD40, a member of the TNF-receptor superfamily, is constitutively expressed on the surface of APC, including B cells, MΦ, and DC [48]. CD40L, a member of the TNF superfamily, undergoes tightly regulated inducible expression on the surface of CD4+ T cells as a result of signals derived from T cell receptor (TcR) stimulation [48]. CD40–CD40L interactions are critical for the induction and regulation of immune responses, something made clear by humans carrying a mutant CD40L gene (the X-linked hyper-IgM syndrome) and by a variety of mouse models. The effect of engagement of CD40 on APC (DC and MΦ) induces profound phenotypic changes and induces the production of immune regulatory mediators, as summarized in Table 1. It also allows for increased APC survival. In addition, CD40–CD40L interactions play a fundamental role in B cell activation and in optimal CD8 induction; however, these topics will not be covered by this review.

CD40 engagement is one of several pathways of maturation and activation of DC (rev. in [73]). However, its unique role is underscored by the inability of TNF-α (a powerful inducer of DC maturation) to rescue DC function in CD40 knockout mice [74]. It is important to note that other molecules from the TNF-receptor superfamily, particularly TRANCE and RANK, are also involved in DC homeostasis and provide signals complementary and/or redundant to CD40L [75–77].

In summary, the importance of CD40–CD40L interactions in DC–T cell cross talk can be summarized as follows (Fig. 1): (1) cognate DC–T cell interactions induce CD40L up-regulation on activated T cells; (2) CD40 triggering on DC promotes DC maturation and activation; (3) interactions between activated DC and activated T cells stabilize and increase CD40L

**TABLE 1. Effects of CD40 Signaling on APC**

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<tr>
<th>Effects</th>
<th>References</th>
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<tr>
<td>1) Morphologic and phenotypic changes</td>
<td>[49]</td>
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<tr>
<td>- dendrite development (DC)</td>
<td></td>
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<tr>
<td>- increased levels of surface MHC Class II</td>
<td>[49–51]</td>
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<tr>
<td>(DC and MΦ)</td>
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<tr>
<td>- increased levels of costimulatory molecules (DC and MΦ)</td>
<td>[35, 49–52]</td>
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<tr>
<td>- switch in expression of chemokine receptors (DC and MΦ)</td>
<td>[35, 53–56]</td>
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<td>2) Production of immuno-regulatory molecules</td>
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<tr>
<td>- cytokines: TNF-α, IL-12, IL-10, IL-15, IFN-α (DC and MΦ)</td>
<td>[35, 50, 51, 56–60]</td>
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<tr>
<td>- chemokines: IL-8, MIP-1α, MIP-1β, RANTES (DC and MΦ)</td>
<td>[35, 50, 52, 61]</td>
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<td>- nitric oxide (MΦ)</td>
<td>[62–65]</td>
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<td>- metalloproteinases (MΦ)</td>
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<td>3) Increased survival (DC and MΦ)</td>
<td>[50, 67–72]</td>
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Expression; (4) CD40 signaling favors survival of mature DC, thus prolonging DC–T cell interactions and increasing T cell activation. Defective activation of APC by T cells that do not optimally express CD40L could thus represent a primary, pivotal event in the establishment of immunosuppression.

Role of CD40–CD40L interactions in cell-mediated immunity in HIV

Consistent with the hypothesis that IL-12 impairment in HIV-infected donors is partially due to a lack of CD4+ T cell help, we have shown that IL-12 production by PBMC from HIV-infected donors can be restored to normal values by the synergistic combination of IFN-γ and CD40L [28]. These results have been confirmed in other studies [38, 78]. In addition, our recent data indicate that the levels of SAC-induced IL-12 production are correlated with CD40L expression (unpublished results). These results directly suggest a role for impaired CD40L expression in defective IL-12 production in HIV-infected donors. Moreover, Mario Ostrowski et al. have shown the importance of CD40L triggering for generation of optimal T cell activation. Arrows accompanied by a plus sign signify a positive activation signal.

CD40L expression is dysregulated in HIV infection

The capacity to upregulate CD40L on purified CD4+ T cells following stimulation through the TcR becomes progressively impaired in HIV infection, in parallel with overall clinical immunosuppression [38, 82]. Interestingly, CD40L expression is much more affected than CD69 expression, implying that impaired CD40L expression is not due solely to an inability to activate T cells [38]. In contrast, expression of CD40L by purified CD4+ T cells stimulated with the combination of calcium ionophore and phorbol ester is comparable in individuals with and without HIV infection [83]. These results suggest that either the signaling steps impaired in HIV infection lie in the events proximal to TcR engagement or that CD40L impairment may be overcome by more vigorous (albeit nonphysiologically) activation. The mechanisms underlying CD40L dysregulation in HIV infection remain unknown. Interestingly, basal (unstimulated) expression of CD40L is increased on CD4+ T cells from HIV-infected donors and has been reported to decrease following HAART [84]. The effect of HAART on impaired CD40L expression on stimulated T cells has not been reported.

CD40L expression is tightly regulated (transcriptionally, post-transcriptionally and/or post-translationally). HIV may thus interfere at several levels. The proximal CD40L promoter contains several sites that bind transcription factor complexes thought to be important in CD40L transcription [35–37] (cf. Fig. 2). CD40L transcription is also regulated by a Rel/NF-KB element in the 3′flanking region that acts in cis to enhance promoter activity [88]. The cooperative activity of all of these transcription factor complexes is likely needed for optimal CD40L transcription. Nuclear translocation of these factors follows the sequential activation of signaling cascades occurring upon TcR engagement and costimulation through the CD28 receptor. The signaling pathways likely to be relevant for

![Fig. 1. Schematic representation of the role of CD40–CD40L interactions in the DC-T cell cross talk. 1: Cognate DC-T cell interaction activates naïve T cells, inducing upregulation of CD40L expression. 2: CD40L on activated T cells interacts with CD40 on DC and such interaction triggers DC maturation, inducing increased surface expression of MHC Class II and costimulatory molecules. 3: Mature DC provide a positive feedback for optimal CD40L expression, through stabilization following B7–CD28 interactions and through production of IL-12, which enhances CD40L expression. 4: CD40 signaling favors survival of mature DC, thus prolonging DC–T cell interactions and increasing T cell activation. Arrows accompanied by a plus sign signify a positive activation signal.

![Fig. 2. Transcriptional regulation of CD40L promoter in T cells. Numbers in parentheses indicate the promoter coordinates of the regulatory elements. The CD28 responsive element (CD28RE) binds a complex that can be equally supershifted by Ab against p50, p65, c-Rel, c-Fos and JunD [36]. The NF-KB binding site binds to p65 [37]. Both proximal and distal NF-AT binding sites are important for CD40L transcription [85].]
CD40L induction are schematically represented in Fig. 3 (adapted from [89, 90]). TcR signaling leads to an early wave of tyrosine kinase activity. This early wave, through the recruitment of adaptor molecules that serve as docking sites, leads to the activation of three main downstream signaling pathways: (1) the phospholipase C (PLC-γ) pathway, leading to induction of NF-AT; (2) the small GTP-binding protein Ras pathway, leading to induction of c-Fos; and (3) the protein kinase C pathway (PKC-θ). This clearly provides a potential mechanism for gp120-mediated alterations of CD40L expression in uninfected control donors [104–107]. In contrast, gp120 pre-engagement of CD4 impairs subsequent TcR-mediated activation of these CD4 + T cells [108, 109]. HIV gp120 is present at a high concentration in tissues [110, 111] and circulates in the blood of HIV-infected donors, on the surface of virions or as free protein [112].

Role of HIV gp120 in CD40L Dysregulation

Published data suggest that the major HIV-1 surface glycoprotein, gp120, interferes with CD40L expression on CD4 + T cells from HIV-uninfected donors [97]. Several in vitro studies have shown that engagement of CD4 by gp120 profoundly alters CD4 + T cell function. In particular, TcR-mediated activation of lymphocytes whose CD4 molecules have previously been engaged by gp120 induces anergy and apoptosis [97–101]. Such in vitro models mirror what is seen in CD4 + T cells from HIV-infected donors [22, 102, 103]. Of note, this model of gp120–CD4 interactions reconciles two apparently conflicting features of CD4 + T cell responses in HIV infection, the simultaneous presence of immune activation and immune suppression. Indeed, gp120, without further TcR engagement, induces partial activation of CD4 + T cells from uninfected
transmission to CD4+ T cells have been shown to be triggered by CD40L activation of plasmacytoid DC, despite the production of large amounts of type I interferons under these conditions [8]. Similarly, enhanced HIV infection of CD40L-treated Langerhans-like cells has been shown, suggesting a mechanism by which inflammatory CD40L+ T cells, if present in mucosal tissue, could lead to increased HIV transmission efficiency [115].

The role of CD40–CD40L interactions in the reactivation of integrated virus in APC was also demonstrated in our HIV-transgenic mouse model, a system in which APCs serve as the major source of inducible HIV expression [116]. Immune activation of integrated HIV could be driven by the costimulatory interaction of activated CD4+ T cells with APCs, and CD40-CD40L interactions played a major role in this process [117, 118]. Because chronic T cell activation, driven by coinfections, as well as HIV itself, is a characteristic of HIV disease, this pathway may be important in sustaining viral expression from APC reservoirs.

CONCLUSIONS

Due to the central role of CD40–CD40L interactions in APC activation, the selective decrease of CD40L-expressing CD4+ T cells in HIV infection may constitute a major mechanism underlying HIV-induced APC dysfunction. Such a defect is expected to have disproportionate consequences, starting a vicious cycle, in which defective APCs fail to give optimal feedback signals to T cells, which, in turn, fail to provide signals critical for APC survival. An acquired deficiency in CD40L would be predicted to impair control, not only of HIV, but also of the many pathogens controlled by strong cellular immunity. The mechanisms by which HIV infection affects CD40L expression remain unclear but appear to involve HIV gp120-mediated engagement of CD4. Clear elucidation of mechanism(s) may well lead to the development of novel immunotherapeutic approaches to HIV infection.

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REFERENCES


