HIV-specific CD8 T cells express low levels of IL-7R alpha: Implications for HIV-specific T cell memory

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HIV-specific CD8 T cells express low levels of IL-7Rα: Implications for HIV-specific T cell memory

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Abstract

Chronic infections in mice can result in defects in memory CD8 T cell properties including low expression of the IL-7Rα (CD127). To determine whether defects in memory CD8 T cell formation exist during human chronic infections and to what extent these defects may be allele- or epitope-specific, we compared influenza (Flu), vaccinia (VV) and EBV-specific CD8 T cells to HIV-specific CD8 T cells, using a panel of 13 HIV tetramers. Compared to Flu, VV or EBV, HIV tetramer+ CD8 T cells expressed significantly lower levels of CD127, and this reduction was pervasive across all epitopes and alleles tested and over a wide range of viral loads and CD4 counts. These results indicate impaired HIV-specific memory CD8 T cell differentiation, regardless of level of control of viremia, epitopes targeted or restricting HLA alleles.

Keywords

HIV; CD8 T cell; Immunological memory; T cell memory; Chronic infection; IL-7rα

Introduction

Chronic viral infections are often associated with loss of virus-specific CD8 T cell effector functions (e.g. cytokine production or cytotoxicity) (Wherry and Ahmed, 2004). Such functional impairment has been observed in mouse models (Fuller et al., 2004; Gallimore et al., 1998; Ou et al., 2001; Wherry et al., 2003; Zajac et al., 1998; Zhou et al., 2002) and also in humans (Goepfert et al., 2000; Greten et al., 1998; Kelleher and Rowland-Jones, 2000; Kostense et al., 2001; Lechner et al., 2000; Reignat et al., 2002; Shankar et al., 2000;
Recent studies in mice have found that virus-specific CD8 T cells during chronic infections also fail to develop normal memory CD8 T cell properties such as antigen-independent persistence and homeostatic proliferation (Fuller et al., 2005; Lang et al., 2005; Obar et al., 2004; Wherry et al., 2004). For example, during chronic LCMV infection, virus-specific CD8 T cells are unable to respond to the homeostatic cytokines IL-7 and IL-15 and their numbers decline when removed from antigen (Wherry et al., 2004). These defects are in stark contrast to the IL-7 and IL-15 dependent self-renewal and maintenance of memory CD8 T cells following resolution of an acute infection (Kaech et al., 2002; Schluns and Lefrancois, 2003; Wherry and Ahmed, 2004). Substantial reduction in the levels of IL-7 and IL-15 receptors during chronic infection may be a central cause for the poor antigen-independent memory T cell properties (Fuller et al., 2005; Lang et al., 2005; Obar et al., 2004; Wherry et al., 2004). It remains unclear, however, whether defects in memory CD8 T cell maintenance pathways also arise during human chronic infections and whether there are allele- or epitope-specific differences in memory CD8 T cell properties including IL-7Rα expression.

In the current study, we have addressed this issue in humans by comparing IL-7Rα (CD127) expression on CD8 T cells specific for non-persisting human viruses (influenza virus (Flu) and vaccinia virus (VV)), a latent infection (EBV) and for HIV-specific CD8 T cells. Our results indicate that CD127 expression is significantly reduced on HIV-specific CD8 T cells compared to CD8 T cells specific for Flu, VV or EBV. Low CD127 expression on HIV-specific CD8 T cells did not correlate with viral load, CD4 count or the magnitude of the epitope-specific CD8 T cell population examined. In addition, low CD127 levels on HIV-specific CD8 T cells were found in diverse patient cohorts from both the U.S. and South Africa regardless of the epitope or restricting HLA allele. These results suggest that reduced IL-7Rα expression on virus-specific CD8 T cells is a pervasive effect of chronic HIV infection. These observations may have important implications for the development of memory CD8 T cells during HIV infection and for vaccination strategies designed to preserve HIV-specific CD8 T cell responses.

**Results and discussion**

To investigate how HIV infection impacts the expression of the IL-7Rα on virus-specific CD8 T cells, we assembled cohorts of healthy controls subjects (HIV-negative; n=15) and HIV-infected subjects from the U.S. (n=12) and South Africa (n=30) (see Table 1). PBMC were screened for HIV-specific CD8 T cell epitopes by ELISPOT as described (Altfeld et al., 2000), and multiple HLA class I/peptide tetramers were then constructed for the most frequently targeted epitopes to identify CD8 T cells specific for HIV (tetramers were constructed as described; Altman et al., 1996). As controls, Flu, VV and EBV responses were identified by screening PBMC from HIV-negative healthy donors.

Using a panel of 17 tetramers (13 HIV, 1 Flu, 1 VV and 2 EBV; see Table 2), virus-specific CD8 T cell phenotype was examined. CD27 is typically downregulated with progressive T cell differentiation and CD27lo T cells may represent effectors cells (Appay et al., 2002; Hamann et al., 1997, 1999). The vast majority of tetramer+ CD8 T cells specific for all 4
viruses expressed high levels of CD27 (Figs. 1A and B), though the percentage of CD27+ cells was lower for both EBV and HIV compared to Flu and VV (p<0.05). CD8 T cell populations specific for HIV and EBV did not differ from each other for CD27 expression. Flu and VV-specific CD8 T cells also expressed high levels of CD62L. CD62L is downregulated upon activation and often remains low for long periods of time. Low levels of this molecule were expressed by EBV and HIV-specific CD8 T cells compared to Flu and VV-specific CD8 T cells (p<0.05; Figs. 1A and B). Levels of CD62L were also significantly lower on HIV-specific CD8 T cells when compared directly to EBV-specific CD8 T cells (p<0.05; Fig. 1B). Thus, HIV-specific CD8 T cells exhibit a largely CD27+ and CD62LLo phenotype consistent with previous studies (Appay et al., 2002; Champagne et al., 2001).

We next evaluated whether human virus-specific CD8 T cells responding to acute versus chronic infections differed in their expression of CD127 (IL-7Rα). Flu and VV-specific CD8 T cells expressed high levels of CD127; for all subjects, 80–97% of the tetramer+ CD8 T cells were CD127+ (Figs. 2A and B). For EBV-specific CD8 T cells, the levels of CD127 were modestly, but significantly (p<0.01) reduced compared to Flu and VV with a mean of ~62% CD127+ (Figs. 2A and B). During HIV infection, the vast majority of tetramer+ CD8 T cells expressed low levels of CD127 (mean of ~25% CD127+, p<0.01 compared to all other groups; Figs. 2A and B). For each of 13 tetramers tested in HIV-infected subjects, less than 50% of the tetramer+ populations expressed high levels of CD127. In fact, it was rare to find an HIV tetramer+ CD8 T cell population that was >50% CD127+ (only 7/78 responses) and none was more than 70% CD127+ in contrast to Flu and VV where the vast majority of tetramer+ CD8 T cells were CD127+.

We next investigated whether there was any relationship between different immunological or virological parameters and CD127 expression by HIV-specific CD8 T cells. There was no clear association between CD127 levels on HIV-specific CD8 T cell populations and viral load (Fig. 3A). Though there was a slight trend towards lower CD127 expression when viral load (VL) was above 1×10^5 copies/ml and higher CD127 expression in patients with undetectable virus, this was not statistically significant and many subjects with suppressed viral replication (VL <50 copies/ml) had predominantly CD127− tetramer+ CD8 T cell populations. Even in patients on antiretroviral therapy (a=10 responses in 8 individuals), the majority of HIV-specific CD8 T cell responses examined were predominantly CD127Lo (filled symbols in Fig. 3). Furthermore, some subjects with higher VL (=>10^4 copies/ml) had >50% CD127+ tetramer+ CD8 T cells. It will be interesting in the future to determine whether the duration of infection or epitope escape mutations plays any role in the levels of CD127 expression on HIV tetramer+ CD8 T cells. There was also no statistically significant correlation between CD127 levels on HIV-specific CD8 T cells and CD4 counts (Fig. 3B) nor between CD127 expression and the magnitude (frequency of tetramer+ CD8 T cells) of the epitope-specific CD8 T cell population (Fig. 3C). Finally, HLA restriction alone did not appear to be a major determinant of CD127 expression on HIV-specific CD8 T cells since there was no significant difference between CD127 expression levels on tetramer+ CD8 T cell populations restricted by 7 different HLA types (Fig. 3D). This was true for HLA types common in both U.S. and South African populations. Together, the data presented indicate that HIV-specific CD8 T cells have dramatically reduced CD127 expression compared to CD8 T cells responding to non-persisting or latent viral infections. Moreover, this low
expression of CD127 by HIV-specific CD8 T cells appears to be pervasive across a range of viral load, CD4 counts, virus-specific CD8 T cell population size and HLA alleles.

The impact of chronic infections on memory CD8 T cell development remains poorly understood. In humans, HIV-specific CD8 T cells have been the subject of considerable attention and studies have found that the memory T cell differentiation state and function of HIV-specific CD8 T cells are altered during HIV infection (Appay et al., 2002; Champagne et al., 2001). Whether or not HIV-specific CD8 T cells develop into bona fide memory T cells capable of self-renewal and other key memory T cell properties is an important, but unresolved, issue. The expression of CD127 (IL-7Rα) plays a key role in antigen-independent memory CD8 T cell homeostasis following acute infection or vaccination (Goldrath et al., 2002; Kaech et al., 2003; Schluns et al., 2000; Wherry et al., 2004). Defects in CD127 expression and IL-7 responsiveness appear to underlie memory T cell defects in mice during chronic infections (Fuller et al., 2005; Lang et al., 2005; Obar et al., 2004; Wherry et al., 2004). Recent studies in humans also suggest that CD8 T cells specific for Flu and RSV, two viruses that are cleared from the host, express higher levels of CD127 than CD8 T cells responding to the latent infections EBV and CMV (van Leeuwen et al., 2005). Low CD127 expression on HIV-specific CD8 T cells has recently been described using a mall number of HLA-A2 or -B8 tetramers (Boutboul et al., 2005; Paiardini et al., 2005). However, neither of these studies directly compared HIV-specific CD8 T cells to virus-specific CD8 T cells responding to acute infections where virus does not persist. In addition, whether low CD127 expression is consistent across multiple HLA alleles and in different human populations remained unclear. The present study confirms and extends these previous reports using 13 different HIV epitopes and 7 different HLA alleles and subjects from diverse geographical regions (e.g. the U.S. and South Africa). We also now provide a direct comparison of HIV-specific CD8 T cells and CD8 T cells specific for Flu and VV, two non-persisting infections. This direct comparison demonstrates dramatically lower levels of CD127 expression on HIV-specific CD8 T cells compared to CD8 T cells specific for acute infections or EBV. Together, our observations and the earlier studies (Boutboul et al., 2005; Paiardini et al., 2005; van Leeuwen et al., 2005) suggest that, during human HIV infection, optimal IL-7Rα expression on virus-specific CD8 T cells is not achieved.

In the current study, we have focused our analysis on only HIV-specific CD8 T cells during HIV infection. An important remaining question is whether or not reduced CD127 expression during HIV infection is restricted to virus-specific CD8 T cells or is rather a global phenomenon affecting all T cells. Both antigen stimulation and IL-7 (or IL-2) signaling can reduce CD127 levels (Fry and Mackall, 2005; Kaech et al., 2003; Park et al., 2004), and loss of CD4 T cells during HIV infection can lead to elevated circulating IL-7 levels in humans (Fry and Mackall, 2005; Kaech et al., 2003; Park et al., 2004). It is unclear how circulating IL-7 levels may impact CD127 expression by HIV-specific CD8 T cells. In HIV+ subjects in the present study, a population of tetramer–CD127+ CD8 T cells is clearly present and in some subjects can represent more than 50% of total CD8 T cells (see Fig. 2 and data not shown), suggesting that the mechanism of low CD127 expression by HIV-specific CD8 T cells may not universally impact all circulating CD8 T cells. However, there is an overall reduction in the percentage of total CD127+ CD8 T cells in HIV+ compared to HIV−subjects (41.1±2.3% versus 71.1±4.0% respectively; p<0.0001). This difference
may reflect the expansion of HIV-specific CD8 T cells, ongoing T cell responses to concomitant or opportunistic infections or a more global effect of HIV infections on T cells. Unfortunately, at the time of this study, reagents to examine CD8 T cell responses to acute infections (e.g. influenza virus) were not available for the South African cohort and too few non-HIV-specific responses were identified in the U.S. subjects to draw definitive conclusions regarding the global or antigen-specific nature of CD127 downregulation. It will be important to address this question in the future by examining CD8 T cells specific for nonpersisting viruses (e.g. Flu, VV, etc) in HIV-infected subjects both in the U.S. and in South Africa.

Finally, our observations may have important implications for the development of CD8 T cell memory during HIV infection since efficient responsiveness to IL-7 is a key property of antigen-independent memory CD8 T cells. Interestingly, several groups have documented a substantial loss of HIV-specific CD8 T cells following antiretroviral therapy or epitope escape mutation where antigen levels are substantially reduced or eliminated (Alter et al., 2003; Jamieson et al., 2003). Whether low levels of CD127 contribute to this poor persistence of HIV-specific CD8 T cells when antigen levels decline is currently unclear. Future studies will be necessary to address how and why CD127 levels remain low during HIV infection and whether low CD127 expression impacts long-term HIV-specific CD8 T cell maintenance.

**Materials and methods**

**Patients**

Healthy control subjects and HIV-infected patients were recruited in Atlanta (Emory University), Boston (Partners AIDS Research/Massachusetts General Hospital/Harvard University) and South Africa (University of KwaZulu-Natal, Durban) with institution review board approval, and all blood was obtained and processed in accordance with institutional human subjects and biosafety requirements. PBMC were obtained from healthy controls ($n=15$) and HIV-infected subjects ($n=42$). For patient characteristics, see Table 1.

**Flow cytometry**

PBMC were stained with HLA class I/peptide tetramers and antibodies to phenotypic markers and analyzed by flow cytometry as described (Kim et al., 2005). For a list of HLA class I/peptide tetramers, see Table 2. HLA class I/peptide tetramers were either purchased from Beckman Coulter or produced as described (Altman et al., 1996).

**Statistical analysis**

Statistical analysis was performed using a pairwise $t$ test or ANOVA. For correlations, linear regression was performed using Prism (GraphPad Software).

**Acknowledgments**

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References


Fig. 1.
Identification and characterization of human virus-specific CD8 T cells. (A) CD27 and CD62L expression on Flu or HIV tetramer+ CD8+ T cells is shown. The percentage of influenza (Flu), vaccinia (VV), EBV or HIV tetramer+ CD8 T cells that expressed CD27 or CD62L is indicated. Horizontal lines indicate the average for each group. For CD27, both EBV and HIV were lower than either Flu or VV (p<0.01 in pairwise t test comparisons), but EBV and HIV were not different from each other (p>0.05). For CD62L, both EBV and HIV were lower than Flu or VV (p<0.01) and HIV was lower than EBV (p=0.03).
Fig. 2.
Expression of CD127 (IL-7Rα) by human virus-specific CD8 T cells. Flu, VV, EBV and HIV-specific CD8 T cells were identified with HLA class I/peptide tetramers and costained for CD127 expression. (A) Representative FACS plots for Flu, VV, EBV and HIV tetramer versus CD127 staining are shown. All plots are gated on CD8+ T cells, and the numbers indicate the percentage of CD127+ tetramer+ CD8 T cells. (B) Summary of CD127 stains for Flu, VV, EBV and HIV tetramer+ CD8 T cells. Lines indicate the average for each group. *p* values for pairwise comparisons are indicated.
Fig. 3.
Relationship of CD127 expression to immunological and virological parameters. Viral load, CD4 counts and HLA typing were performed in routine clinical evaluation as described (Altfeld et al., 2000), and these data were compared to CD127 expression on HIV tetramer+ CD8 T cells in each subject. CD127 expression (% of tetramer+ CD8 T cells) was plotted versus viral load (A), CD4 T cell counts (B) or percent of CD8 T cells that were tetramer+ (C). For parts A–C, lines indicate the best fit linear regression analysis. In all cases, no significant correlation was observed. For part D, the percent CD127$^{\text{Hi}}$ tetramer+ CD8 T cells was plotted for each of the 7 HLA alleles studied. No significant difference was observed for any HLA allele ($p>0.05$ for all pairwise comparisons). Filled symbols indicate the subjects treated with antiretroviral therapy.
### Table 1

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### Table 2

Tetramers used in this study

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