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HIV-Specific IL-21 Producing CD4+ T Cells Are Induced in Acute and Chronic Progressive HIV Infection and Are Associated with Relative Viral Control

Feng Yun Yue,*† Calvin Lo,*† Ali Sakhdari,*† Erika Yue Lee,*† Colin M. Kovacs,‡ Erika Benko,‡ Jun Liu,*† Haihan Song,*† R. Brad Jones,*† Prameet Sheth,*† Duncan Chege,*† Rupert Kaul,*† and Mario A. Ostrowski*†‡

We examined the role of CD4+ T cell IL-21 production in viral control of HIV infection. HIV-infected individuals had greater circulating IL-21–producing CD4+ T cells in blood compared with uninfected volunteers. HIV-specific IL-21–producing CD4+ T cells were detected in blood during untreated acute and chronic HIV infection, and elevated frequencies of these cells correlated with relative viral control. These cells had an effector memory or end effector phenotype and expressed CXCR5. HIV-specific CD8+ T cells are induced in viremic HIV infection and likely contribute to viral control by affecting CD8+ T cell maintenance. The Journal of Immunology, 2010, 185: 498–506.

In chronic viral infections, especially those that have a tendency to become persistent, CD4+ T cells have been shown to be necessary for the maintenance of an effective ongoing CD8+ CTL response (1), and to mature neutralizing Ab responses to viruses (2). In the lymphocytic choriomeningitis virus (LCMV) murine model (3–7), CD4+ T cells, although not required for the initial induction of primary CTL responses, are necessary to maintain CTL function during the chronic phase of infection (3, 4, 7). In chronic persistent infections, such as in certain strains of LCMV, and HIV, virus-specific CD8+ T cells are shown to have an exhausted phenotype characterized by an inability to produce cytokines, perforin, and granzyme, or proliferate in response to cognate Ag, and have been shown to upregulate the exhaustion markers, PD-1, LAG-3, 2B4, and Tim-3 (8–12). Recently, IL-21, a member of the γ-chain cytokines depending on Ag abundance. Thus, IL-21–producing CD4+ T cells and prostate high levels of IL-21R, indicating sensitivity to IL-21. Low or aviremic long-term nonprogressors, however, showed absent or low HIV-specific IL-21 CD4+ T cells, but more easily detectable HIV-specific IL-2–producing CD4+ T cells, suggesting changing requirements for particular γ-chain cytokines depending on Ag abundance. Thus, IL-21–producing CD4+ T cells are induced in viremic HIV infection and likely contribute to viral control by affecting CD8+ T cell maintenance.

Materials and Methods

Ags used

Yeast-derived p55 of HIV (Austral Biologicals, San Ramon, CA) at 5 µg/ml, yeast cytochrome c protein (Sigma-Aldrich, St. Louis, MO) control at 5 µg/ml was used as a negative control as previously performed (20), and CMV lysate and control lysates were used at a 1/200 final dilution (Virion, Rüschlikon, Switzerland) as previously (20); and staphylococcal enterotoxin B (SEB) was used at a final concentration of 1 µg/ml (Toxin Technologies, Sarasota, FL). Overlapping HIV Clade B Gag pooled peptides were obtained from the National Institutes of Health AIDS Research and Reference Reagent Program. Stimulations were performed with final concentrations of 10 µg/ml/pool.

Indicated abbreviations:

Abbreviations used in this paper: HAART, highly active antiretroviral therapy; LCMV, lymphocytic choriomeningitis virus; LTNP, long-term nonprogressor; SEB, staphylococcal enterotoxin B; Th, follicular Th; VL, viremic load.
Multicolor cytokine flow cytometry

PBMCs were incubated with Ag for 6 h in the presence of brefeldin A and 1 μg/ml of anti-CD49d and CD28 Ab for costimulation (BD Biosciences), and then, cells were washed, permeabilized, and stained by a panel of conjugated Abs (FITC, PE, PerCP, and allophycocyanin). The following Abs in various combinations were used: IFN-γ–PE, IL-2–FITC, IL-4–PE, IL-10–PE, FoxP3–FITC, IL-17–FITC, IL-21–allophycocyanin, CD27–FITC, CD28–allophycocyanin, CD4–PerCP, CXCR5–PE, CCR7–PE, CD3–allophycocyanin, and respective isotype controls. Cells were fixed in 1% paraformaldehyde/PBS and then analyzed on a FACSCalibur (BD Biosciences), and data were acquired by CellQuest software (BD Biosciences) and analyzed with FlowJo (TreeStar, San Carlos, CA). At least 100,000 events in the lymphocyte gate were acquired per sample.

Tetramer stains

For tetramer analysis, 10^6 PBMC were stained with the following tetramers pools to HIV (HLA-A*0201-SLYNTVATL[Gag], HLA-B*0801-FLKEKGGL[Nef], HLA-B*0702-TPGPVRYRL[Nef], HLA-A*0801-GEIYKRWII[Gag], HLA-A*0201-ILKEPVHGV[Pol]), or CMV (HLA-B*0801-ELRRLKMMYM, HLA-A*0201-NLVPMVATV, HLA-A*0702-TPRVTGGGAM, all from PP65) (Beckman Coulter, Fullerton, CA).

IL-21 ELISA

Frozen PBMCs were thawed and plated at 2 × 10^6 cells in 200 μl complete medium and stimulated with p55 Ag (5 μg/ml), and supernatants were harvested at 7 d. IL-21 levels were detected using the IL-21 ELISA MAX kit from BioLegend, according to the manufacturer’s instructions. RNA samples were diluted to 10 ng/μl in diethyl pyrocarbonate-treated water. Reverse transcription and PCR reactions were performed on an ABI Prism 7900HT instrument (Applied Biosystems, Foster City, CA) using the Taqman RNA-to-CT 1-Step Kit (Applied Biosystems), following the manufacturer’s instructions and with the following cycle conditions: 48°C–15 min, 95°C–10 min; 40 cycles of: 95°C–15 s, 60°C–1 min. Primer/probe mixes were Hs00427620_m1 (TBP) and Hs01055421.g1 (IL-21) (Applied Biosystems). Real-time PCR was monitored and analyzed with Sequence Detection System version 2.0 (Applied Biosystems) Reverse transcriptase negative controls were performed in parallel and showed no amplification. Relative quantitations were assigned based on a standard curve generated by serial dilution of RNA from SEB stimulated PBMCs from an HIV-uninfected subject. All samples

Table I. Clinical characteristics of subjects

<table>
<thead>
<tr>
<th>Clinical Stage</th>
<th>n</th>
<th>Infection Period</th>
<th>CD4+ T Cells/mm^3 Mean</th>
<th>CD4+ T Cells/mm^3 Range</th>
<th>VL Copies/ml Mean</th>
<th>VL Copies/ml Range</th>
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<tr>
<td>Acute infection</td>
<td>13</td>
<td>&lt;6 mo</td>
<td>611</td>
<td>310–1130</td>
<td>20,262</td>
<td>375–71,061</td>
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<tr>
<td>Chronic infection</td>
<td>12</td>
<td>&gt;1 y</td>
<td>535</td>
<td>250–960</td>
<td>61,217</td>
<td>2214–373,631</td>
</tr>
<tr>
<td>LTNP</td>
<td>11</td>
<td>&gt;10 y</td>
<td>880</td>
<td>780–1030</td>
<td>60</td>
<td>49–162</td>
</tr>
<tr>
<td>Progressors on HAART</td>
<td>11</td>
<td>&gt;1 y</td>
<td>406</td>
<td>140–540</td>
<td>54</td>
<td>49–100</td>
</tr>
</tbody>
</table>

Undetectable VLs (<50 copies/ml branched DNA) were scored as “49 copies/ml” for calculations of mean VL. Eleven HIV negative subjects were included in the study as well.

Cell stimulation

For PBMC stimulation, 1 × 10^6 PBMCs were cultured with IL-21 at 100 ng/ml or IL-2 at 50 U/ml or in medium alone and harvested at 120 h, and cells were stimulated with a peptide pool spanning HIV Gag protein (eBioscience).

Quantitative RT-PCR for IL-21

PBMCs were cultured in RPMI 1640 media supplemented with 10% FBS and antibiotics. Cells were plated at 1 × 10^6 per well in a 96-well flat-bottom plate and stimulated with SEB (1 μg/ml), HIV p55 Ag (5 μg/ml), or medium control for 6 h. Cells were lysed with RLT Plus buffer (Qiagen), DNA removal and total RNA isolation was performed with the RNeasy Plus Mini Kit (Qiagen) as per manufacturer’s instructions. RNA samples were diluted to 10 ng/μl in diethyl pyrocarbonate-treated water. Reverse transcription and PCR reactions were performed on an ABI Prism 7900HT instrument (Applied Biosystems, Foster City, CA) using the Taqman RNA-to-CT 1-Step Kit (Applied Biosystems), following the manufacturer’s instructions and with the following cycle conditions: 48°C–15 min, 95°C–10 min; 40 cycles of: 95°C–15 s, 60°C–1 min. Primer/probe mixes were Hs00427620_m1 (TBP) and Hs01055421.g1 (IL-21) (Applied Biosystems). Real-time PCR was monitored and analyzed with Sequence Detection System version 2.0 (Applied Biosystems) Reverse transcriptase negative controls were performed in parallel and showed no amplification. Relative quantitations were assigned based on a standard curve generated by serial dilution of RNA from SEB stimulated PBMCs from an HIV-uninfected subject. All samples

FIGURE 1. Viral-specific IL-21–producing cells are detectable in HIV infection. Representative data from an HIV-infected acute seroconverter, a chronically infected individual, and an HIV-uninfected individual (top panel), in which ex vivo PBMCs were stimulated 6 h with either medium control, HIV p55 Ag, CMV lysate, or SEB and then stained for CD4/IL21/IFN-γ/IL-2. The frequencies of Ag-specific cells producing IL-21 (upper panel) or IFN-γ (lower panel) for comparison with the same Ags in a given subject are indicated as percentage of total CD4+ T cells.

![Figure 1](http://www.jimmunol.org/)
FIGURE 2. Viral-specific IL-21–producing cells are detectable in HIV infection. In A, summary data of IL-21–producing CD4+T cells in response to HIV p55 Ag, CMV lysate, and SEB, and stained as described in Fig. 1. The x-axis represents clinical group. LTNP with VL <200 copies/ml. The y-axis represents % CD4+T cells producing IL-21. In B, PBMCs from a normal volunteer (neg) (OM611), an HIV-infected LTNP (OM25, plasma VL <50 copies/ml), two chronic progressors (C) (OM 2, VL = 5000 copies/ml; OM7, VL = 373,631 copies/ml), and two acute seroconverters (A) (OM5029, VL = 12,832 copies/ml; OM5018, VL = 29,000) were cultured in the presence of p55 Ag in plain medium and were assessed for IL-21 by ELISA at 7 d. A low VL during acute/chronic HIV infection was defined as <20,000 copies/ml. In C, comparisons between IL-21 responses to HIV p55 Ag were performed in the same HIV-infected individuals listed in (B) by ELISA and intracellular flow cytometry. In D, IL-21 mRNA is demonstrated from PBMCs (read from left to right) that were treated with either 1 μg/ml SEB, 5 μg/ml HIV p55, or medium control for 6 h, lysed, and RNA was extracted as described in the Materials and Methods. RNA samples from these various subjects and stimulation conditions were diluted to 10 ng/ml and quantitated by one-step Taqman RT-qPCR as described in the Materials and Methods. Relative quantitations of IL-21 and TBP (housekeeping gene) mRNA were assigned by comparison with a standard curve that was generated by serial dilutions of RNA from SEB stimulated PBMCs taken from an HIV-uninfected subject. All samples were analyzed in quadruplicate. Shown are mean ratios of IL-21/TBP relative quantitations expressed as arbitrary units. Error bars represent SE. PBMCs from the following subjects were examined: OM2 (chronic progressor, VL = 5000 copies/ml, C low), OM4 (LTNP, VL = 49 copies/ml, LTNP low), OM14 (chronic progressor, VL = 7000 copies/ml, C low), OM442 (acute seroconverter, VL = 26,389 copies/ml, A high), OM5037 (acute seroconverter, VL = 13,948 copies/ml, A low), OM 5037 (HIV-uninfected, neg). Subjects OM2, OM4, OM14, and OM5037 had HIV p55 IL-21–producing CD4+ T cells >0.03%; whereas, OM442 and 5037 did not have detectable frequencies of >0.02%. In E, summary data of cytokine producing CD4+T cells in the HIV cohort in response to HIV p55 Ag. The x-axis shows specific cytokine response to HIV. IL-2 and IFN-γ represents cells producing both cytokines in response to Ag, which are subsets of IFN-γ and IL-2 responses. Comparisons with statistical significance are shown as ***p < 0.005; **p < 0.05.
FIGURE 3. Phenotypic characterization of IL-21–producing CD4+ T cells. Ex vivo PBMCs from an HIV-infected and -uninfected volunteer were stimulated with Ag for 6 h, and then intracellularly stained for IL-21/IFN-γ/IL-2/IL-17/IL-10/FoxP3/IL-4. Coexpression of IL-21 on Ag-specific cells for IFN-γ/IL-2/IL-17/IL-10/FoxP3 are shown in (A) and IFN-γ/IL-4 in (B). Med = medium control conditions. In C, ex vivo PBMCs from 10 HIV-infected acute seroconverters, 10 chronic progressors, and 10 LTNP were stimulated for 6 h with HIV p55 Ag, and then intracellularly stained for IL-21/IFN-γ/IL-2. Summary data show frequencies of HIV p55-specific CD4+ T cells producing IL-21 alone, IL-21 and IFN-γ, IL-21 and IL-2, and IL-21 and IL-2 and
were run in quadruplicate and IL-21 relative quantitations were standardized to TBP relative quantitations.

Statistical analysis
To determine whether two groups were statistically different for a given variable, we used the Wilcoxon rank sum test (two-tailed) or the Student t test. For correlations of different variables within a group, we calculated Spearman’s correlation coefficient and tested whether it was statistically different from 0 by using an asymptotic normal approximation.

Results
HIV-specific IL-21–producing CD4+ T cells are detected in PBMCs from HIV-infected individuals
To determine the role of IL-21–producing CD4+ T cells in virological control of HIV, we studied 47 HIV infected individuals at various clinical stages of infection (Table I) that included the following: 1) acute/early untreated infection, defined as infected for <6 mo, (6/13 individuals had evolving Western blots and 7/13 were Western blot seropositive with a negative serology in the prior 6 mo); 2) chronic untreated HIV infection defined as seropositivity for >1 y; 3) long-term nonprogressors (LTNPs) who were viral controllers, defined as HIV-seropositive >10 y with no evidence of CD4 decline below 500/mm3, in the absence of treatment, and with a plasma viral load consistently <200 copies/ml; and 4) chronically infected individuals who had progressed and were treated with highly active antiretroviral therapy (HAART) for at least 1 y. In addition, 11 HIV-uninfected normal volunteers were studied as controls. PBMCs were stimulated with Ags ex vivo and examined for IL-21 and other cytokine expression after 6 h by intracellular cytokine flow cytometry. Representative data from an HIV-uninfected and two HIV-infected individuals are shown in Fig. 1, which confirms the presence of virus-specific IL-21–producing CD4+ T cells (data not shown in B).
HIV-infected individuals (in response to CMV and HIV Ags). The majority of IL-21 production from PBMCs, as previously shown (15), was observed in CD4+ T cells (data not shown). Ex vivo CD4+ T cells from HIV-infected individuals, regardless of disease stage or therapy, showed greater IL-21 production in response to SEB when compared with HIV-uninfected individuals, with the following mean frequencies in blood (± SE): during acute/early infection, 0.51 ± 0.07%; during chronic infection, 0.52 ± 0.08%; for LTNP, 0.14 ± 0.03%; for HAART-treated progressors, 0.30 ± 0.08%; and for HIV negative, 0.05 ± 0.02% of total CD4+ T cells, respectively (Fig. 2A). This indicates that HIV infection is associated with greater circulating numbers of IL-21–producing CD4+ T cells in blood. This was also confirmed by IL-21 ELISA, in which PBMCs from some HIV-infected acute and chronic progressors produced greater amounts of IL-21 in response to HIV p55 Ag in culture, compared with that from a LTNP or an HIV seronegative volunteer (Fig. 2B). In addition, there was a direct correlation between HIV-specific IL-21 production by intracellular flow cytometry and supernatant ELISA of p55 stimulated cells taken from the same individual (Fig 2C). There was also a correlation between IL-21 production to SEB and p55 stimulated cells by intracellular flow cytometry and mRNA IL-21 levels (Fig 2D). For example, individuals who had undetectable frequencies (≤0.02%) of IL-21–producing CD4+ T cells by flow cytometry after p55 stimulation also had no detectable IL-21 mRNA after p55 stimulation; whereas, those who had >0.03% frequencies of p55-specific IL-21–producing CD4+ T cells showed inducible IL-21 mRNA (Fig. 2D). IL-21–producing CD4+ T cells responding to HIV p55 Ag were most frequently detected from acute and chronically infected individuals as follows (means ± SE): during acute/early infection, 0.17 ± 0.04%; during chronic infection, 0.06 ± 0.03%; for LTNP, 0.03 ± 0.01%; HAART-treated progressors, 0.03 ± 0.01% of total CD4+ T cells, respectively (Fig. 2E). There was considerable heterogeneity in the range of detection of IL-21–producing HIV-specific cells in the HIV-infected cohort in that LTNP- and HAART-treated individuals had significantly less frequent detection of IL-21–producing HIV-specific cells than participants during acute untreated HIV infection and tended to have less compared with untreated chronic HIV infection (Fig. 2A). CMV-specific IL-21–producing CD4+ T cells were also detectable in HIV-infected and -uninfected individuals (Figs. 1, 2A). IL-21–producing CD4+ T cells tended to be less frequent than those that produced IFN-γ in response to any Ag tested (Figs. 1, 2E). Specifically, when we compared the frequency of HIV-specific cells producing IFN-γ and/or IL-2 to those that produced IL-21 (Fig. 2E), we found that HIV-specific CD4+ T cells producing IFN-γ were more frequent and more easily detectable at all disease stages than those producing IL-2 or IL-21, although these differences were not always statistically significant due to the wide ranges of responses to these cytokines observed in this cohort. For example, in 10 participants with acute/early HIV infection studied, the mean frequencies of HIV p55 Ag-specific CD4+ T cells producing IFN-γ were 0.27 ± 0.07%, 0.11 ± 0.04% for IL-2, 0.12 ± 0.04% for IL-21, and 0.05 ± 0.02% for combined IL-2/IFN-γ (Fig. 2D).

IL-21–producing CD4+ T cells demonstrate an effector memory and end effector phenotype in HIV infection and express CXCR5

We further defined the phenotype of IL-21–producing CD4+ T cells by examining coexpression of cytokines, including IL-2, IFN-γ, IL-17, IL-10, IL-4, and with FoxP3 (Fig. 3A, 3B). IL-21–expressing CD4+ T cells were generally IL-10, IL-4, and FoxP3 negative, expressing IL-21 alone, although small subpopulations did coexpress IFN-γ, with even lesser coexpression of IL-2 and/or IL-17 (Fig. 3A). This was also demonstrated for IL-21–producing HIV-specific CD4+ T cells taken from HIV-infected individuals (Fig. 3A–C), in which 60–70% of the IL-21 response was due to IL-21 single-expressing cells. CXCR5 had been shown to be a marker of IL-21–producing Th1 cells (16). We were able to confirm that IL-21–producing CD4+ T cells from HIV-infected and -infected individuals were similarly enriched in CXCR5 expression when compared with CD4+ T cells that produced IL-17, IL-2, or IFN-γ in response to Ag (Fig. 3D). CD4+ T cells expressing IL-21 in response to SEB or HIV Ags (Fig. 4) showed a predominant CCR7+/CD45RA+ phenotype, consistent with an “effector memory cell” phenotype (21) (Fig. 4). In addition, IL-21–producing CD4+ T cells from HIV-infected individuals also contained a CCR7+/CD45RA+ population consistent with a “late
effector phenotype,” which has been noted to be increased in chronic viral infections (21).

HIV-specific IL-21–producing CD4+ T cells correlate with relative control in acute and chronic HIV infection

Individuals with long-term nonprogression and who controlled plasma virus <200 copies/ml had the lowest detectable frequencies of HIV-specific IL-21–producing CD4+ T in the HIV-infected cohort (Fig. 2B, 2D). Thus, long-term nonprogression with viral control (viral load [VL] <200 copies/ml) was not associated with continuing IL-21–producing responses against HIV Ags. However, when we compared the frequencies of ex vivo IL-21–producing HIV-specific CD4+ T cells from individuals with untreated acute and chronic HIV infection, we observed a significant negative correlation between HIV viral load and HIV 55-specific IL-21 responses measured by intracellular IL-21 flow cytometry (Fig. 5A, 5B) or by supernatant ELISA (Fig. 5C). That is, individuals with relatively low plasma VLs (>200, but <20,000 copies/ml) demonstrated stronger HIV-specific IL-21–inducing responses, than those with plasma VLs ≥20,000 copies/ml.

FIGURE 6. HIV-specific CD8+ T cells express high levels of IL-21Rα. In A, a representative experiment is shown. PBMCs from an HIV-infected chronic progressor were stained with a pool of HIV- or CMV-specific tetramers and costained for Abs to CD8 and IL-21Rα as described in the Materials and Methods. Upper panel shows HIV-specific tetramers, and lower panel, CMV-specific, by IL-21Rα, respectively. In B, summary data of IL-21Rα expression on viral-specific tetramers from seven HIV-infected individuals are shown. In C, PBMCs from HIV-infected and -uninfected individuals were stained for Abs to CD8 and IL-21Rα, and summary data of total CD8 IL-21Rα expression are shown. In D, PBMCs from three HIV-infected individuals were cocultured in medium or IL-21 or IL-2 and then assessed at 120 h for frequency of HIV-Gag pool specific CD8+ T cells expressing IFN-γ by intracellular flow cytometry (see Materials and Methods).
HIV-specific CD8+ T cells express high levels of IL-21 receptor in acute and chronic infection and expand in the presence of IL-21

To determine whether IL-21–producing CD4+ T cells from HIV-infected individuals could influence HIV-specific CD8+ T cells, we compared IL-21 receptor expression from HIV- and CMV-specific CD8+ T cells. HIV- and CMV-specific CD8+ T cells from the same HIV-infected individuals were identified by tetramer analysis and costained for Abs to the IL-21 α-chain. Representative and summary data are shown in Fig. 6A and 6B, respectively. HIV-specific CD8+ T cells showed significantly greater IL-21 receptor expression when compared with those that were CMV specific. In addition, ex vivo CD8+ T cells from individuals during acute and chronic untreated infection demonstrated higher levels of IL-21 receptor expression than those from individuals with long-term nonprogression with viral control (VL <200 copies/ml) or from CD8+ T cells from HIV-uninfected individuals (Fig. 6C). In addition, ex vivo HIV-specific CD8+ T cells from chronic progressors could be easily expanded and survived in cultures if cocultured with IL-21, although, this was similar to stimulation by IL-2 (Fig. 6D).

Discussion

Our findings provide insight into the role of IL-21 in chronic virus infections in humans, namely, HIV infection. Viral-specific IL-21–producing cells are detectable from blood in acute and chronic HIV infection and demonstrate an effector memory, or end effector phenotype. In addition, they are enriched for CXCR5 expression, and tend not to coexpress other cytokines, providing evidence for a unique phenotype, previously described as Th17 (17). Previous studies have demonstrated that another γ-chain cytokine, IL-2, produced by HIV-specific CD4+ T cells correlates with HIV viral control and nonprogression (22, 23); however, it was unclear whether IL-2 was responsible for viral control or was induced by memory T cells only after virus was controlled (23). We find that IL-21–specific T cells are mainly induced when Ag is easily detectable in the plasma. We had also observed increased frequencies of IL-21–producing SEB-stimulated CD4+ T cells in acute and chronic HIV infection, which may reflect an increased frequency of viral-specific CD4+ T cells, whose TCR use similar Vβ chains that respond to SEB, and that have expanded to produce IL-21 in response to HIV. The fact that higher frequencies of IL-21–producing HIV-specific T cells correlate with better control of plasma virus, is consistent with the notion that IL-21 is an effector cytokine that is induced only in the presence of large amounts of Ag, and that once Ag is reduced or cleared, as in the situation of viral control/long-term nonprogression, the need for IL-21 production is reduced. At this point, it is probable that IL-2–producing CD4+ T cells play a larger role in the maintenance of memory. Our findings are also reflected in the LCMV model, in which, after clearance of acute LCMV-Armstrong infection, mice had low levels of detectable IL-21–producing cells, but high levels of IL-2–producing cells; whereas, the presence of chronic persistent LCMV (CI-13) was associated with high levels of IL-21–producing cells and low IL-2 production (15). In addition, in IL-21 receptor-deficient mice, their CD8+ T cells were impaired in self-maintenance suggesting a requirement of CD8+ T cells in chronic virus infection for IL-21 (14). Our observation that HIV-specific CD8+ T cells express high levels of IL-21 receptor during viremia suggests that viral-specific CD8+ T cells require IL-21 to maintain their survival and function, as also recently demonstrated in vitro (24). Taken together, these observations suggest a critical role of IL-21 in the control of chronic HIV infection, as was demonstrated in the LCMV model. Not all HIV-infected individuals had IL-21–specific CD4+ T cells, and these individuals had the highest viral loads. In fact, we noted a threshold effect of VL, in that, individuals with plasma VLs >20,000 copies/ml had infrequent or undetectable HIV-specific IL-21–producing CD4+ T cells. Our findings are consistent with those of Iannello et al. (25) who also demonstrated the presence of HIV-specific IL-21–producing CD4+ T cells in viremic HIV-infected individuals, which however, were less frequent than CMV-specific IL-21–producing CD4+ T cells seen in normal volunteers. In that study, however, relationships between HIV VLs and HIV-specific IL-21–producing CD4+ T cells were not characterized. The reason for lack of IL-21 production in individuals with higher VLs is unclear; however, direct infection and killing by HIV is a possibility that could be examined. Preferential direct infection of IL-21–producing CD4+ T cells by virus would likely reduce help of virus-specific CD8+ T cell responses resulting in a vicious cycle of increasing viral replication, and CD4+ T cell destruction.

In summary, we demonstrate that IL-21 is induced during acute and chronic HIV-infection and correlates with relative control of virus. One caveat of our data is that we were only able to examine CD4+ T cells taken from peripheral blood, and because the majority of HIV replication occurs in lymphoid tissues, it would be important to corroborate such findings from cells taken from lymphoid tissues. Also, it is still unclear, however, whether high levels of HIV viremia that directly lead to CD4+ T cell exhaustion also result in reduced IL-21 production, or whether host-specific abilities or polymorphisms influence the ability to produce IL-21 early and rapidly to control virus. Treatment strategies aimed at replenishing IL-21 during acute infection should be considered as they would address these questions.

Acknowledgments

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Disclosures

The authors have no financial conflicts of interest.

References


Corrections


In Fig 2A, the statistical symbols should not be placed in the CMV Ag conditions, as the differences between groups are not statistically significant.

In Fig 2E, the statistical symbols have been corrected from *** to ** for IFN-γ versus IL-2 in HIV/chronic and for IFN-γ versus IL-2 for HIV/LTNP. The symbols were removed from IFN-γ versus IL-2 for HIV/acute.

The corrected figure is shown on the following page. The published figure legend is correct but is shown here for reference.

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FIGURE 2. Viral-specific IL-21–producing cells are detectable in HIV infection. In A, summary data of IL-21–producing CD4\(^{+}\)T cells in response to HIV p55 Ag, CMV lysate, and SEB, and stained as described in Fig. 1. The x-axis represents clinical group. LTNP with VL <200 copies/ml. The y-axis represents % CD4\(^{+}\) T cells producing IL-21. In B, PBMCs from a normal volunteer (neg) (OM611), an HIV-infected LTNP (OM25, plasma VL <50 copies/ml), two chronic progressors (C) (OM 2, VL = 5000 copies/ml; OM7, VL = 373,631 copies/ml), and two acute seroconverters (A) (OM5029, VL = 12,832 copies/ml; OM5018, VL = 29,000) were cultured in the presence of p55 Ag in plain medium and were assessed for IL-21 by ELISA at 7 d. A low VL during acute/chronic HIV infection was defined as <20,000 copies/ml. In C, comparisons between IL-21 responses to HIV p55 Ag were performed in the same HIV-infected individuals listed in (B) by ELISA and intracellular flow cytometry. In D, IL-21 mRNA is demonstrated from PBMCs (read from left to right) that were treated with either 1 \(\mu\)g/ml SEB, 5 \(\mu\)g/ml HIV p55, or medium control for 6 h, lysed, and RNA was extracted as described in the Materials and Methods. RNA samples from these various subjects and stimulation conditions were diluted to 10 ng/\(\mu\)l and quantitated by one-step Taqman RT-qPCR as described in the Materials and Methods. Relative quantitations of IL-21 and TBP (housekeeping gene) mRNA were assigned by comparison with a standard curve that was generated by serial dilutions of RNA from SEB stimulated PBMCs taken from an HIV-uninfected subject. All samples were analyzed in quadruplicate. Shown are mean ratios of IL-21/TBP relative quantitations expressed as arbitrary units. Error bars represent SE. PBMCs from the following subjects were examined: OM2 (chronic progressor, VL = 5000 copies/ml, C low), OM4 (LTNP, VL = 49 copies/ml, LTNP low), OM14 (chronic progressor, VL = 7000 copies/ml, C low), OM442 (acute seroconverter, VL = 26,388 copies/ml, A high), OM5037 (acute seroconverter, VL = 13,948 copies/ml, A low), OM 5037 (HIV-uninfected, neg). Subjects OM2, OM4, OM14, and OM5037 had HIV p55 IL-21–producing CD4\(^{+}\) T cells >0.03%, whereas OM442 and 5037 did not have detectable frequencies of >0.02%. In E, summary data of cytokine producing CD4\(^{+}\) T cells in the HIV cohort in response to HIV p55 Ag. The x-axis shows specific cytokine response to HIV. IL-2 and IFN-\(\gamma\) represents cells producing both cytokines in response to Ag, which are subsets of IFN-\(\gamma\) and IL-2 responses. Comparisons with statistical significance are shown as ***p < 0.005; **p < 0.05.