Published in final edited form as:

Cell Immunol. 2014 ; 288(0): 24–30. doi:10.1016/j.cellimm.2014.01.009.

T cell interleukin-15 surface expression in chimpanzees infected with human immunodeficiency virus

Annette R. Rodriguez^{1,2}, Vida Hodara², Kruthi Murthy³, LaShayla Morrow², Melissa Sanchez², Amy E. Bienvenu², and Krishna K. Murthy²

¹Center for Research & Training in the Sciences, Research Centers at Minority Institutes Program- Biophotonics Core, University of Texas at San Antonio, San Antonio, TX, 78249

²Department of Virology and Immunology, Texas Biomedical Research Institute, San Antonio, TX, 78245

³Department of Microbiology and Immunology, The University of Texas Health Science Center at San Antonio, San Antonio, TX, 78229

Abstract

Interleukin-15 (IL-15) contributes to natural killer cell development and immune regulation. However, IL-15 and interferon-gamma (IFN- γ) production are significantly reduced during progression to AIDS. We have previously reported that HIV infected chimpanzees (*Pan troglodytes*) express CD3-CD8+ IFN- γ + natural killer (NK) cells with an inverse correlation to plasma HIV viral load. To expand on our initial study, we examined a larger population of HIV infected chimpanzees (n=10). Whole blood flow cytometry analyses showed that recombinant gp120 (rgp120) or recombinant IL-15 induces specific CD3-CD8+ IFN- γ + NK cells at higher levels than CD3+CD8+ IFN- γ + T cells in HIV infected specimens. Interestingly, peripheral blood T cells exhibited 0.5 to 3% IL-15 surface Tcell/NKT cell phenotypes, and rIL-15 stimulation significantly ($P<0.007$) up-regulated CD4+CD25+ T cell expression. Importantly, these data demonstrate novel T cell interleukin-15 expression and indicate a plausible regulatory mechanism for this cell-type during viral infection.

Keywords

Interleukin-15; Natural Killer Cell; NKT cell; interferon-gamma; HIV; AIDS; tumor necrosis factor-alpha

© 2014 Elsevier Inc. All rights reserved.

Corresponding author: Annette R. Rodriguez, Ph.D., Center for Research & Training in the Sciences, Research Centers at Minority Institutes Program, Biophotonics Core, University of Texas at San Antonio, One UTSA Circle, San Antonio, Texas 78249, Fax: (210) 688-9840, Annette.Rodriguez@utsa.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

INTRODUCTION

Chimpanzees share approximately 98% homology with humans, however, few HIV infected chimpanzees have been shown to progress to AIDS-like symptoms [1, 2]. Although this valuable animal model has been successfully used to evaluate resistant mechanisms [3–10], biomedical research using chimpanzees has been substantially reduced as a result of federal government decision and most have been retired to facilities such as the Louisiana Chimp Haven.

In accordance with chimpanzee resistance to AIDS, we have demonstrated that HIV infected chimpanzees maintain robust production of IFN- γ which is influenced by IL-15 [8]. Interleukin-15 (IL-15), interferon-gamma (IFN- γ), and tumor necrosis factor alpha (TNF- α) production are significantly reduced during progression to AIDS [11, 12]. Interleukin-15 plays a crucial role in regulating immune responses by influencing NK cell [13, 14] and NKT cell activity [15], in addition to promoting memory CD8+T cell survival [16, 17]. Interleukin-15 is produced by dendritic cells [18] and monocytes/macrophages [19–21]. Importantly, surface IL-15 is critical for function rather than soluble [19]. Additionally, it has been proposed that IL-15 expression and secretion from the same cell that express the IL-15 receptor-alpha is vital for function [19]. While mRNA has been detected in normal T cells [22], IL-15 protein expression in T cells has been primarily studied in T cell lines [20–22]. Recently, Miranda-Carus and colleagues showed that surface T cell IL-15 expression from normal healthy humans can be induced after three to five days of cell culture [23]. The inability of anti-retroviral therapy to eradicate HIV from infected individuals has prompted an interest in alternative therapeutic and vaccine strategies utilizing cytokines such as IL-2 and IL-15 [24–26]. In view of the fact that IL-15 function is important in inhibition of disease progression in humans [27], we designed a study to further evaluate T cells, NK cells, and the contribution of IL-15 in chimpanzees infected with HIV-1. Surface expression of IL-15, in addition to IFN- γ , and TNF- α production from T cells and NK cells were analyzed in blood specimens from HIV-1 infected chimpanzees. The results indicated that IFN- γ and TNF- α were primarily expressed in CD3-CD8+ NK cells, while IL-15 surface expression was found on CD4+ and CD8+ T cells, NKT cells, CD8+ granulocytes, and CD4 positive monocytes in peripheral blood samples. Additionally, IL-15 stimulation *in vitro* was shown to upregulate CD25 expression on CD4+ T cells. Together, these findings suggest that IL-15 surface expression on peripheral blood T cells and a subpopulation of NKT cells may influence IFN- γ production and modulate CD4 cell subpopulations during HIV infection. This study provides critical insight into the control of progression to AIDS in this animal model. Importantly, the dynamics of IL-15 surface expression expands beyond the aspects of monocyte and dendritic cell contributions. This study shows a new dimension of the T cell/NKT cell repertoire and their effect on viral infection and lack of disease progression in the chimpanzee model.

RESULTS

IFN- γ and TNF- α expression is robust in NK cells during HIV infection

T cell and NK cell IFN- γ expression was evaluated in specimens collected from ten HIV infected and three naïve chimpanzees. Cells were examined within the lymphocyte gate

(Fig. 1a), CD3 positive, CD3 negative gates for CD3+CD8+ and CD3-CD8+ populations. Whole blood controls (PBS) and recombinant gp120 (rgp120) stimulated specimens from HIV+ chimpanzees revealed that CD3-CD8+ CD56- IFN- γ + NK cells were significantly higher than CD3+CD8+ IFN- γ + T cells ($p < 0.009$) [Fig. 1b]. These findings were in agreement with our previous report that included analysis from a smaller group of three chimpanzees during a six month study [8]. As expected, samples from naïve chimpanzees did not result in an increase of IFN- γ (data not shown) following rgp120 stimulation. Additionally, blood samples from three HIV positive chimpanzees were assessed for TNF- α and IFN- γ expression after stimulation of whole blood with rgp120 at three 4-week intervals. CD3-CD8+ NK cell expression of TNF- α and IFN- γ was higher (2.2 %–3.7 % and 1.8 % – 2.9 %, respectively), than CD3+CD8+ T cells (0.2 – 0.3 %) for two of the chimpanzees. Blood samples from one chimpanzee demonstrated CD3+CD8+ TNF- α cell population slightly above 1 %, and IFN- γ at approximately 0.8 % (Fig. 2).

Since IL-15 has been shown to influence NK cell and T cell IFN- γ production, whole blood specimens from three HIV+ and three naïve chimpanzees were analyzed after 15 h stimulation at 37 °C with rIL-15 (20 ng ml⁻¹) or PBS (control). IFN- γ expression increased from 3 to 10-fold with rIL-15 stimulation (Fig. 3). CD3-CD8+ NK cell was greater than the CD56+ NK cells or CD8+ T cell IFN- γ expression. However, the expression from naïve animal samples was variable, with one sample demonstrating a similar percentage of CD3-CD8+ NK cells and CD3+CD8+ T cells expressing IFN- γ . These results demonstrate the prominent role of NK cell specific response to gp120 and the influence of IL-15.

IL-15 expands CD4+CD25+ T cells

To further study the effects of IL-15, fresh PBMCs were isolated from eight additional chimpanzees during routine physicals and cultured with rIL-15 or PBS (control), and analyzed for expression of CD25 on CD4+ T cells and CD8 T+ cells after 24h incubation Table 1. The percentage of CD4+T cells expressing CD25 ranged from 7 to 16 % of the lymphocyte population in IL-15 stimulated cultures, a 2–6 fold increase compared to control samples (Fig. 4a), demonstrating the influence of IL-15 regulation of CD25 expression. The activation marker CD69 was also evaluated on CD4 and CD8 T cells. CD69 was minimally expressed on CD4 T cells, however was increased in CD8 T cells for Chimpanzees 2–8. CD8+ T cells minimally expressed CD25 (data not shown). Further analysis of CD154 (CD40L), important for CD4 T cell interaction with antigen presenting cells, revealed that surface expression from all three HIV positive chimpanzee PBMC samples up-regulated CD154 on CD4+T cells (16.3 %, 52.3 % and 47.8 %) compared to < 3 % of CD8+ T cells with SEB stimulation. Chimpanzee-1 (4X0124) with the lowest CD154 expression has also maintained a low CD4 population (14 to 17 %) with persistent viremia for over ten years (Fig. 4b), while the other two HIV+ chimpanzees have maintained CD4 populations of 35 to 45 % of total lymphocytes.

To examine the viral loads in the HIV positive chimpanzees, we used RT-PCR to assess the plasma viral RNA. The viral loads were below detectable limits for the chimpanzees throughout the study, with the exception of chimpanzee 4X0124. The viral load ranged from 100 copies to over 25,000 copies per mL. The animals used in this study have previously

shown to be positive for viremia sporadically over the past ten years during routine physicals, with the exception of chimpanzee 4X0124, which was consistently positive as previously reported [8].

IFN- γ is secreted after antigen stimulation of whole blood

Total lymphocyte IFN- γ expression was shown to be robust in HIV infected animals and correlated inversely with viral load [8]. Therefore, IFN- γ mRNA was further assessed in PBMCs collected from ten HIV infected chimpanzees and compared to IFN- γ mRNA from five naïve chimpanzees. Real-time RT-PCR analysis revealed that IFN- γ mRNA expression level was not significantly different between infected and uninfected animals (Fig. 5a). However, IFN- γ was not detected by ELISA cytokine analysis in the plasma samples frozen at matching time-points (data not shown). Detection by ELISA was possible with whole blood stimulation of samples (without addition of GolgiPlug™) in parallel with intracellular staining, followed by plasma separation (Fig. 5B). Stimulation with rgp120 resulted in an increase in plasma IFN- γ from three to four-fold in two HIV+ chimpanzees, and less than a 2-fold increase for one chimpanzee with a high baseline level of plasma IFN- γ ($> 400 \text{ pg mL}^{-1}$). SEB stimulation of whole blood increased plasma IFN- γ to over 1000 pg mL^{-1} in all three infected animal specimens. Whole blood from the naïve chimpanzee did not exhibit an increase in plasma IFN- γ with rgp120; however an increase to over 1200 pg mL^{-1} was noted with SEB stimulation compared to less than 300 pg mL^{-1} for PBS control samples. The results demonstrate that while IFN- γ mRNA is similar in HIV+ and naïve chimpanzees, this cytokine is secreted at higher levels in response to specific rgp120 stimulation in HIV infected animals.

Interleukin-15 is expressed on the surface of T cells

Given the significant influence of IL-15 on the production of IFN- γ , and the finding that IL-15 mRNA is produced in T cells [22], surface IL-15 was further assessed in HIV positive and HIV negative chimpanzee whole blood. Analysis was performed within the lymphocyte, monocyte and granulocyte gates; a representative scatter plot and dot plots are shown (Fig. 6A). Interestingly, a portion of CD3+ cells were shown to express surface IL-15 as well as CD16. The populations were subsequently evaluated within the CD3 lymphocyte gate (Fig. 6B). These cells were CD3+CD4+CD16+IL15+, CD3+CD4+CD16-IL15+ and CD3+CD8+CD16-IL15+. However, these populations were CD25 negative. In addition, a large granulocyte CD8 population outside the lymphocyte gate was shown to express surface IL-15 (data not shown); however, they did not express CD83. After the 15 h stimulation with rgp120 or SEB, surface IL-15 expression was up-regulated on HIV+ chimpanzee cells, with CD3+CD8+ T cells exhibiting a 3-fold increase in one specimen with rgp120 stimulation, and a 2-fold increase with SEB (Fig. 6C). Staphylococcal enterotoxin B (SEB) interacts with TCR V β regions [28, 29]. Specimens from naïve animals showed an increase of IL-15 expression on T cells from three to six fold following SEB stimulation. These results demonstrate the diverse population of T cells/NKT cells expressing IL-15, and up-regulation of IL-15 surface expression in response to antigenic stimulation.

DISCUSSION

In this study we have shown that a subpopulation of T cells/NKT cells and monocytes express surface IL-15 and that rIL-15 treatment increases CD4+CD25+ T cells and enhances IFN- γ and TNF- α production in CD8+ NK cells and CD8+ T cells. CD8+ NK cells expressed IFN- γ at significantly higher levels than CD8+ T cells in all blood specimens collected from HIV infected chimpanzees after *in vitro* stimulation with rIL-15. Surface IL-15 was expressed on CD4+ and CD8+ T cells as well as CD3-CD4+ monocytes. The IL-15 expressing subpopulations were not detectable on PBMCs processed by histopaque gradient. This may be the primary reason for the reduced IFN- γ expression in purified PBMCs (data not shown) when compared to whole blood stimulation. However, others have analyzed PBMCs after five to seven days of stimulation with PMA or ionomycin added to cultures for successful T cell analysis. Although this may be adequate for T cell expansion, NK cells require IL-15 for survival and activity. Miranda-Carus and colleagues have suggested that culturing fresh PBMCs at high density improve T cell expansion and surface expression of IL-15 [23].

Our previous report [8] showed that addition of rIL-15 or rgp120 increased IFN- γ production, and that rgp120 + antiIL-15 abrogated IFN- γ production by more than 50% in whole blood collected from HIV infected specimens. Additionally, IFN- γ production showed an inverse correlation to viral load which was primarily mediated by CD3-CD8+ IFN γ + NK cells. Throughout our studies, we used rIL-15 at 20 ng ml⁻¹ for the chimpanzee whole blood system, because IFN- γ production increased with addition of rIL-15 (1 to 20 ng ml⁻¹) in a dose dependent manner, and showed a sharp decrease at 100 ng ml⁻¹ (data not shown).

Considering the increased interest in formulation of a vaccine or therapy utilizing IL-15 [24–27, 30], these findings provide information which should be considered during vaccine and therapy assessment. A recent vaccine study in macaques using SHIV antigen with an IL-15 plasmid as adjuvant demonstrated reduced virus loads in animals compared to control groups without the IL-15 plasmid adjuvant. The immunized animals exhibited strong IFN- γ production and a reduction of IL-8 and metalloproteinase-9 [26]; the latter has been shown to be associated with apoptosis and autoimmune diseases [31].

In vivo analysis has also revealed that IL-15 increases CD8+ NK cells and CD8+ T cells in cynomologous macaques; however, CD4+ T cells and viral loads were not altered [24]. The results may suggest that the function of IL-15 *in vivo* is also dependent on cross presentation [19]. Also, it is important to note that in a separate study, IL-15 treatment during acute infection was shown to increase viral load compared to untreated SIV infected Rhesus Macaques. Although viral specific NK and CD8+ T cells were noted, there was an increase in IFN- γ production, not TNF- α [30]. Interleukin-15 bioavailability and heterodimeric IL-15/sIL15R α forms have recently been investigated [19, 32]. Bergamaschi and colleagues have suggested that the stability of the heterodimeric IL-15/sIL15R α and membrane associated IL-15 may provide an important regulation mechanism for long distance and local function respectively [32]. However, additional mechanisms have also been shown to influence cytokine stability and function [33, 34]. Therefore, the cell surface IL-15 noted on

circulating T cells/NKT cells in this study may provide an essential function in HIV resistance via immune cell modulation, mobilization or activation. Our study, and the additional investigations cited, focus the importance of gaining insight into the cellular and molecular mechanisms of T cell IL-15 expression in humans as well as non-human primate models to determine the influence on viral infection and disease progression.

Relevant studies in humans have identified a rare population of individuals that maintain consistently low HIV viral loads ($< 50\text{--}100$ RNA copies mL^{-1}); these individuals are denoted as HIV controllers (HIC) or “elite” controllers [35–37]. Human HIV non-progressors exhibit higher production of IFN- γ , TNF- α , and IL-2 compared to reduced production in progressors [12]. In this study, rIL-15 increased CD25, the IL2 receptor. Mechanisms of resistance ascribed to “elite” controllers include NK cell activity, HIV specific CD8 T cells [38], CD4 T cells that express IFN- γ and IL-2, higher specificity but lower T cell activation [39], and low T regulatory cells in lymphoid tissues [39].

In this study, rIL-15 also upregulated CD25 expression on CD4 T cells. The activation marker CD69 was minimally expressed on CD25+CD4+ T cells, but was notably increased in the CD8 T cell population for all but one chimpanzee specimen (Fig. 4b). CD69 is a C type lectin that inhibits sphingosine 1 phosphate and lymphocyte egress from lymphoid organs [41]. IL-15 induced the highest CD4+CD25+ T cell increase on the specimen collected from chimpanzee #5, which induced the highest increase in CD3+CD8+CD69+ T cells; the overall positive correlation between the two population groups was only moderate ($r=.68$, Fig. 4). Additional CD4T cell functional analysis and T cell regulatory assessment is required. However, the results may further suggest that IL-15 may also influence interactions of CD8+T cells by retention in the lymphoid tissues, in contrast to CD4+CD25+T cells, which may represent an increase in circulating CD4+CD25+T cells.

Conclusions

While humans tend to exhibit lymph node destruction, chimpanzees do not normally exhibit the lymph node alteration [4] progression to AIDs. The role of CD4+CD25+ T cells collected from lymph nodes versus peripheral blood CD4+CD25+ T cells and HIV specific response requires further evaluation. Dysregulation of CD4+CD25+ populations, and diminished IFN- γ and TNF- α production are markers for progression to AIDS. Although soluble IL-15 concentration is often used to monitor this cytokine, surface IL15 is an essential component [40, 30] of IL-15 functional properties. Our findings advocate further assessment of CD4+CD16+IL-15+ and CD8+IL-15+ NKT/T cell regulation and function. Importantly, T cell surface interleukin-15 may influence NK cell specificity and provide a novel adaptive-innate immune cell connection.

MATERIALS AND METHODS

Chimpanzees

Heparinized blood and clotted blood samples were collected from five naïve chimpanzees and ten HIV infected chimpanzees during routine physicals. Chimpanzees were cared for in

accordance with protocols and procedures approved by the Institutional Animal Care and Use Committee and housed at the Texas Biomedical Institute.

Intracellular Staining

Heparinized blood specimens were aliquoted to 1 mL volumes in polypropylene tubes for intracellular staining (ICS). ICS was conducted utilizing Cytofix/Cytoperm Plus™ kit with GolgiPlug™ (BD Biosciences Pharmingen) as previously described (Rodriguez et al., 2007). Briefly, whole blood was incubated with PBS for controls or stimulated with 10 µg ml⁻¹ of rgp120 or rIL-15 (20 ng ml⁻¹, R&D) and incubated at 37 °C with 5 % CO₂ for 1 hour. GolgiPlug™ (1 µl) was added to all samples, mixed well and incubated for an additional 14 to 15 hours at a slant and processed for surface and intracellular staining (ICS). The fluorescent conjugated antibodies included: CD3 fluorescein isothiocyanate (FITC, Clone SK7, BD Biosciences Pharmingen), CD4 PC5 (phycoerythrin-cyanin 5.1, Clone: 13B8.2, Beckman Coulter), CD8 PC5 (Clone: B9.11, Beckman Coulter), IL-15 PE (Clone: 34559, R&D), CD16 FITC (Clone), and CD56 PC5 (Clone: N901, Beckman Coulter) for surface staining and IFN-γ PE (Clone: 4S.B3, BD Biosciences Pharmingen), or TNF-α for ICS. Samples were acquired by FACScan immediately. Twenty to thirty thousand events within the gated lymphocyte population were collected for IFN-γ and TNF-α analysis and 50,000 events were collected for IL-15 analysis. CD8+, CD4+ and CD16+ populations within the lymphocyte gate were further analyzed by using forward scatter versus CD8, CD4 or CD56; CD4 positive cells were also analyzed within the monocyte gate. Additional flow cytometry included four-color analysis using IFN-γ FITC (Clone: 4S.B3, BD Biosciences Pharmingen), CD3 PerCP (Clone Sp34-2, BD Biosciences Pharmingen), TNF PE (Clone: N901, Beckman Coulter), and CD8 APC (Clone 3B5, Caltag) with collection by CYAN flow cytometer. In a separate experiment, peripheral blood mononuclear cells (PBMCs) were incubated with rIL-15 or PBS (control) for 24 to 48 h then analyzed by flow cytometry for CD3, CD4, CD8 and CD25 by surface staining as previously described. Additionally, HIV positive chimpanzee PBMCs were stimulated with SEB (1 µg ml⁻¹), gp120 (10 µg ml⁻¹), gag (10 µg ml⁻¹) or tat (10 µg ml⁻¹), with PBS for controls, and analyzed for CD154 expression on CD4+ and CD8+ T cells.

Enzyme Linked Immunoassay for IFN-γ Serum

Plasma or culture supernatants were collected and frozen at -80 °C. Samples were analyzed according to manufacturer's procedure for IFN-γ analysis.

Isolation and Storage of PBMCs

EDTA and Heparinized blood were processed for plasma with centrifugation at 700 g and PBMCs were isolated by density gradient using Histopaque 1077. Plasma aliquots were frozen at -80 °C for RT-PCR and cytokine analysis. PBMCs recovered from heparinized blood were utilized cytokine analysis and flow cytometry.

RNA Assessment

TaqMan Real-Time RT-PCR—Standards were developed as previously described [8]. Briefly, gag primers G00-5'GACTAGCGGAGGCTAGAAG3' and

G01-5'AGGGGTCGTTGCCAAAGA3' (34) were used to amplify the gag region of plasmid pBKHB10S (provided by the AIDS Research and Reference Reagent Program) by polymerase chain reaction (PCR) and inserting the PCR product into TOPO TA cloning vector pCRII-TOPO® (Invitrogen). Primers and probe (Gag primers 6F 5'CATGTTTTTCAGCATTATCAG AAGGA3'; 84R 5'TGCTTGATGTCCCCCACT3'; Probe 5'FAM-CCACCCCACAAGATTTAAACACCAT GCTAA-Q3'), in combination with SuperScript III Platinum One-Step qRT-PCR Kit (Invitrogen) were used for detection with the 7700 Thermocycler.

RNA standards for IFN- γ Real-Time RT-PCR were similarly prepared using IFN- γ plasmid by InvivoGen. SuperScript III Platinum One-Step qRT-PCR Kit (Invitrogen) was used in conjunction with primers for IFN- γ (TaqMan® Gene Expression Assay) and GAPDH (TaqMan® Endogenous control) by Applied Bioscience. Reverse transcription was performed at 45 °C for 30 minutes, followed by 95 °C for 10 minutes and 40 cycles at 95 °C for 15 seconds and 60°C for 1 minute with the 7700 Thermocycler.

Statistical Analysis

Data were analyzed utilizing GraphPad Prism® 4 software for *t-test* with values of $P < 0.05$ considered as significant.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Luis Barbosa of NHLBI for his enthusiastic support for this project. This work was supported by NHLBI contract NO1-HB-27091 to Dr. Krishna K. Murthy, and by the Computational System Biology and Core facilities funded by the National Institute on Minority Health and Health Disparities (G12MD007591) from the National Institutes of Health.

REFERENCES

1. Novembre FJ, Saucier M, Anderson DC, Klump SA, O'Neil SP, Brown CR, Hart CE, Guenther PC, Swenson RB, McClure HM. Development of AIDS in a chimpanzee infected with human immunodeficiency virus type 1. *Journal of Virology*. 1997; 71:4086–4091. [PubMed: 9094687]
2. Davis IC, Girard M, Fultz PN. Loss of CD4+ T cells in human immunodeficiency virus type-1 infected chimpanzees is associated with increased lymphocyte apoptosis. *Journal of Virology*. 1998; 72:4623–4632. [PubMed: 9573225]
3. Castro BA, Homsy J, Lennette E, Murthy KK, Eichberg JW, Levy JA. HIV-1 expression in chimpanzees can be activated by CD8+ Cell depletion of CMV infection. *Clinical Immunology and Immunopathology*. 1992; 65:227–233. [PubMed: 1333378]
4. Heeney J, Jonker R, Koornstra W, Dubbes R, Niphuis H, Di Rienzo AM, Gougeon ML, Montagnier L. The resistance of HIV-infected chimpanzees to progression to AIDS correlates with absence of HIV-related T-cell dysfunction. *Journal of Medical Primatology*. 1992; 22:194–200.
5. ten Haaf PJF, Murthy KK, Verstrepen BE, Eichberg JW, Heeney JL. Intact CCR5 coreceptors in HIV infected chimpanzees. *AIDS*. 1997; 11:1291–1293. [PubMed: 9256948]
6. Nehete PN, Schapiro SF, Johnson PC, Murthy KK, Satterfield WC, Sastry KJ. A synthetic peptide from the first conserved region in the envelope protein gp160 is a strong T-cell epitope in HIV-infected chimpanzees and humans. *Viral Immunol*. 1998; 11:147–158. [PubMed: 9918406]
7. Gonzalez E, Kulkarni H, Bolivar H, Mangano A, Sanchez R, Catano G, Nibbs RJ, Freedman BI, Quinones MP, Bamshad MJ, et al. The Influence of CCL3L1 Gene-Containing Segmental Duplications on HIV-1/AIDS Susceptibility. *Science*. 2005; 307:1434–1440. [PubMed: 15637236]

8. Rodriguez AR, Arulanandam BP, Hodara VL, McClure HM, Cobb EK, Salas MT, White R, Murthy KK. Influence of IL-15 on CD8+ NK cells in Human immunodeficiency virus type 1 infected chimpanzees. *J Gen Virol*. 2007; 88:641–651. [PubMed: 17251583]
9. ten Haaf P, Murthy K, Salas M, McClure H, Dubbes R, Koornstra W, Niphuis H, Davis D, van der Groen G, Heeney J. Differences in early virus loads with different phenotypic variants of HIV-1 and SIVcpz in chimpanzees. *AIDS*. 2001; 15:2085–2092. [PubMed: 11684927]
10. Voevodin A, Samilchuk E, Dashti S. A survey for 32 nucleotide deletion in the CCR-5 chemokine receptor gene (deltaccr-5) conferring resistance to human immunodeficiency virus type 1 in different ethnic groups and in chimpanzees. *Journal of Medical Virology*. 1998; 55:147–151. [PubMed: 9598936]
11. Ahmad R, Sindhu ST, Toma E, Morisset R, Ahamad A A. Studies on the production of IL-15 in HIV infected/AIDS patients. *J. Clin. Immunol*. 2003; 23:81–90. [PubMed: 12757260]
12. Ostrowski SR, Gerstoft J, Pedersen BK, Ullum H. Impaired production of cytokines is an independent predictor of mortality in HIV-1-infected patients. *AIDS*. 2003; 17:521–530. [PubMed: 12598772]
13. Chuang SS, Kumaresan PR, Mathew PA. 2B4 (CD244)-mediated activation of cytotoxicity and IFN-gamma release in human NK cells involves distinct pathways. *Journal of Immunology*. 2001; 167:6210–6216.
14. Ohteki T, Suzue K, Maki C, Ota T, Koyasu S. Critical role of IL-15-IL-15R for antigen-presenting cell functions in the innate immune response. *Nature Immunology*. 2001; 2:1138–1143. [PubMed: 11702064]
15. Castillo EF, Schluns KS. Regulating the immune system via IL-15 transpresentation. *Cytokine*. 2012; 59:479–490. [PubMed: 22795955]
16. Carson WE, Ross ME, Baiocchi RA, Marien MJ, Boiani N, Grabstein K, Caligiuri MA. Endogenous production of interleukin 15 by activated human monocytes is critical for optimal production of interferon-gamma by natural killer cells in vitro. *Journal of Clinical Investigation*. 1995; 96:2578–2582. [PubMed: 8675621]
17. Waldmann TA, Tagaya Y. The multifaceted regulation of interleukin-15 expression and the role of this cytokine in NK cell differentiation and host response to intracellular pathogens. *Annual Review of Immunology*. 1999; 17:19–49.
18. Kuniyoshi JS, Kuniyoshi CJ, Lim AM, Wang FY, Bade ER, Lau R, Thomas EK, Weber JS. Dendritic cell secretion of IL-15 is induced by recombinant huCD40LT and augments the stimulation of antigen-specific cytolytic T cells. *Cellular Immunology*. 1999; 193:48–58.
19. Sandau MM, Schluns KS, Lefrancois L, Jameson SC. Cutting edge: transpresentation of IL-15 by bone marrow-derived cells necessitates expression of IL-15 and IL-15R alpha by the same cells. *Journal of Immunology*. 2004; 173:6537–6541.
20. Azimi N, Brown K, Bamford RN, Tagaya Y, Siebenlist U, Waldmann TA. Human T cell lymphotropic virus type I Tax protein trans-activates interleukin 15 gene transcription through an NF-kappaB site. *Proceedings of the National Academy of Sciences of the United States of America*. 1998; 95:2452–2457. [PubMed: 9482906]
21. Grabstein KH, Eisenman J, Shanebeck K, et al. Cloning of a T cell growth factor that interacts with the beta chain of the interleukin-2 receptor. *Science*. 1994; 264:965–968. [PubMed: 8178155]
22. Musso T, Calosso L, Zucca M, et al. Human Monocytes Constitutively Express Membrane-Bound, Biologically Active, and Interferon- Upregulated Interleukin-15. *Blood*. 1999; 93:3531–3539. [PubMed: 10233906]
23. Miranda-Carus M, Binito-Miguel M, Llamas MA, Balsa A, Martin-Mola E. Human T cells constitutively express IL-15 that promotes ex vivo T cell homeostatic proliferation through autocrine/juxtacrine loops. *Journal of Immunology*. 2005; 175:3656–3662.
24. Mueller YM, Petrovas C, Bojczuk PM, Dimitriou ID, Beer B, Silvera P, Villinger F, Cairns JS, Gracely EJ, Lewis MG, Katsikis PD. Interleukin-15 increases effector memory CD8+ T cells and NK Cells in simian immunodeficiency virus-infected macaques. *Journal of Virology*. 2005; 79:4877–4885. [PubMed: 15795273]

25. AKutler M, Robinson TM, Chattergoon MA, et al. Coimmunization with an optimized IL-15 plasmid results in enhanced function and longevity of CD8 T cells that are partially independent of CD4 T cell help. *Journal of Immunology*. 2005; 175:112–123.
26. Boyer JD, Robinson TM, Kutzler MA, et al. Protection against simian/human immunodeficiency virus (SHIV) 89.6P in macaques after coimmunization with SHIV antigen and IL-15 plasmid. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104:18648–18653. [PubMed: 18000037]
27. Kottlil S, Chun TW, Moir S, Liu S, McLaughlin M, Hallahan CW, Maldarelli F, Corey L, Fauci AS. Innate immunity in human immunodeficiency virus infection: effect of viremia on natural killer cell function. *Journal of Infectious Diseases*. 2003; 187:1038–1045. [PubMed: 12660917]
28. Hudson KR, Robinson H, Fraser JD. Two adjacent residues in staphylococcal enterotoxins A and E determine T cell receptor V beta specificity. *J Exp Med*. 1993 Jan 1; 177(1):175–184. [PubMed: 8418198]
29. Hayball JD, Robinson JH, O'Hehir RE, Verhoef A, Lamb JR, Lake RA. Identification of two binding sites in staphylococcal enterotoxin B that confer specificity for TCR V beta gene products. *Int Immunol*. 1994 Feb; 6(2):199–211. [PubMed: 8155597]
30. Mueller YM, Do DH, Altork SR, Artlett CM, Gracely EJ, Katsetos CD, Legido A, Villinger F, Altman JD, Brown CR, Lewis MG, Katsikis PD. IL-15 treatment during acute Simian Immunodeficiency Virus (SIV) Infection Increases viral set point and accelerates disease progression despite the induction of stronger SIV-specific CD8+ T cell responses. *Journal of Immunology*. 2008; 180:350–360.
31. Matache C, Stefanescu M, Dragomir C, Tanaseanu S, Onu A, Ofiteru A, Szepli G. Matrix metalloproteinase-9 and its natural inhibitor TIMP-1 expressed or secreted by peripheral blood mononuclear cells from patients with systemic lupus erythematosus. *Journal of Autoimmunity*. 2003; 20:323–331. [PubMed: 12791318]
32. Bergamaschi C, Bear J, Rosati M, Beach RK, Alicea C, Sowder R, Chertova E, Rosenberg SA, Felber BK, Pavlakis GN. Circulating IL-15 exists as heterodimeric complex with soluble IL-15R α in human and mouse serum. *Blood*. 2012; 120:1–8. [PubMed: 22767572]
33. Lortat-Jacob H, Baltzer F, Grimaud JA. Heparin decreases the blood clearance of interferon-gamma and increases its activity by limiting the processing of its carboxyl-terminal sequence. *J Biol Chem*. 1996 Jul 5; 271(27):16139–16143. [PubMed: 8663206]
34. Münch J, Janardhan A, Stolte N, Stahl-Hennig C, Ten Haaft P, Heeney JL, Swigut T, Kirchhoff F, Skowronski J. T-cell receptor:CD3 down-regulation is a selected in vivo function of simian immunodeficiency virus Nef but is not sufficient for effective viral replication in rhesus macaques. *Journal of Virology*. 2002; 76:12360–12364.
35. Deeks SG, Walker BD. Human Immunodeficiency Virus Controllers: mechanisms of durable virus control in the absence of antiretroviral therapy. *Immunity*. 2007; 27:406–416. [PubMed: 17892849]
36. Saez-Cirion A, Lacabaratz C, Lambotte O, Versmisse P, Urrutia A, Boufassa F, Barre-Sinoussi F, Delfraissy J, Sinet M, Pancino G, Benet A. HIV controllers exhibit potent CD8 T cell capacity to suppress HIV infection ex vivo and peculiar cytotoxic T lymphocyte activation phenotype. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104:6776–6781. [PubMed: 17428922]
37. Emu B, Sinclair E, Favre D, Moretto WJ, Hsue P, Hoh R, Martin JN, Nixon DF, McCune JM, Deeks SG. Phenotypic functional and kinetic parameters associated with apparent T-cell control of human immunodeficiency virus replication in individuals with and without antiretroviral treatment. *Journal of Virology*. 2005; 79:14169–14178. [PubMed: 16254352]
38. Loke P, Favre D, Hunt PW, Leung JM, Kanwar B, Martin JN, Deeks SG, McCune JM. Correlating cellular and molecular signatures of mucosal immunity that distinguish HIV controllers from noncontrollers. *Blood*. 2010; 115:20–32.
39. Kinter A, McNally J, Riggin L, Jackson R, Roby G, Fauci AS. Suppression of HIV-specific T cell activity by lymph node CD25+ regulatory T cells from HIV-infected individuals. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104:3390–3395. [PubMed: 17360656]

40. Schluns KS, Williams K, Ma A, Zheng XX, Lefrançois L. Cutting edge: requirement for IL-15 in the generation of primary and memory antigen-specific CD8 T cells. *J Immunol.* 2002; 168(10): 4827–4831. *J Immunol.* (2002). [PubMed: 11994430]
41. Shiow LR, Rosen DB, Brdi ková N, Xu Y, An J, Lanier LL, Cyster JG, Matloubian M. CD69 acts downstream of interferon- α/β to inhibit S1P₁ and lymphocyte egress from lymphoid organs. *Nature.* 2006; 440:540–544. [PubMed: 16525420]

HIGHLIGHTS

- Chimpanzee peripheral blood Tcell/NKT cells expressed surface IL-15.
- Recombinant gp120 and IL-15 up-regulated CD3-CD8+ IFN- γ +TNF- α +NK cells.
- Staphylococcal enterotoxin B increased T cell interleukin-15 surface expression.
- T cell surface interleukin-15 may modulate multiple immune parameters.

Fig. 1. Interferon- γ expression in HIV positive chimpanzee NK and T cells

Whole blood from ten HIV+ chimpanzees was analyzed by flow cytometry. (A) A representative scatter plot is shown for the lymphocyte gate with CD8 versus IFN- γ and CD3 versus IFN- γ dot plots. (B) CD3-CD8+CD56- IFN γ + NK cells were significantly higher than CD3+CD8+ T cells ($P < 0.009$, $n=10$) with rgp120 stimulation ($10 \mu\text{g ml}^{-1}$) of whole blood. Percentages of total lymphocyte population are shown for IFN- γ .

A.

B.

Fig. 2. IFN- γ and TNF- α are expressed from the same CD3-CD8+ populations

Whole blood from infected chimpanzees was stimulated with rgp120 ($10 \mu\text{g ml}^{-1}$) and analyzed for CD3-CD8+IFN- γ +, CD3+CD8+ IFN- γ +, CD3-CD8+ TNF- α +, and CD3+CD8+TNF- α + cells by flow cytometry. (A) Representative dot plots: CD4 T cells, CD8 T cells, and CD8 NK cells. (B). Analysis of CD8+ cells within lymphocyte gate are shown for controls (PBS) and rgp120 stimulated samples; rgp120 increased IFN- γ and TNF- α from CD3-CD8+ NK cells > CD3+CD8+ T cells.

Fig. 3. Recombinant IL-15 increases IFN- γ expression primarily in CD8+ NK cells from HIV positive chimpanzees

Whole blood from three HIV positive and three HIV negative chimpanzees was incubated with rIL-15 (20 ng mL⁻¹) or PBS (controls) and analyzed by flow cytometry for IFN- γ intracellular staining. The CD3-CD8+, CD3-CD56+, and CD3+CD8+ populations were analyzed within the lymphocyte gate; 20K–30K events were collected. The naïve chimpanzee lymphocytes demonstrated variability in CD3-CD8+NK cells and CD3+ CD8+ T cells: Samples from HIV+ animals exhibited CD3-CD8+IFN- γ + cells > CD3+CD8+IFN- γ + cells > CD3-CD56+IFN- γ + cells within the lymphocyte gated region.

A.

B.

C.

Fig. 4. Interleukin-15 expands the CD4+CD25+ T cell population

(A) PBMCs from eight chimpanzees were incubated at 37°C for 24 hours with rIL-15 (20ng mL⁻¹). Expression of CD4+CD25+ T cells increased in all eight chimpanzees examined with expression ranging from 7 % to 15 % of the total lymphocyte populations ($P < 0.007$). (B) CD69 expression on CD4 and CD8 T cells is shown as a percent change (PBS to rIL-15) in lymphocyte CD69 expression for the corresponding eight chimpanzees in figure A. (C) Incubation of PBMCs from HIV+ chimpanzees with SEB (1 μ g mL⁻¹), gp120 (10 μ g mL⁻¹),

gag 10 $\mu\text{g ml}^{-1}$), (tat 10 $\mu\text{g ml}^{-1}$) or PBS (control) demonstrated an increase of CD154 expression on CD4+T cells compared to minimal expression on CD8+ T cells.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 5. IFN- γ mRNA in naïve and HIV positive chimpanzee lymphocytes are similar

(A) IFN- γ mRNA from HIV+ and HIV- chimpanzee PBMCs was analyzed by Taqman RT-PCR. IFN- γ mRNA was not significantly different in the two sets of animals examined. (B) Plasma samples separated from whole blood following stimulation with rgp120 ($10 \mu\text{g mL}^{-1}$), rIL-15 (20 ng mL^{-1}), SEB ($1 \mu\text{g mL}^{-1}$), or PBS (control) for 15 h were analyzed by ELISA. Whole blood from HIV+ chimpanzees incubated with rgp120 resulted in approximately 2-fold to 5-fold increase in plasma IFN- γ . SEB stimulation increased plasma IFN- γ to over 1000 pg mL^{-1} .

Fig. 6. CD4 and CD8 T cell/NKT populations express surface IL-15

Whole blood from HIV positive and naïve chimpanzees was analyzed after stimulation with rgp120 ($10 \mu\text{g ml}^{-1}$), SEB ($1 \mu\text{g ml}^{-1}$), or PBS (control). (A) A representative scatter plot with the lymphocyte gate is shown. Samples were analyzed for CD3, CD4, CD8 and CD16. (B) Populations were further analyzed in the CD3 positive or the CD3 negative gate. Representative dot plots are shown. (C) Stimulation of whole blood with rgp120 ($10 \mu\text{g ml}^{-1}$) or SEB ($1 \mu\text{g ml}^{-1}$) for 15 h. Percent of total lymphocytes shown for IL-15 surface expression (< 2-fold to 3-fold increase) on T cell populations from HIV positive animals. T cells from naïve animals increased IL-15 surface expression from 3 to 6-fold with SEB stimulation.

Table 1

Chimpanzee Peripheral Blood Surface CD4 Expression

Chimpanzee	Treatment	CD3	
		CD4+ (%)	CD4+CD25+ (%)
1	PBS IL-15	38.62	3.63
		36.28	7.19
2	PBS IL-15	35.02	2.15
		31.95	7.8
3	PBS IL-15	36.99	1.65
		37.72	11.8
4	PBS IL-15	27.64	2.12
		26.1	6.57
5	PBS IL-15	35.42	4.52
		41.95	15.32
6	PBS IL-15	50.83	6.62
		55.64	12.43
7	PBS IL-15	36.37	2.45
		37.69	9.05
8	PBS IL-15	45.89	4.66
		46.31	13.56

* PBMCs 5×10^6 † Recombinant IL-15 (20ng mL⁻¹)