Poster #329

Association of Bacteria-Induced IL-23 and Th17 Frequencies in HIV-1$^+$ Individuals

Jennifer Manuzak*[1-3], Marco Iannetta[1-3], Sonia Amraoui[1-3], Jean-Baptiste Guillerme[1-3], Nipa Decroix[4], Pierre Loulergue[3-5], Odile Launay[3-5], Lene Vimeux[1-3], Anne Hosmalin[1-5]

[1]Inserm U1016, Institut Cochin, Paris, France
[2]Centre National de la Recherche Scientifique Unite Mixte de Recherche 8104, Paris, France
[3]Universite Paris Decartes, Paris, France
[4]Centre d'Investigation Clinique CIC 1417, INSERM-AP-HP, Hopital Cochin, Paris, France
[5]Assistance Publique-Hopitaux de Paris (AP-HP), Hopital Cochin, Paris, France

*Jennifer A. Manuzak, Ph.D
Unite INSERM 1016, Institut Cochin
Bâtiment Gustave Roussy, 8ème étage
27, Rue du Fg St Jacques
75014, Paris, France
Tel: +33 1 40 51 65 18
Jennifer.manuzak@inserm.fr

Abstract (Updated)

Background: Cytokine imbalanced in HIV-1 infection could be mediated in part by the innate immune response to translocated intestinal microbes. Previous work showed that PBMC from HIV-1-infected patients (HIV-1$^+$) produce significantly more IL-23 in response to bacterial stimulation as compared to uninfected controls (HIV-1$^-$). Overproduction of IL-23 could skew T cell responses towards a Th17 phenotype. However, the relationship between microbial translocation, cytokine production and Th17 cell frequencies in the context of *in vivo* HIV-1 infection is not well understood. We compared IL-23 production and the frequency of Th17 cells *ex vivo* in response to bacterial and TCR stimulation during HIV infection.

Methods: PBMC from 19 chronically infected HIV-1$^+$ and 20 HIV-1$^-$ donors were utilized. The frequency of IFN-γ, IL-17A, IL-17F, IL-22 and IL-4 producing cells within memory CD4$^+$ T cells (CD3$^+$CD4$^+$CD45RA$^-$) of total PBMC was assessed either *ex vivo* on fresh PBMC or *in vitro* after stimulation with *E. coli* or *P. aeruginosa* using either frozen HIV-1$^+$ PBMC or fresh PBMC from uninfected controls by multi-parameter flow cytometry. Levels of IL-23 in PBMC culture supernatants after bacterial stimulation were assessed by ELiSA. Non-parametric statistical tests (Mann-Whitney, Wilcoxon Rank Test and Spearman's Tests) were used to determine statistical significance between groups.

Results: The frequency of IL-17A$^+$IL-17F$^+$ (Th17) cells *ex vivo* was significantly increased in HIV-1$^+$ (n=13) as compared to HIV-1$^-$ (n=11; p=0.009). The median levels of IL-23 or IL-6 production in HIV-1$^+$ (n=11) did not reach statistical significance over HIV-1$^-$ (n=11) after *in vitro* stimulation with *E. coli* (p=0.31 and 0.6, respectively) or *P. aeruginosa* (p=0.28 and 0.21, respectively). Although no differences in cytokine producing memory T cell frequencies were detected in response to bacterial stimulation as compared to the media only control in HIV-1$^+$ PBMCs, elevated frequencies of IL-17A+ and IL-17A +IFN-γ+ memory T cells were detected in response to *E. coli* and *P. aeruginosa* stimulation in PBMCs from uninfected controls. Finally, the frequency of IL-17A+IFN-γ+IL-22+ memory cells significantly correlated with the level of *E. coli*-induced IL-23 production (p=0.02).

Conclusions: We found increased frequencies of peripheral blood Th17 cells in chronic HIV-1$^+$ patients. This may be due to elevated cytokine production in response to translocated bacteria, as suggested by the relationship between IL-23 levels and Th17 frequencies after bacterial stimulation in uninfected controls. Th17 cells are preferentially depleted in the intestinal mucosa of HIV-1$^+$, likely due to infection and killing of these cells. Our data suggest a potential mechanism by which the innate immune response to HIV-1-induced microbial translocation could promote the expansion, activation, viral infection and destruction of Th17 cells in the gut, thus contributing to HIV-1 disease pathogenesis.

Background

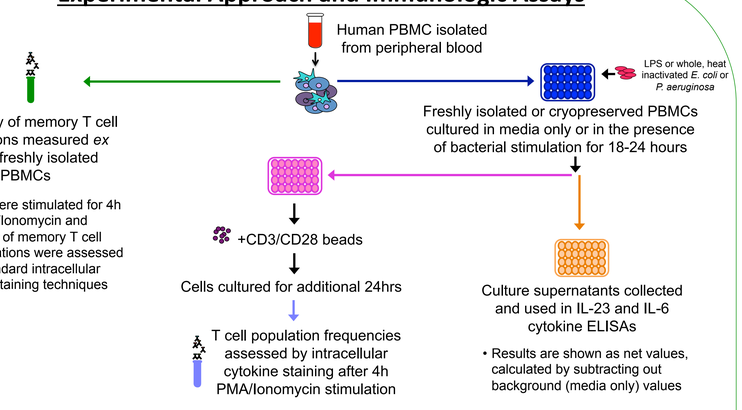
- Microbial translocation from the intestinal lumen occurs at elevated rates in HIV-1$^+$ individuals and correlates with markers of systemic immune activation, a predictive marker of HIV-1 disease progression and pathogenesis
- Dysfunctional innate immune responses to translocated bacterial antigens may contribute to the development and persistence of systemic immune activation
  - Increased pro-inflammatory cytokine production by dysfunctional dendritic cells (DCs) and CD16$^+$ monocytes in response to microbial antigens has been documented in HIV-1$^+$ individuals[1,2]
- Increased production of Th17 polarizing cytokines, such as IL-23 and IL-6, by dysfunctional DCs and CD16$^+$ monocytes could impact T cell polarization and activation in HIV-1$^+$ individuals
  - Th17 cells are preferentially infected by HIV-1[3]
  - Th17 cells are preferentially depleted in the intestinal mucosa of HIV-1$^+$[4]
  - Studies investigating peripheral blood Th17 dynamics have yielded varying results[5]

A relationship between translocated microbes, cytokine production and Th17 cell polarization in the context of HIV-1 infection is not yet completely defined.

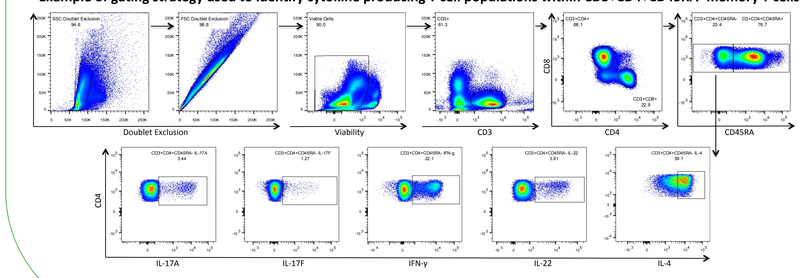
Hypothesis

Production of pro-inflammatory cytokines, particularly IL-23 and IL-6, by PBMC in response to stimulation with commensal enteric bacteria, contributes to elevated Th17 expansion in HIV-1-infected individuals and may reflect in the peripheral blood

Experimental Approach and Immunologic Assays



Example of gating strategy used to identify cytokine producing T cell populations within CD3+CD4+CD45RA- memory T cells



• Analysis and presentation of distributions was performed using SPICE version 5.1, downloaded from http://exon.niaid.nih.gov.[6]

Study Subject Characteristics

	Uninfected	HIV-1$^+$
Total Subjects (Male, Female)	20 (10 Male, 10 Female)	19 (8 Male, 11 Female)
Age (Range)	44 years (24 – 59 years)	33 years (24 – 64 years)
CD4$^+$ T cell count (cells/μl)	n/a	541.08 (310.73 – 869.08)
Viral Load (HIV-1 RNA/ml plasma)	n/a	16,287 (39.81 – 72,443.6)

Heparinized blood samples were obtained from untreated, chronically HIV-1$^+$ patients (n=19) in the Prevac Cohort (Clinical Investigation Center of the Cochin Hospital, Paris). Prevac was approved by the "Ile-de-France 4" Ethics Committee (#2010-13NICB, Paris, France). Healthy, HIV-1 uninfected adult blood samples (n=20) from were collected at the Etablissement Français du Sang of the Saint-Vincent de Paul and Saint Antoine Crozatier Hospitals (Paris, France) through an ethics convention with INSERM. All donors gave informed consent before inclusion. Data are presented as median (range). n/a = not applicable

Results

Increased frequency of IL-17-producing memory T cells *ex vivo* in HIV-1$^+$ individuals compared to uninfected controls

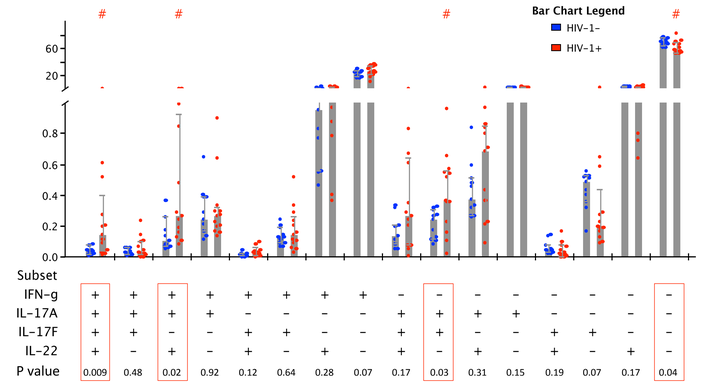


Figure 1. Frequency of memory T cells *ex vivo* producing combinations of the cytokines IFN-γ, IL-4, IL-17A and IL-22 within freshly isolated PBMCs from HIV-1$^+$ individuals (n=13) and uninfected controls (n=11). Thick horizontal bars represent median values. Whiskers represent interquartile ranges. Statistical significance calculated using a Mann-Whitney test. Red boxes indicate cytokine producing populations that are statistically different between the two groups.

Similar levels of bacteria-induced IL-23 and IL-6 production by cryopreserved PBMCs from HIV-1$^+$ individuals compared to uninfected controls

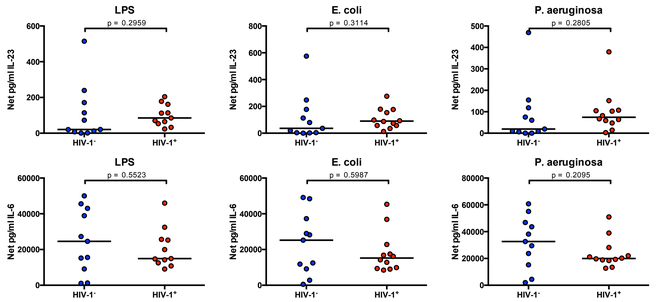


Figure 2. Production of IL-23 and IL-6 by cryopreserved PBMCs from HIV-1$^+$ individuals (n=11) and uninfected controls (n=11) in response to *in vitro* stimulation with LPS, *E. coli* or *P. aeruginosa*. Horizontal bars represent median values. Statistical significance calculated using a Mann-Whitney test.

References

(1) Manuzak et al. JVI. 2013; 87(24): 13252 **(2)** Louis et al. J. Leuko. Biol. 2010; 87(4): 645-653. **(3)** Dillon et al. J. Immunol. 2012; 189: 885-896. **(4)** Brenchley et al. Blood. 2008; 112: 2826-2835. **(5)** Klatt, et al. Curr. Opin. HIV AIDS. 2010; 5(2): 135-140. **(6)** Roderer et al. 2011. Cytometry Part A, 79A: 167-174.

Acknowledgements

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Frequency of IL-17-producing memory T cells in cryopreserved PBMCs from HIV-1$^+$ patients after bacterial stimulation

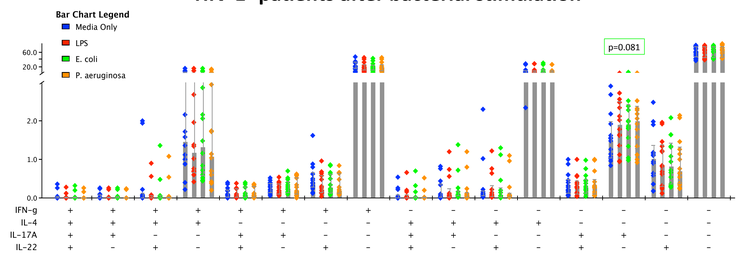

Figure 3. Frequency of memory T cells producing combinations of the cytokines IFN-γ, IL-4, IL-17A and IL-22 within cryopreserved PBMCs from HIV-1$^+$ individuals (n=14) in response to *in vitro* stimulation with LPS, *E. coli* or *P. aeruginosa.* Each diamond represents an individual data point. Vertical bars represent median value for each condition. Whiskers represent interquartile ranges. Statistical significance between the stimulation conditions and the media only control calculated using a Wilcoxon Rank Test.

Elevated frequency of IL-17-producing memory T cells in fresh PBMCs from uninfected controls after bacterial stimulation

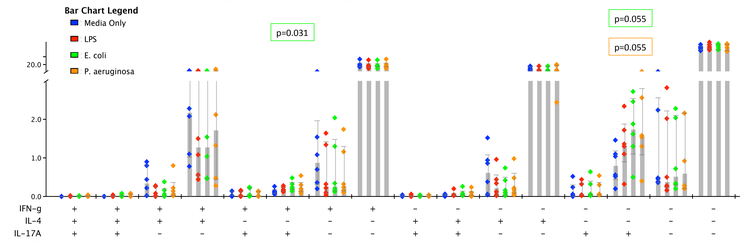

Figure 4. Frequency of memory T cells producing combinations of the cytokines IFN-γ, IL-4, IL-17A and IL-22 within freshly isolated PBMCs from uninfected controls (n=6) in response to *in vitro* stimulation with LPS, *E. coli* or *P. aeruginosa.* Data and statistical analysis presented as in Figure 3.

Significant correlation between IL-23 levels and IL-17A$^+$IFN-γ$^+$IL-22$^+$ T cell frequencies in response to bacterial stimulation in fresh PBMCs from uninfected controls

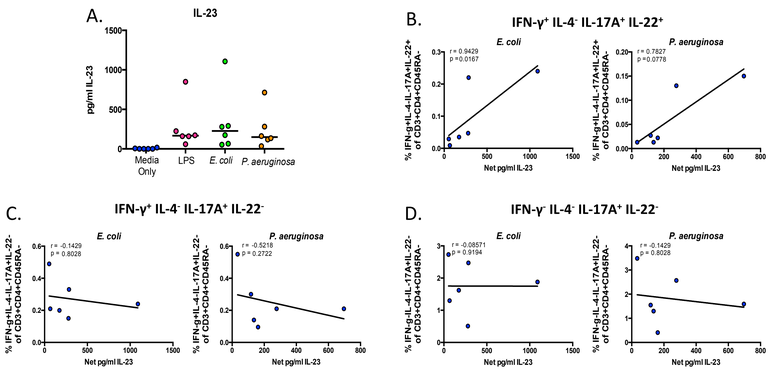

Figure 5. (A) Production of IL-23 by fresh PBMCs from uninfected controls (n=6) in response to *in vitro* LPS, *E. coli* and *P. aeruginosa* stimulation. Horizontal bars represent median values. (B-D) Correlation of *E. coli* and *P. aeruginosa*-induced IL-23 production in uninfected controls with frequencies of memory T cells producing (B) IFN-γ$^+$ IL-4$^-$ IL-17A$^+$ IL-22$^+$, (B) IFN-γ$^+$ IL-4$^-$ IL-17A$^+$ IL-22$^-$, and (C) IFN-γ$^-$ IL-4$^-$ IL-17A$^+$ IL-22$^-$. Statistical significance calculated using a Spearman's test.

Discussion and Conclusions

- Memory multi-functional circulating Th17 cells were elevated in this cohort of chronically infected HIV-1$^+$ individuals
- Bacterial stimulation of freshly isolated PBMCs from uninfected controls lead to:
  - Increased frequencies of IL-17A$^+$ T cells in response to *E. coli* and *P. aeruginosa*
  - Significantly increased frequencies IL-17A$^+$IFN-γ$^+$ T cells in response to *E. coli*
  - Correlation between IL-23 production and IL-17A$^+$IFN-γ$^+$IL-22$^+$ T cells in response to *E. coli*
- The use of cryopreserved, not fresh HIV-1$^+$ PBMCs[1,2] could explain the lack of statistically significant increases in cytokine production after bacterial stimulation as compared to uninfected controls, and therefore a lack of correlation with bacteria-induced Th17 frequencies after *in vitro* culture
- **Possible clinical implication**: Inhibiting microbial translocation or suppressing bacterial-induced cytokine production, such as IL-23, could aid in reducing the frequency of highly infectable Th17 cells, which could help to limit HIV-1 disease pathogenesis.