HIV alters innate immune response to BCG, which differs from SIV infected mangabeys

Melanie A. Gasper[1,2] David. R. Sherman[1,2] and Donald L. Sodora[1,2]

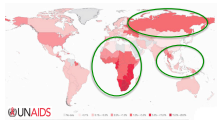
Seattle Biomedical Research Institute[1] and University of Washington School of Medicine, Department of Global Health Seattle, Washington, USA

Abstract & Background

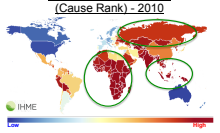
BACKGROUND: Coinfection with *M. tuberculosis*, as well as other *Mycobacteria*, is a leading cause of morbidity and mortality for HIV-infected patients. Here we set out to understand functional immune responses to an opportunistic pathogen, *M.bovis BCG*, during both pathogenic HIV and non-pathogenic SIV infections (natural SIV host sooty mangabeys). *M. Bovis BCG* is a live-attenuated vaccine strain of Mycobacteria, which has demonstrated opportunistic potential.
METHODS: Multiparameter flow cytometry and gene expression analysis, utilizing a Nanostring platform (248 genes), was used to evaluate the ability of monocytes and peripheral blood mononuclear cells (PBMC) to respond to 4 hour *ex vivo* BCG stimulation. KEGG analysis was used to identify pathways altered by changes in gene expression. Uninfected and HIV/SIV infected humans and sooty mangabeys were analyzed.
RESULTS: HIV infection was associated with a reduced percentage of whole blood monocytes producing IL-12 in response to BCG (1.8% compared to 4% in uninfected donors; p=0.02). In contrast, SIV+ mangabeys had a higher percentage of monocytes producing IL-12 and TNF-α in response to BCG compared to uninfected mangabeys, suggesting a maintained immune response. In addition, principal component analysis showed that although both species mounted a robust response to BCG, HIV-infected donors had a unique BCG-induced gene expression profile that distinguished them from uninfected age-matched donors. In contrast, SIV-infection status did not alter the gene expression clustering pattern between SIV+ and SIV- mangabeys. KEGG analysis demonstrated that genes with increased expression at baseline in HIV+ donors were significantly enriched within the NK cell cytotoxicity pathway (hypergeometric test; p=0.007), this difference was not seen between SIVneg and SIV+ mangabeys. Additionally, a statistical interaction between HIV disease status and the response to BCG was found in a number of genes related to NK cell function, including NKp46, IFNg and perforin, suggesting that HIV-infection altered the NK cell response to bacterial stimulation.
CONCLUSION: Overall, these data provide evidence that innate immune responses (including monocyte IL-12 production and NK cell activity) to the BCG opportunistic pathogen are likely to be critical for resistance to opportunistic infections following lentiviral (HIV/SIV) infection.

HIV and Tuberculosis: A Synergistic Pandemic:

Worldwide HIV Prevalence - 2009



Deaths by Tuberculosis (Cause Rank) - 2010



- Tuberculosis is a leading cause of death in HIV+ patients *(Mayer and Hamilton CID 2010)*
- HIVneg individuals: 5-10% **lifetime** risk of developing TB; HIV+ individuals: 10% **annual** risk *(Havlir DV 1998 NEJM; Jones BE 1993 Am Rev Respir Dis)*
- BCG is a vaccine administered against *M. tuberculosis*; causes disseminated disease in severely immunocompromised patients

Aim: Compare differences in response to *Mycobacteria* between HIV+ and HIV negative humans to differences between SIV+ and SIV negative mangabeys.

Goal: To assess pathogenic and nonpathogenic HIV/SIV infections to identify differential innate immune directed toward an opportunistic mycobacteria (BCG) that could lead to immune therapeutic approaches to improve immune responses to mycobacteria.

Study Populations

Human Cohort Inclusion Criteria

- HIV+ blood from Madison Clinic; healthy blood from Seattle BioMed
- Antiretroviral (ART) naive or off of ART treatment for >1 year
- PPD-negative and not presenting with TB symptoms
- Recent HIV viral load and CD4+ T cell count available

Median CD4+ T cell count:	Median Viral Load:
585 cells/ul	**4062 copies/mL**



Sooty mangabey (model of non-progressive SIV infection)

- Whole blood from SIVnegative and SIV+ **sooty mangabeys** (model of nonpathogenic infection) was obtained from the Yerkes National Primate Research Center
- No ART or evidence of mycobacterial infections

Methods

Mycobacteria Culture

- *BCG (Russia) grown to log growth phase*
- *Calculate MOI based on OD*



Species	Biosafety	Replication (h)
M. smegmatis (non-pathogenic)	BSL-1	3
BCG (opportunistic)	BSL-2	16-18
M. bovis BCG	BSL-3	20-24
M. tuberculosis (pathogenic)		

Whole Blood Monocyte Functional Analyses

- 6h BCG MOI 10 exposure in the presence of Brefeldin-A
- Intracellular cytokine staining for TNFα and IL-12

Monocytes

- TNFα: pleiotropic, critical for containment of *M. tuberculosis* *(Bean et al. 1999 JI; Gardam 2003 Lancet Inf Dis)*
- IL-12: pleitropic, important for responses to MTb, drives Th1 response *(Sieffer et al. 1993 Blood; Hsieh et al Science 1993)*



Pathogenic infection



Non-pathogenic infection

Data Analysis

- Monocyte flow cytometric data was collected on an LSR2 (BD) analyzed
- Statistics were generated in GraphPad Prism using a Mann-Whitney test

Gene Expression Analysis

- Whole **PBMC** exposed/stimulated with BCG MOI 10 in 96-well plate for 4h.
- 7 HIVneg, 7 HIV+, 4 SIVneg, 4 SIV+)
- Triplicates pooled for RNA isolation (Qiagen)

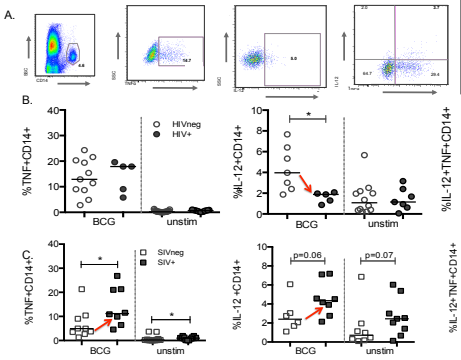


- **Nanostring platform**

- Color coded hybridization and capture probes, determines absolute transcript levels
- Normalized to housekeeping controls GAPDH, ALAS1
- Background cutoff for detection: average copy # neg controls +/- 2SD

1. RESULTS – Flow Cytometric Analysis of monocytes (TNF-alpha & IL-12)

To evaluate ability of peripheral blood monocytes from both humans (HIV-infected and uninfected) and mangabeys (SIV-infected and uninfected) to respond to BCG utilizing a flow cytometric assay.



Whole blood monocyte proinflammatory cytokine production in HIV/SIVneg or HIV+/SIV+ donors/mangabeys following stimulation with *M. bovis* BCG. Representative flow cytometry plots and gating strategy of cytokine-producing monocytes, which were defined as being live, CD3 negative, CD14+, and producing TNFα, IL-12, or both following 6h exposure to BCG (A). Humans (B) or sooty mangabeys (C) that are either HIV/SIVneg (unfilled circles) or HIV/SIV+ (filled circles) monocytes evaluated for expression of TNFα, IL12 or both cytokines after 6 hr BCG exposure (B). Lines indicate the medians for each group (*p<0.05, Mann-Whitney).

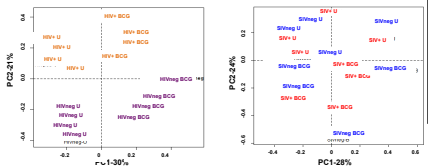
Results: Our flow cytometric analysis determined that monocytes from HIV infected patients have a lower IL12 response to BCG compared to uninfected counterparts. In contrast, SIV infection of mangabeys resulted in monocytes that were more responsive to BCG with a higher percentage producing both Il-12 and TNF-alpha (p=0.06).
We conclude that SIV infection of mangabeys primes monocytes to make them more responsive when exposed to mycobacteria.

2. RESULTS – Nanostring Transcriptomic Analysis (PBMC)-PCA

Differential transcriptomic responses to BCG were first assessed between humans and mangabeys using Principal Component Analysis (PCA).
Summary: BCG exposure defines a pattern response for both humans and mangabeys. HIV status also identified a differential pattern response for humans. However, SIV-infection of mangabeys did not result in a differential response when compared to uninfected mangabeys.

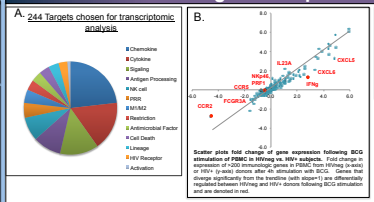
Human PCA

Mangabey PCA



PCA reveals hierarchical clustering pattern unique to pathogenic HIV infection. Semi-supervised clustering via PCA demonstrates that BCG stimulation is a key component to differential gene regulation (PCA-1 for humans, A; PCA 2 for mangabeys, B.). Pathogenic HIV status also defines response patterns (PCA-2 for humans); Nonpathogenic SIV status does not define BCG response patterns for mangabeys.

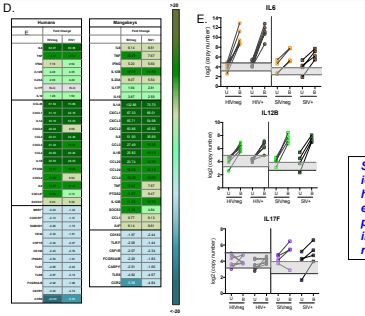
3. Results – Nanostring Transcriptomic Analysis (PBMC)



Scatter plots fold change of gene expression following BCG stimulation of PBMC in HIVneg vs. HIV+ subjects. Fold change in expression of >200 immunologic genes in PBMC from HIVneg (x-axis) or HIV+ (y-axis) donors after 4h stimulation with BCG. Genes that diverge significantly from the trendline (with slope=1) are differentially regulated between HIVneg and HIV+ donors following BCG stimulation and are denoted in red.

Graph depicts Log2 copy number of Interferon-gamma mRNA in an unstimulated (Unstim) or matched BCG-exposed (BCG) PBMC obtained from uninfected and HIV+ humans (C). This graph identifies the differential response that is depicted for interferon-gamma in the scatter plot (B).

Summary: Transcriptomic analysis of PBMC identified 9 genes that are differentially expressed between HIV+ and uninfected people in response to BCG exposure.

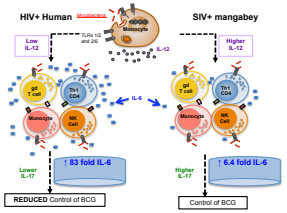


Whole PBMC cytokine gene regulation in HIVneg or HIV+ donors following stimulation with *M. bovis* BCG. Table depicts fold change associated with BCG stimulation of PBMC from HIVnegative or SIVnegative (HIVneg/SIVneg) or HIV+/SIV+ humans/mangabeys (D) Graphs depict Log2 copy number of RNA in an unstimulated control (U) or matched BCG-exposed (B) samples in either humans (circles) or mangabeys (squares) for IL6, IL12B and IL17F (E). The lower bound of the gray shaded portion of the graph represents the mean of the negative controls, and the upper bound represents two standard deviations above the mean, which ultimately determined the cutoff for expression. FC: fold change; BD: below detection.

Summary: Transcriptomic analysis of PBMC identified differential immune responses between humans and mangabeys in response to BCG exposure. Differences in IL-6, IL-12 and IL-17 were particularly interesting as these proteins are important for immune activation and immune responses toward mycobacterial infections.

4. Summary– Model

Model for how BCG specific innate immune responses differ between HIV+ patients and SIV+ mangabeys.



Model depicts four different immune cells that play roles in mycobacterial responses (Monocytes, NK cells , Th1 T cells and gamma delta T cells). Regarding cytokine expression, Nanostring analysis identified three cytokines in particular IL-12, IL-6 and IL-17 that were differentially regulated in HIV+ humans compared to SIV+ mangabeys. Flow cytometric analysis indicated that monocytes from the two different species respond to BCG differently with regard to IL-12 production, mangabeys make more while humans make less. IL-6 is strongly upregulated in BCG-stimulated PBMC from HIV-infected patients, but only modestly upregulated in the mangabeys. And IL-17 is produced in higher levels in mangabeys, compared to humans.

These findings provide evidence for differential cytokine responses to BCG in SIV+ mangabeys that we hypothesize plays a key role for the overall outcome of the infection in these two species.

Conclusions

1. HIV infection either directly or indirectly (via immune activation) impacts the ability of monocytes and other immune cells to respond to an exposure of a Mycobacterial opportunistic pathogen.

- In contrast to HIV+ patients, monocytes from HIV+ people exhibited a reduced expression of IL-12 in response to BCG (compared to an unstimulated control)
- Differences were also observed between expression of 9 genes in PBMC from HIV-infected patients (compared to uninfected people) in response to BCG (including Interferon-gamma).

2. SIV infection of sooty mangabeys results in a less activated immune response (compared to HIV+ people) and a more robust monocyte response following exposure to a Mycobacterial opportunistic pathogen.

- Monocytes from mangabeys appear to be 'primed' to respond to a Mycobacterial exposure producing more IL-12 and TNF-alpha (p=006).
- Transcriptomic analysis revealed a number of differential responses in the mangabeys to BCG, in comparison to the humans. Some of the more interesting differences include lower levels of IL-6 expression and higher levels of both IL-12 and IL-17 expression within the PBMC of mangabeys in response to BCG. We hypothesize these differences play a role in the improved immune responses observed in SIV-infected mangabeys (compared to humans).

Acknowledgements:

Sodora laboratory
Bridget Fisher

David Sherman's laboratory
Reiling Liao
Tracey Day

Yerkes Primate Center
Stephanie Ehnert

University of Washington CFAR
Madison Clinic study personnel
CFAR statistics core

- Patients and healthy donors
- Sooty Mangabey donors

Funding: This work was supported in part by the Viral Pathogenesis Training Grant (NIH T32- AI083203-01) and the Seattle Biomedical Research Institute Innovation Grant