Monocyte and NK Cell Dysfunction Responses to Mycobacteria During Chronic HIV-1 Infection
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ABSTRACT & BACKGROUND

Background: Confrontion with M. tuberculosis (Mt) and other Mycobacteria is a leading cause of morbidity and mortality in HIV+ patients. While neither monocytes nor NK cells are directly infected with HIV, several of their phenotypic and functional properties become altered during chronic infection, and both cell types are important to the control of Mt. We hypothesized that HIV-induced cell dysfunction may result in impaired monocyte-NK cell crosstalk, which we will evaluate in the context of infection.

Methods: To assess monocyte cytokine production, whole blood from HIV+ naïve patients and healthy donors was stimulated with Mycobacteria for 6h (in the presence of Brefeldin-A) and followed by intracellular cytokine staining. For cellular functional assays, cells were isolated from PBMC via magnetic bead isolations. Monocytes were infected with fluorescent (mCherry) BCG (opportunistic pathogen) or Mt at MOI=10 for 6h to assess phagocytosis. Autologous NK cells were added to assess cell killing.

Results: We observed a decrease in IL-12 (p=0.017), but similar TNF alpha production, by blood monocytes from HIV+ individuals following a 6h stimulation with Mt. Monocytes infected with fluorescent BCG (opportunistic pathogen), compared to uninfected donors. As IL-12 is known to regulate NK cell functionality, we were also interested in evaluating NK cell function in our cohort. While developing a flow-based functional assay to measure NK cell killing of autologous Mt-infected monocytes, we found that NK cell killing from HIV+ donors exhibited reduced killing of target K562 cells compared to controls (p=0.04). Further evaluation established that monocytes from the same HIV+ patients in which we identified lower NK cell killing also exhibited decreased phagocytosis of fluorescently labeled BCG (p=0.016) but similar phagocytosis of Mt compared to uninfected donors.

Conclusions: Our data demonstrate a reduction in both monocyte and NK cell functionality in the same HIV+ patients and is suggestive of synergism in the dysfunction of two innate cell types. The assays described have the potential to result in novel therapeutic targets to enhance innate cellular function in HIV+ patients.

RESULTS

Deaths by Tuberculosis (Cause Rank) - 2010

Tuberculosis is a leading cause of death in HIV+ patients (Mayer and Hamilton CID 2010)

HIV+ vs. HIV- individuals: 5.10% lifetime risk of developing TB; HIV+ individuals: 1.0% annual risk (HIV+ associated with increased risk of TB)

BCG is a vaccine administered against M. tuberculosis; causes disseminated disease in severely immunocompromised patients

SPECIFIC AIMS

Overall Goal: Elucidate differences in innate responses to Mycobacteria between HIV- and HIV-negative humans that reveal novel, key elements in HIV pathogenesis and increased vulnerability to opportunistic infections.

Am 1. Characterize innate immune gene expression during pathogenic and non-progressive HIV/SIV infections following stimulation with Mycobacteria

Am 2. Assess monocyte functional responses to Mycobacteria, including cytokine production and phagocytosis during pathogenic HIV and non-progressive SIV infection

Am 3. Functionally assess the ability of NK cells to kill infected targets during HIV infection.

STUDY POPULATION

Human Cohort Inclusion Criteria

• HIV-blood from Madison Clinic; healthy blood from Seattle Blood

• Anti-retroviral (ART) naïve or off of ART treatment for ≥1 year

• PPDR-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

• ≤ 500 viral copies/mL

HIV coinfection

SIV coinfection

HIV/SIV coinfection

Mt coinfection

SIV coinfection (model of non-progressive SIV infection)

Figure 1. Principal Component Analysis (PCA) reveals hierarchical clustering of patients. Semi-supervised clustering via PCA demonstrates that BCG stimulation is a key component to differential gene regulation (PCA1) in HIV- and HIV+ patients. B: Pathogens. HIV+ status also defines response patterns. PCA2: for human. Nonpathogenic SIV status does not define BCG response patterns for monocytes.

Figure 2. Analysis of differentially-expressed gene baseline in HIV+ donors. Of 224 targets (A), 3D genes were selected to be differentially expressed during HIV infection at baseline; these genes were submitted for KEGG analysis and found to be significantly enriched for two pathways: antigen processing and cell-mediated toxicity (B). Individual dot plots display differences in NK cell gene expression before (C) and after (D) BCG stimulation.

Figure 3. Genes involved in antigen processing pathways have higher baseline expression levels (A) than pathogens. Pathogenic BAC1 and class II antigen processing molecules showed significantly higher gene expression in HIV+ infected donors. This supports our hypothesis that we would find increased basal immune activation in these donors, and may contribute to increased NK cell activation and subsequent lack of NK cell response to BCG.

Figure 4. NK cells from HIV+ donors have reduced capacity to kill MHC-I deficient K562 target cells at an effectortarget ratio of 4:1 following 4h coculture compared to HIV-uninfected controls. The ability of NK cells to kill MHC-I deficient K562 cells was measured in a flow-cytometry-based assay (A). Dot plots display the mean for each patient; medians were used to compare killing between the groups at E:T (effectortarget) 1:1 and 4:1 (p<0.05; Mann-Whitney).

Figure 5. Whole blood monocyte inflammatory cytokine production following 4h stimulation with BCG. A similar proportion of blood monocytes from HIV+ patients produce TNFα (A) while a lower proportion produce IL-12 (B) compared to uninfected donors following stimulation with opportunistic BCG (A: p<0.005; B: Mann-Whitney).

CONCLUSIONS AND FUTURE DIRECTIONS

Non-naïve analysis reveals several genes that become differently regulated upon PBMC stimulation with opportunistic Mt, and that SIV status may not be a key factor in these responses, while HIV is a key factor in response patterns.

In baseline HIV-infected donors displayed higher levels of antigen processing (inducer of immunity activators) and higher levels of NK cell-related gene expression. These pathways were found to be significantly enriched in our gene set. SIV-infected naïve monocytes/gene expression was similar to uninfected controls.

We found decreased levels of mRNAs associated with activated NK cells in healthy donors (activating KIRs, NKLG2, HPA4) following BCG stimulation.

Despite having a proportion of inflammatory monocytes, HIV+ patients display a lower proportion of IL-12 producing monocytes compared to uninfected donors following stimulation with opportunistic BCG.

In contrast to HIV+ patients, monocytes from HIV-infected donors stimulate IL-12 in response to BCG (compared to an unstimulated control) responses to LPS were similar.

Monocytes from both HIV- and HIV+ donors produce TNFα response to both BCG and LPS; this does not differ between the groups.

NK cells from HIV+ patients in our Seattle-based cohort have reduced capacity to kill MHC-I deficient K562 target cells compared to HIV-uninfected controls

These findings may provide insight into potential future therapies that enhance innate cellular function in HIV+ patients, which may ultimately stymie the reemergence of HIV-related complications.

Future Directions:

• Validate differentially-expressed targets (non-naïve) via real-time PCR

• Examine innate cell interactions in response to Mycobacteria in hosts of pathogenic and non-pathogenic infections

• Monocyte phagocytosis

• NK cell killing

• Assess the role of cytokines and the cytokine milieu in responses to Mycobacteria

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