Gut Microbiota enriched in HIV-Infected Subjects Induce Inflammatory Cytokines but not T Regulatory Cells
Neff CP, Fontenot AP, Rhodes ME, Mazmanian S, Lozupone C, and Palmer BE
Division of Allergy and Clinical Immunology, Department of Medicine, University of Colorado, Aurora, CO
Department of Biological Sciences, California Institute of Technology, Pasadena, CA

Abstract

Background: We co-exist with an abundant microbiota that cooperate with and help regulate our immune system. However, during HIV infection the microbial community in the gut is altered to a distinctive composition which is not consistently restored to that of seronegative subjects by ART. Recently, our group illustrated that in contrast to uninfected subjects, the microbial community in HIV-infected subjects exhibits increased phylogenetic diversity and is Prevotella-rich and Bacteroides-poor. Here we seek to understand the impact of the change in gut microbiota on HIV infection by characterizing innate and adaptive immune responses, including T regulatory cells, to selected gut bacteria that changed with HIV infection.

Methodology: Based on our 16S rRNA sequencing from HIV+ and HIV- subjects we chose four characteristic bacteria; Bacteroides dorei, Bacteroides fluxus, which were decreased, and Prevotella copri, Eubacterium biforme, which were elevated with HIV infection.

Results: Levels of T regulatory cells increased with all bacteria compared with the uninfected control, although levels were not significantly different in regard to HIV status. Median levels of T regulatory cells from PBMC cultured with B. fluxus (4.14) and B. dorei (0.34) were greater compared to that of P. copri (2.73) and E. biforme (2.55). Although these values did not reach statistical significance (p = 0.08). In regard to IL-10 production, higher levels were observed in PBMCs stimulated with B. fragilis species that is known to stimulate IL-10 production by Tregs using a molecular checker polysaccharide A (PSA). Stimulation with B. fluxus, a species that we predicted to have this same immune phenotype based on the presence of PSA encoding genes in its genome, also resulted in high IL-10 levels. These levels were significant when compared to B. fluxus to non-PSA producing B. dorei (p = 0.008) to E. biforme (p = 0.001) and P. copri (p = 0.002). Additionally, B. dorei compared to E. biforme with a PSA gene knockedout was also significant (p = 0.008). Helios, a marker of thymically derived Tregs, was significantly reduced (p = 0.04) in PBMC stimulated with B. fluxus compared to E. biforme, L-10 production was confirmed to not be derived from mononuclears.

Conclusions: These results illustrate that HIV infection is associated with a distinct microbial signature that is more inflammatory. Furthermore, Bacteroides species which are lost during HIV infection are better adapted for inducing T regulatory cells than P. copri and E. biforme that are enriched in the gut during infection. Within the Bacteroides species, genome PSA operon predict high L10 production from Tregs. By evaluating bacterial mediated immune responses these studies will provide important new understanding about the mechanisms and functional aspects of these bacteria in regulating the immune system and their role in HIV disease.

Background

• Commensal bacteria in the human gastrointestinal tract play multiple roles including, but not limited to: aiding in digestion, providing colonization resistance for pathogens and stimulation of the immune system
• Our group illustrated that in contrast to uninfected subjects, the microbial community in HIV-infected subjects exhibits increased phylogenetic diversity and is Prevotella-rich and Bacteroides-poor (Fig. 2). These highly characteristic changes in the gut community may play a role in gut-linked disease that increase co-infection with HIV infection.
• Previously we showed that E. biforme had a significantly higher TNF-α/IL-10 ratio compared to the other bacteria isolates.
• The use of anti-retroviral drug therapy does not consistently restore the gut microbiota to that of a seronegative subject.
• In vivo studies in mice indicate that Bacteroides fragilis (B. fragilis) polysaccharide A (PSA) intermediates the levels of CD4+ T cells, including Tregs, and inflammatory gut response to pathogens.

Schematic of Treg interaction with symbiotic bacteria

Fig. 1. Certain symbiotic bacteria, such as B. fragilis, require CD4+ T cells interacting molecular factors for persistence in the gut. By inducing production of the anti-inflammatory IL-10 cytokine from Tregs, B. fragilis can limit the activity of both effector T-cells and innate immune cells (panel A). Although both effector and regulatory T-cells are specifically targeted and killed by HIV, innate immune cells are not. The loss of CD4+ T cells with HIV infection may thus result in killing of beneficial bacteria by innate immune cells (panel B).

Methodology

• Based on our 16S rRNA sequencing from HIV+ and HIV- subjects we chose four characteristic bacteria; Bacteroides dorei, Bacteroides fluxus, which were decreased, and Prevotella copri, Eubacterium biforme, which were elevated with HIV infection.
• To predict which bacteria can produce PSA we BLASTed the genes in the B. fragilis strain 9343 PSA operon against protein coding genes from 8065 bacterial genomes. To test the role of PSA in generating Tregs and IL-10 production, B. fragilis, which expresses PSA, was modified with a PSA gene knockout (B. fragilis KO).
• PBMCs from HIV-infected and non-infected subjects were incubated with lysates of these bacteria. Levels of anti-inflammatory cytokine, IL-10, was evaluated by ELISA, and percentage of Tregs was enumerated by flow cytometry at 3 days.
• To determine if generated Tregs were thymically derived or peripherally induced, cells were stained for Helios and Ki-67, markers of thymic derived Tregs and proliferation, respectively.
• To verify that IL-10 production was not non-specific derived, monocytes were depleted from PBMC by adherence to plastic or by CD14 magnetic bead selection prior to the addition of the stimuli.
• Statistical significance was determined using Mann–Whitney T tests or ANOVA.

References