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Alveolar T-helper 17 responses to *streptococcus pneumoniae* are preserved in ART-untreated and treated HIV-infected Malawian adults

Chikondi Peno [a,*], Dominic H. Banda [a], Ndaru Jambo [a], Anstead M. Kankwatira [a], Rose D. Malamba [a], Theresa J. Allain [b], Daniela M. Ferreira [c], Robert S. Heyderman [a,d], David G. Russell [e], Henry C. Mwandumba [a,b,c], Kondwani C. Jambo [a,c,*]

[a] *Malawi-Liverpool-Wellcome Trust Clinical Research Programme, University of Malawi College of Medicine, Blantyre, Malawi*
[b] *Department of Medicine, University of Malawi College of Medicine, Blantyre, Malawi*
[c] *Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK*
[d] *Division of Infection & Immunity, University College, London, UK*
[e] *Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, New York, USA*

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Summary *Objective:* We explored if HIV infection is associated with impaired T-Helper 17 responses against *Streptococcus pneumoniae* in the lung.
Methods: We recruited 30 HIV-uninfected healthy controls, 23 asymptomatic HIV-infected adults not on ART, and 40 asymptomatic HIV-infected adults on ART (Median time 3.5yrs), in whom we collected bronchoalveolar lavage fluid. We measured alveolar $CD4^+$ T cell immune responses following stimulation with pneumococcal cell culture supernatant using flow cytometry-based intracellular cytokine staining.
Results: We found that the proportion of alveolar $CD4^+$ T cells producing IL-17A following stimulation with pneumococcal cell culture supernatant (CCS) was similar between HIV-uninfected controls and ART-naïve HIV-infected adults (0.10% vs. 0.14%; $p = 0.9273$). In contrast, the proportion and relative absolute counts of $CD4^+$ T cells producing IL-17A in response to pneumococcal CCS were higher in ART-treated HIV-infected adults compared HIV-uninfected controls (0.22% vs. 0.10%, $p = 0.0166$; 5420 vs. 1902 cells/100 ml BAL fluid; $p = 0.0519$). The increase in relative absolute numbers of IL-17A-producing alveolar $CD4^+$ T cells in ART-treated individuals was not correlated with the peripheral blood $CD4^+$ T cell count ($r=-0.1876$, $p = 0.1785$).

* Corresponding authors. Malawi-Liverpool-Wellcome Trust Clinical Research Programme, P.O. Box 30096, Blantyre 3, Malawi.
E-mail addresses: cpeno@mlw.mw (C. Peno), kjambo@mlw.mw (K.C. Jambo).

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Conclusion: Alveolar Th17 responses against *S. pneumoniae* are preserved in HIV-infected adults. This suggests that there are other alternative mechanisms that are altered in HIV-infected individuals that render them more susceptible to pneumococcal pneumonia.

Introduction

Invasive pneumococcal disease (IPD), in the form of pneumonia and bacteraemia is a leading cause of mortality worldwide.[1,2] HIV-infected adults are 60 times more likely to suffer IPD than age-matched HIV-negative persons.[3–5] Initiation of antiretroviral therapy (ART) has led to a reduction in the incidence of IPD in HIV-infected individuals.[4] Nevertheless, the risk of pneumococcal pneumonia is still 30-fold higher in HIV-infected persons on ART compared to HIV-uninfected individuals.[6,7] It is not clear what factors are behind the persistent high risk of pneumococcal pneumonia in HIV-infected individuals on ART.

We have previously shown that IFN-γ- and TNF-producing alveolar $CD4^+$ T cell responses to *Streptococcus pneumoniae* are maintained in asymptomatic chronic HIV-infected individuals.[8] This suggested that the increased risk to pneumococcal pneumonia in these individuals might not be due to depletion of these important $CD4^+$ T cell subsets in the alveoli. Recently, IL-17A-producing $CD4^+$ T cells in the lung have been shown to be critical in conferring protection in murine models of pneumococcal lung infection.[9,10] In humans, our data from an experimental pneumococcal nasal carriage model showed that pneumococcal carriage leads to increased frequency of pneumococcal-specific Th17 cells in the lung, and that alveolar macrophages exhibited enhanced killing of opsonised pneumococci upon stimulation with recombinant human IL-17A.[11] Furthermore, children that are prone to acute otitis media have been shown to have reduced proliferation and differentiation of pneumococcal-specific IL-17A-producing $CD4^+$ T cells in peripheral blood compared with non-infection prone children.[12] Taken together, these studies suggest that Th17 cells may play an important role in conferring protection against mucosal infection in adults. However, the link between pneumococcal-specific Th17 immunity and increased risk of pneumococcal pneumonia in HIV-infected individuals has not yet been substantiated.

We therefore explored the possibility that Th17 responses against *S. pneumoniae* in the lung are impaired in HIV-infected adults and are not reconstituted with ART. We determined the proportion of alveolar $CD4^+$ T cells producing IL-17A, TNF and IFN-γ after stimulation with pneumococcal cell culture supernatant (CCS), in HIV-uninfected controls compared to untreated or ART-treated asymptomatic HIV-infected adults.

Results

Participant characteristics

We recruited 30 HIV-uninfected healthy controls (median age [range] 39[18–44]; male:female, 22:8) and 63 asymptomatic HIV-infected adults (median age [range] 32[20–46]; male:female,16:47), 23 of whom were ART-naive and 40 were receiving ART (median time on ART [range] 3.5yrs [0.7–9.8]). Two-thirds (30/40) of the ART-treated participants were receiving stavudine/lamivudine/nevirapine therapy, while one-third (10/40) were on tenofovir/lamivudine/efavirenz therapy according to national treatment guidelines. The peripheral blood CD4 count of the ART-naïve HIV-infected adults was lower than that of HIV-uninfected controls (399 vs. 818 cells/μl, $p < 0.0001$). Similarly, the peripheral blood CD4 count of the ART-treated HIV-infected adults was lower than that of HIV-uninfected controls (450 vs. 818 cells/μl, $p < 0.0001$). However, the peripheral blood CD4 count of the ART-naïve HIV-infected adults was not statistically significantly different from ART-treated HIV-infected adults, but the viral load was lower in the ART-treated HIV-infected adults, with 85% (34/40) of the individuals having a plasma HIV viral load of <150 copies/ml. The main characteristics of the participants are summarized in Table 1.

Alveolar CD4+ T cell cytokine responses against *S. pneumoniae* are preserved in HIV-infected adults

Flow cytometry-based intracellular cytokine staining for IL-17A, TNF and IFN-γ was used to detect $CD4^+$ T cell responses following stimulation of BAL cells with pneumococcal cell culture supernatant (Fig. 1). We found comparable proportions of IL-17A-producing alveolar $CD4^+$ T cells between ART-naïve HIV-infected adults and HIV-uninfected controls (Median 0.14% [Interquartile range 0.05–0.30] vs. 0.10% [0.02–0.20]; $p = 0.9273$) (Fig. 2A). Similarly, there were no significant differences in the proportions of TNF- and IFN-γ-producing alveolar $CD4^+$ T cells between ART-naïve HIV-infected adults and HIV-uninfected controls (0.31% [0.05–0.78] vs. 0.10%[0.05–0.34]; $p = 0.5206$ and 0.51% [0.15–1.48] vs. 0.37%[0.12–0.90]; $p > 0.9999$, respectively) (Fig. 2B and 2C).

ART is associated with increased proportion of IL-17A/ TNF-producing alveolar $CD4^+$T cells against *S. pneumoniae* in HIV infected adults

We then investigated the impact of ART on IL-17A, TNF and IFN-γ-producing alveolar $CD4^+$ T cells, by stimulating BAL cells obtained from ART-treated HIV-infected adults with pneumococcal CCS and determining the proportions of the responding cells using flow cytometry-based intracellular cytokine staining. We found that the proportion of IL-17A-producing alveolar $CD4^+$ T cells was higher in ART-treated HIV-infected adults compared to HIV-uninfected individuals (Median 0.22% [Interquartile range 0.08–0.50] vs. 0.10%[0.02–0.20]; $p = 0.0166$) (Fig. 2A). Similarly, the proportion of