DETECTION OF IL-22 IN RESPONSE TO MYCOBACTERIA

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Background
IL-22 is a member of IL-10 family of cytokines. A distinct subset of CD4+ T cells producing IL-22 has been identified, termed 'Th22' cells. IL-22 functions to preserve mucosal barriers and induce antimicrobial peptides, and forms a component of protective immunity to a range of extracellular and intracellular bacterial infections. A recent study in IL-22-deficient mice described a protective role for IL-22 during the chronic stage of *Mycobacterium tuberculosis* (*Mtb*) infection. In humans, soluble IL-22 has been detected at sites of extra-pulmonary tuberculosis (TB), and a polymorphism in the IL-22 promoter has been linked to TB susceptibility. These data suggest a potential role for IL-22 in *Mtb* control, but to date mycobacteria-specific IL-22 production has been understudied in humans.

Methods
In this study, we determined the frequency of Th22 cells in healthy *Mtb*-exposed adults (n=24) in response to mycobacterial antigens using a whole blood assay, followed by flow cytometric analyses. Additionally, we investigated the secretion kinetics of IL-22 by ELISA, and explored the production of IL-22 in peripheral blood mononuclear cells (PBMC).

Results
We tested a range of antibody clones specific for IL-22, and identified those that were optimal for use in flow cytometry experiments. Furthermore, we detected peak IL-22 production at 24 hours after *Mtb* stimulation in whole blood, similar to the kinetics of IFN-g and IL-17 secretion. Using optimised methodology, we observed that the frequency of mycobacteria-specific CD4+ T cells producing IL-22 in the blood of healthy *Mtb*-exposed adults was greater than the magnitude to CD4+ T cells producing IFN-g (median 0.91% and 0.55%, respectively; p=0.024). The majority of CD4+ T cells produced IL-22 alone (79% [IQR: 71-84]), with low frequencies co-producing either IFN-g (12% [IQR: 6-18]) or IL-17 6% [IQR: 4-10], demonstrating that Th22 cells are the main producers of IL-22. Compared to a standard whole blood assay, very low to no IL-22 was detectable in PBMC (median 0.378% vs 0.048%; p=0.008, n=8). We show that addition of fluorescently-labelled PBMC to autologous whole blood followed by *Mtb* stimulation led to recovery of similar frequencies of CD4+ IL-22+ cells in PBMC, indicating that low IL-22 detection in PBMC was not due to loss of IL-22-producing cells during cell isolation. Moreover, conditioned media from *Mtb*-stimulated whole blood was able to partially restore IL-22 detection in PBMC, by a median of 40% compared to whole blood. Additional requirements for optimal detection of IL-22 in PBMC are currently being investigated, including specific cell subsets that may be important in antigen presentation to Th22 cells.

Conclusion
These results confirm that IL-22 is produced mainly by a distinct subset of CD4+ T cells, and contributes significantly to the immune response to mycobacteria. Our novel findings highlight the potential importance of IL-22 in immune control of mycobacteria. The requirements for detection of IL-22 appear to differ between whole blood and PBMC, and additional studies are underway to investigate this further.