PHENOTYPIC HETEROGENEITY OF MUCOSAL ASSOCIATED INVARIANT T (MAIT) CELL CLONES FROM BRONCHOALVEOLAR LAVAGE FLUID

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Background

Bacterial lung infections, including tuberculosis (TB) are the leading causes of morbidity and mortality worldwide. The lung's mucosal response to bacterial exposure is still poorly understood, leading to an obstacle in the development of effective vaccines, primarily in the case of TB. Mucosal Associated Invariant T (MAIT) cells, an innate-like group of lymphocytes, represent an attractive potential universal vaccine target because they recognize vitamin B metabolites from diverse bacteria including *Mycobacterium tuberculosis* (*Mtb*), when presented by the invariant MHC-related protein 1 (MR1) molecule. In the periperhal blood, MAIT cells have a consistent phenotype, CD3+ CD4- MR1 tetramer+ (5-OP-RU loaded) $CD26^{hi}$ and $CD161^{hi}$. The phenotypic and functional characteristics of MAIT cells in the lung, however, is currently unknown.

Method

Paired bronchoalveolar lavage (BAL) fluid and peripheral blood samples were collected from a patient with non-TB pneumonia. Samples were stained with 5-OP-RU loaded MR1 tetramer along with antibodies against CD26 and CD161 and subpopulations sorted based on their expression of the latter two markers. Analysis of MR1 tetramer+ MAIT cells revealed surprising heterogeneity of CD161 and CD26 expression in the lung compartment compared to the peripheral blood. In peripheral blood, MR1 tetramer+ cells were predominantly $CD26^{hi}$ $CD161^{hi}$, whereas in the BAL fluid MR1 tetramer+ cells were $CD26^{hi}$ $CD161^{hi}$, $CD26^{lo}$ $CD161^{lo}$ as well as $CD26^{lo}$ $CD161^{hi}$. MAIT cell subpopulations were then cloned by limiting dilution, phenotypically and functionally confirmed to be MAIT cells and further characterized.

Results

MAIT cell clones were cultivated from the 3 different phenotypic populations of the BAL fluid. Subpopulations sorted from the peripheral blood, however, failed to yield viable clones which warrants further investigation. Regardless of the phenotypic population of origin, all BAL-derived clones displayed MR1 restricted production of INF-γ in response to *M. smegmatis* stimulation. These BAL-derived clones demonstrated heterogeneous expression of CD8 expression as well as heterogenous MR1 tetramer binding affinity.

Conclusions

MAIT cells from the BAL fluid display previously unreported phenotypic heterogeneity. This work has confirmed functionally that these cells demonstrate MR1 restricted anti-bacterial cytokine production. Ongoing work into the phenotypic and functional characterization of the BAL fluid-derived clones may provide a better understanding of the heterogeneity seen in BAL-resident MAIT cells during active pulmonary infection.

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