**INTRODUCTION**

Tuberculosis is the second worldwide leading cause of death from an infectious disease after HIV infection and we still know very little about immunity to Mtb. In contrast to Mtb-specific CD4 T-cells, the role of Mtb-specific CD8 T-cells remains highly controversial. Indeed, several studies performed in mice and nonhuman models of TB have suggested a role of Mtb-specific CD8 T-cells in the control of Mtb infection (Stijn et al. 1992; Litaudon et al. 1999, Brightling and Anderson 2010). In these models, the production of IFN-γ and perforin by Mtb-specific CD8 T-cells was required to protect mice from Mtb infection. Other studies performed in human reached the same conclusions (Renna et al. 2009; Cossio 2009). However, the mechanisms underlying DTH T-cell priming and recruitment remain unclear. Of note, one recent study performed in children below 5 years old showed that Mtb-specific CD8 T-cells were present in children with active TB disease but not in healthy children recently exposed to Mtb, despite similar frequencies of Mtb-specific CD4 T-cell responses in both groups (Lauto et al., 2012). The authors concluded from this observation that Mtb-specific CD8 T-cell responses were generated upon exposure to high bacilli loads and could therefore be used as an immunologic biomarker of primary Mtb infection resulting in TB disease. Whether this finding is also relevant for adults is unknown. Overall, these data indicate that the current understanding of the immune factors involved in the control of Mtb require further investigation.

**METHODOLOGY**

We performed a comprehensive characterization of Mtb-specific CD8 T-cell responses in 273 subjects with either latent Mtb infection (LTBI, n = 206) or active TB disease (TB, n = 67). We assessed their functional (production of IFN-γ, IL-2 and TNF-α; proliferation capacity and cytokinicity) and phenotypic (T-cell differentiation and exhaustion) profiles in cells isolated from peripheral blood correlated these profiles with distinct clinical presentations.

PBMCs were stimulated with Mtb-derived peptide pool covering ESAT-6 and CFP-10 proteins. Ex-vivo Mtb-specific CD8 T-cell responses were identified and subsequently profiled by intracellular cytokine staining (ICS). Proliferation capacities were evaluated after six days of in vitro T-cell expansion. Data acquisition was performed on an LSR II four-laser (405, 488, 532 and 633 nm) and analyzed using FlowJo version 8.8.6 (Tree Star inc.). Analysis and presentation of distributions was performed using SPICE version 5.1.

**RESULTS**

**I. Frequency of detection and magnitude of Mtb-specific CD8 T-cell responses in LTBI and TB patients.**

(A) Proportion of LTBI subjects and TB patients with Mtb-specific CD8 T-cell responses defined by the presence of IFN-γ, TNF-α and/or Mtb-specific CD8 T-cell responses defined by the presence of IFN-γ, TNF-α and/or Mtb-specific CD8 T-cell responses.

(B) Magnitude of Mtb-specific CD8 T-cell responses defined by the presence of IFN-γ, TNF-α and/or Mtb-specific CD8 T-cell responses.

**II. T-cell differentiation and exhaustion of Mtb-specific CD8 T cells in LTBI subjects and TB patients.**

Representative flow cytometry examples of the expression of CCR7 and CD38RA (A and of CD4+ T cells from LTBI subjects and TB patients. Mtb-specific CD8 T-cell responses were defined as IFN-γ-producing cells following stimulation with ESAT-6 and/or CFP-10 peptide pools.

**III. Functional profile of Mtb-specific CD8 T cells**

Representative flow cytometry profile (A) and cumulative analysis (B) of Mtb-specific CD8 T-cell responses in LTBI (n = 55) and TB (n = 51) patients. Profiles are gated on live CD3+CD8+ T cells and the various combinations of IFN-γ, IL-2 or TNF-α are shown following stimulation with ESAT-6 or CFP-10.

**IV. Cytotoxic potential of Mtb-specific CD8 T-cell responses in LTBI and TB patients**

Flow cytometry profiles show perforin, granzyme B, Grmα, B and granulysin (Gls) expression on Mtb-specific CD8 T-cells in representative LTBI and TB patients (C). Perforin and granzyme B expression levels were significantly higher in LTBI patients than in TB patients (D).

**V. Associations between Mtb-specific CD8 T-cell responses and clinical presentation.**

(A) Cumulative analysis of the expression of perforin, granzyme B, and CD69 in Mtb-specific CD8 T-cells from LTBI and TB patients (C and D).

(B) Cumulative analysis of the expression of perforin, granzyme B, and CD69 in Mtb-specific CD8 T-cells from LTBI and TB patients (C and D).

**CONCLUSION**

1. Mtb-specific CD8 T-cells responses are more abundant in active TB disease than in LTBI.
2. Neither the magnitude nor the cytokine profile was significantly different between the two groups of patients.
3. Significant differences were observed regarding the level of T-cell exhaustion and stimulation and the cytokine profiles.
4. Frequency, magnitude and proliferative capacities were significantly associated to clinical presentation in TB patients.

These observations suggest distinct dynamics between the Mycobacteria, the CD8 T-cell response and the clinical outcome.

These data provide potential biomarkers for the diagnosis of active TB.