Dominant TNF-α Mycobacterium Tuberculosis-Specific CD4 T-Cell Responses Discriminate Between Latent Infection and Active Disease

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Abstract

Background: Diagnosis of Mtb infection remains complex and requires several clinical, radiological, histo-pathological, bacteriological and molecular parameters. IFN-γ-release assays (IGRAs), i.e. QuantiFERON and ELISpot, measure responses to antigens (e.g. ESAT-6 or CFP-10) that are mainly limited to Mtb, and discriminate infection from immunity induced by vaccination with Bacille Calmette-Guérin. IGRAs however do not discriminate between active TB disease and latent Mtb infection.

Methods: Mtb-specific T-cell responses were investigated in a test cohort of 283 subjects with known diagnosis of latent Mtb infection and active TB disease and subsequently in a validation cohort of 114 subjects with blinded clinical status. Mtb-specific T-cell responses were analyzed by polychromatic flow cytometry using peptide pools (15-mers overlapping by 11 aa) encompassing proteins ESAT-6 and CFP-10 but also PPD antigens. In particular, Mtb-specific CD4 T-cell responses were analyzed for the simultaneous expression of IFNγ, TNFa and IL-2.

Results: Mtb-specific IFNγ ELISpot responses were not different between patients with active TB disease or latent Mtb infection. In contrast, the functional profile of Mtb-specific CD4 T-cell responses was significantly different between active TB disease and latent Mtb infection (ESAT-6, CFP-10 or PPD, all P<0.0001) in the test cohort. Overall, Mtb-specific CD4 T-cell responses from patients with latent Mtb infection were polyfunctional (i.e. mostly composed of cells producing simultaneously TNFa+IFNγ+IL-2) while single TNFa-producing Mtb-specific CD4 T-cell responses were observed in patients with active TB disease. We then investigated the possibility to use this parameter (i.e. % of single TNFa-producing cells) as a diagnostic tool in an independent cohort of 101 patients with blinded TB diagnosis. The concordance between the clinical and the cytokines profile in predicting active TB disease and latent Mtb infection diagnosis was confirmed in >90% of cases thus validating the use of the profile of single TNFa-producing CD4 T-cell response in the timely diagnosis of active TB disease.

Conclusions: These results indicate that analysis of cytokines profiles in Mtb-specific CD4 T-cells by polychromatic flow cytometry is a strong immunological measure discriminating between active and latent Mtb infection. Therefore, polychromatic flow cytometry is a novel and reliable laboratory tool for the timely diagnosis of active Mtb infection.

Figure 1. Quantitative and qualitative analysis of Mtb-specific T-cell responses in the Test Cohort. a) IFNγ ELISpot responses following stimulation with ESAT6 or CFP-10 peptide pools in a cohort of 283 participants with latent Mtb infection (n = 272) or active TB disease (n = 11). Shown are the numbers of spot-forming units (SFU) per 10⁶ mononuclear cells. Statistical significance (P values) of the results was calculated by unpaired two-tailed student t test using GraphPad Prism 5. Bonferroni correction for multiples analyses was applied. b) Qualitative analysis of Mtb-specific CD4 T-cell responses by polychromatic flow cytometry. Shown are representative flow cytometry analysis of the functional profile of Mtb-specific CD4 T-cell responses in participants with either latent Mtb infection (VCBL, left panels) or active TB disease (VCBL, right panels). Profiles are gated on live CD3⁺CD4⁺ T-cells and the various combinations of IFNγ, IL-2 and TNFa are shown following stimulation with ESAT-6 and CFP-10 peptide pools or PPD. c) Simultaneous analysis of the functional profile of Mtb-specific CD4 T-cells on the basis of IFNγ, IL-2 or TNFa production: ESAT-6, CFP-10, and PPD-specific CD4 T-cell responses are shown from 49 and 8 participants with latent Mtb infection or active TB disease, respectively. Representative examples from PwL5 and A2/3 shown in figure 1b are also identified. All the possible combinations of the different functions are shown on the x axis whereas the percentages of the distinct cytokine-producing cell subsets within Mtb-specific CD4 T-cells are shown on the y axis. The pie charts summarize the data, and each slice corresponds to the proportion of Mtb-specific CD4 T-cells positive for a certain combination of functions. d) Distribution of CFP-10- and ESAT-specific CD4 T-cell responses among subjects with latent Mtb infection or active TB disease.

Figure 2. Analysis of Mtb-specific T-cell responses in the Validation Cohort following unblinding of the clinical status. a) IFNγ ELISpot responses following stimulation with ESAT-6 or CFP-10 peptide pools. Shown are the numbers of SFU per 10⁶ mononuclear cells. Statistical significance (P values) of the results was calculated by unpaired two-tailed student t test using GraphPad Prism 5. Bonferroni correction for multiples analyses was applied. b) Analysis of Mtb-specific IFNγ ELISpot T-cell responses in patients enrolled in Switzerland (CH) and Republic of South Africa (RSA). c) Distribution of CFP-10- and/or ESAT-specific CD4 T-cell responses among patients from the Validation Cohort with positive and concordant Mtb-specific CD4 T-cell responses.

Figure 3. Percentages of CFP-10- or ESAT-6-specific single TNFα-producing CD4 T-cells of the 94 subjects from the Validation Cohort with concordant responses against CFP-10 and ESAT-6. Dashed line represents the cutoff of 37.4% of single TNFα-a. a) Subjects with active disease or latent infection are identified with blue and red dots, respectively. b) Subjects from the Republic of South Africa (RSA) or Switzerland (CH) are identified with orange and green dots, respectively.

Conclusions: The fundamental role of TNF-α in the control of Mtb infection in both humans and mice is well established and this is also supported by the increased risk of Mtb reactivation in rheumatoid arthritis patients receiving anti-TNF-α therapy. However, the dominant single TNFα CD4 T-cell response observed during active TB disease may rather reflect and be a marker of the elevated degree of inflammation rather than of protection. These results indicate that the analysis of cytokines profiles in Mtb-specific CD4 T-cells by polychromatic flow cytometry is an important immunological measure discriminating between active and latent Mtb infection. Therefore, polychromatic flow cytometry is a novel and reliable laboratory tool for the timely diagnosis of active Mtb infection.

Reference

Harari et al, Nature Medicine 2011