Centrum för Infektionsmedicin

Institutionen för medicin, Huddinge

LOCAL IMMUNE RESPONSES IN TUBERCULOSIS: CYTOLYTIC EFFECTOR FUNCTIONS AT THE SITE OF *MYCOBACTERIUM TUBERCULOSIS* INFECTION

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Abstract

Despite recent advances in tuberculosis (TB) research, shortage of knowledge still exists that limits the understanding of host-pathogen interactions in human TB. Cell-mediated immunity has been shown to confer protection in TB, although the relative importance of cytolytic T cells (CTLs) expressing granule-associated effector molecules perforin and granulysin is debated. A typical hallmark of TB is granuloma formation, which includes organized collections of immune cells that form around *Mycobacterium tuberculosis* (Mtb)-infected macrophages to contain Mtb infection in the tissue. This thesis aimed to increase insights to the immunopathogenesis involved in the progression of clinical TB, with an emphasis to explore antimicrobial effector cell responses at the local site of Mtb infection.

A technological platform including quantitative PCR and *in situ* computerized image analysis was established to enable assessment of local immune responses in tissues collected from lung or lymph nodes of patients with active pulmonary TB or extrapulmonary TB. The results from this thesis revealed enhanced inflammation and granuloma formation in Mtb-infected organs from patients with active TB disease. CD68+ macrophages expressing the Mtb-specific antigen MPT64 were abundantly present inside the granulomas, which suggest that the granuloma is the main site of bacterial persistence. Macrophages expressed nitric oxide, while the antimicrobial peptide LL-37 was very low in TB lung lesions compared to distal lung parenchyma. Mtb-infected tissues and particularly the granulomas were enriched with CD3+ T cells, CD4+ T cells and FoxP3+ regulatory T cells (Treg), while the numbers of CD8+ CTLs expressing perforin and granulysin were very low inside the granulomatous lesions. We further observed that mRNA expression of important Th1/Th17 cytokines were not up-regulated in the Mtb-infected tissues. Instead, IL-13 and TGF-β were elevated in lymph node TB, which may suggest a shift of the cytokine response towards a Th2 or immunoregulatory profile. We also detected elevated levels of the B cell stimulatory cytokine IL-21, but also IL-10 in TB lesions from patients with pulmonary TB. Accordingly, chronic TB was associated with an increased expression of CD20+ B cells and IgG-secreting cells as well as FoxP3+ Treg cells in the TB lung lesions. This may suggest that adverse immune responses in progressive TB disease involve enhanced activities of plasma B cells and Treg cells. Next, our findings of impaired CTL responses in human TB were applied to evaluate a novel TB vaccine candidate in a non-human primate model of TB. Our *in situ* technology was used to show that CD8+ T cells as well as perforin, granulysin and the survival cytokine IL-7, were induced locally in the lungs but also spleens of animals that were primed with the novel TB vaccine before Mtb challenge. Thus, immune correlates of protection discovered in human TB could be used as potential biomarkers to evaluate the immunogenicity of novel TB vaccine candidates.

Taken together, our results provide evidence of an impaired CD8+ CTL response at the site of Mtb infection that involves deficient expression of perforin and granulysin. Instead, chronic TB is associated with enhanced levels of antibody-producing B cells with little documented protection in TB. We propose that the induction of Th2 or immunoregulatory cytokines and FoxP3+ Treg cells represents potential immunopathogenic processes that may contribute to impaired cytolytic and antimicrobial effector cell responses in human TB.

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