ImmunoTools *special* Award 2015

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Signaling pathways that regulate IFN-γ production in human tuberculosis

Tuberculosis (TB) remains a substantial global health problem despite current drug treatment. TB causes nearly 9 million new cases and 1.7 million deaths annually, and is among the most common causes of morbidity and mortality in patients with HIV. The resurgence of TB and the appearance of multidrug-resistant *M. tuberculosis* (*Mtb*) have reaffirmed this disease as a global public health problem. BCG, the only available vaccine, is of variable efficacy, especially in TB-endemic regions. To develop a more effective vaccine it is necessary to gain a better understanding of the human immune response to this pathogen. During the course of infection with *Mtb,* innate immune responses control the spread of the bacteria, but T lymphocyte recruitment to the lung is required for containment of *Mtb* in granulomas. Protective immunity against mycobacterial infection requires the generation of Th1 cytokine responses, and IFN-γ, a macrophage-activating cytokine produced by T cells, is crucial in immunity to *Mtb.* Most persons infected with *Mtb* are healthy tuberculin reactors with protective immunity against exogenous infection, and their peripheral blood mononuclear cells (PBMC) produce high concentrations of IFN-γ. In contrast, many TB patients have severe disease and ineffective immunity, and their *Mtb*-stimulated PBMC produce modest IFN-γ. The degree of reduction in IFN-γ production by PBMC is a marker of disease severity. In fact, we have previously reported two groups of TB patients in Argentina, based on *in vitro* T cell responses to *Mtb* antigen (Ag): high responder (HR) patients are individuals that display significant IFN-γ production in response to the Ag, whereas low responder (LR) patients are individuals who exhibit low IFN-γ secretion. Moreover, we demonstrated that immunological features paralleled common clinical parameters analyzed in patients with TB in Argentina: HR patients had significant higher percentages of total lymphocytes compared with LR patients; HR patients exhibited higher purified protein derivative diameters than LR patients; and LR individuals had severe pulmonary lesions, a striking loss of weight, and had been ill longer than HR individuals. Furthermore, the proportion of $CD4^+IFN\text{-}\gamma^+IL\text{-}17^+$ cells is elevated in LR patients who exhibit weak cell-mediated immunity against *Mtb*, and $CD4^+IFN\text{-}\gamma^+IL\text{-}17^+$ lymphocyte ratio is directly related to clinical parameters, indicating disease severity. Elucidation of the mechanisms for reduced IFN-γ production in individuals that develop the disease will allow the improvement of the strategies raised against the pathogen, advancing the understanding of immunity to other mycobacteria and intracellular pathogens. To understand these mechanisms, it is important to delineate how Th precursor cells activate the IFN-γ gene and become committed to the Th1 phenotype Therefore, to gain insight into the mechanisms involved in the enhancement of cell mediated immunity responses to *Mtb*, we investigate signaling pathways that lead to enhanced or reduced transcription of IFN-γ in patients with active TB.

Thus, the main goal of our laboratory is to understand the signaling pathways that control IFN-γ secretion in response to *Mtb* in healthy donors (HD), and to identify the abnormalities that occur in tuberculosis (TB) patients, specifically analyzing the potential abnormalities between HR and LR TB patients. For this, we use serum and leukocytes obtained from TB patients, BCG-vaccinated HD and individuals with latent TB (LTBI) in physiologically relevant model systems to understand the immune mechanisms that control *Mtb* infection. Based on published, we study how *Mtb* infection is controlled or not by the immune system of the host. In particular, and to unravel the role of IFN-γ in TB infection, we study the immunological status of individuals with active TB (HR and LR patients), HD and LTBI by analyzing: i) the presence of soluble factors (cytokines, chemokines and serpins) in the serum; and ii) the proportion and function of CD4 leukocytes populations and subsets; iii) the expression and role of costimulatory molecules in lymphocytes from the different groups under study. Furthermore, we also investigate the immune response of subjects with and without LTBI to specific *Mtb* Ags.

For our current work we use recombinant cytokines as IFN-γ, IL-17, IL-2, IL-4, IL-1β, TNF-α, among others. We also use blocking antibodies for these cytokines in order to regulate de cytokine microenvironment. We also perform ELISA and intracellular flow cytometry for surface markers and intracellular proteins. We use antibodies against CD4, CD3, CD8, markers for memory T cell population, transcription factors as T-bet, GATA-3, CREB. Besides, we are working with macrophages to see the impact of some signalling pathways and costimulatory molecules (SLAM, PD-1, ICOS, TIM3, among others) on macrophage activation and microbicidal activity.

The use of antibodies against some cell markers, the ELISA kits for the determination of cytokines and the recombinant cytokines will provide us with excellent tools that will help in the study of the immune response of TB patients, LTBI individuals and healthy donors .Get the ImmunoTools Award will be of great help for our research and will allow us to move forward with the project our laboratory at the IQUIBICEN-Department of Biological Chemistry, University of Buenos Aires School of Sciences.

ImmunoTools *special* AWARD for **Veronica Garcia** includes 25 reagents

FITC - conjugated anti-human CD3, CD4, CD25, Control-IgG1, Annexin V,

PE - conjugated anti-human CD14, IFN-gamma, TNFa, Control-IgG1,

PerCP - conjugated anti-human CD3, CD4, CD8, Control-IgG1,

APC - conjugated anti-human CD14, Control-IgG1,

human IL-4 ELISA-set for 96 wells, (each 3 reagents),

recombinant human cytokines: rh IFNgamma, rh IL-1beta /IL-1F2, rh IL-13, rh IL-2, rh IL-4, rh IL-17A, rh TNFα DETAILS more AWARDS