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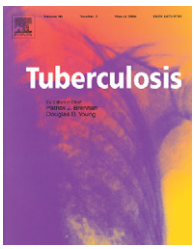

Influence of HLA-DRB1 alleles on Th1 and Th2 cytokine response to *Mycobacterium tuberculosis* antigens in pulmonary tuberculosis

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Summary
The influence of human leukocyte antigens (HLA) on the immune response is well established. We investigated the regulatory role of HLA-DRB1 alleles on cytokine response to live *M. tuberculosis* and its culture filtrate antigen (CFA) in normal healthy subjects (NHS) and pulmonary tuberculosis (PTB) patients. Th1 (IFN-γ and IL-12p40), Th2 (IL-4 and IL-5), pro-inflammatory (IL-6 and IL-8) and anti-inflammatory (TGF-β and IL-10) cytokines were measured by ELISA in 72-h-old peripheral blood mononuclear cell culture supernatants from 58 NHS and 48 PTB patients. HLA-DRB1 genotyping was carried out by polymerase chain reaction and dot-blot hybridization with biotinylated sequence-specific oligonucleotide probes and detection by chemiluminescence. In response to live *M. tuberculosis* and CFA, significantly increased levels of IL-6, IL-8 and TGF-β and decreased IFN-γ, IL-12p40 and IL-10 were seen in PTB patients compared to NHS. We observed a significantly increased IFN-γ response in HLA-DRB1*03-positive NHS ($p = 0.03$) and decreased IFN-γ response in HLA-DRB1*15-positive patients ($p = 0.04$) than respective allele-negative individuals. An increased level of IL-12p40 in DRB1*10 ($p = 0.02$) and IL-10 in DRB1*12- ($p = 0.03$) positive NHS and an increased level of IL-6 in DRB1*04- ($p = 0.02$) positive patients were observed. The study suggests that HLA-DRB1 alleles differentially modulate the various cytokine responses to *M. tuberculosis* antigens, which may influence the cellular and humoral immune responses to *M. tuberculosis* infection in a susceptible host.

Introduction

A perplexing yet unsolved feature of tuberculosis (TB) is that less than 10% of infected individuals develop the disease. Whether a person develops TB depends on host pathogen

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interactions dictated partly by the host genetic factors. Resistance to *M. tuberculosis* infection is known to be conferred by T cell-mediated immune mechanisms. Among the T cells, CD4+ T helper cells play a crucial role in protective immunity to TB.[1]

Upon antigenic stimulation, CD4+ Th cells differentiate into Th1 cells, which secrete cytokines that are involved in cell-mediated immune response (IFN-γ and IL-12) or Th2 cells that secrete mediators of humoral immunity (IL-4 and IL-5).[2] Other pro-inflammatory (IL-6 and IL-8) and anti-inflammatory cytokines (TGF-β and IL-10) also regulate immune response to TB. A pathway of T cell activation, initiated by TGF-β and IL-6 resulting in responsiveness to IL-23, results in antigen-specific T cells that produce IL-17 (Th17).[3] Th17 cells have a crucial role in induction of autoimmune tissue injury and are regulated by IFN-γ.[4] IFN-γ, the signature Th1 cytokine, activates infected macrophages to eliminate intracellular pathogens. IL-12, which comprises of p35 and p40 subunits, directs the development of Th1 cells, while IL-4, the principal Th2 cytokine, suppresses the Th1 response.[5] IL-10 secreted by alternatively activated macrophages and T cells is known to downregulate IL-12 production.[6] IL-8 (CXCL8) is a chemokine secreted by macrophages and T cells that attracts neutrophils and T cells.[7] TGF-β is implicated in suppression of T cell and anti-bacterial immune responses in TB.[8] IL-6 secreted by T cells and macrophages regulates various cell types.[3,9]

CD4+ T cells are activated by the recognition of pathogen-derived peptides in the context of HLA class II molecules presented by antigen presenting cells. The highly polymorphic nature of human leukocyte antigens (HLA) allows it to bind a repertoire of peptides that are specific for each HLA molecule, thereby influencing T cell polarization and hence the profile of cytokines secreted.[10] The major histocompatibility complex linked control of CD4+ T cell activation with distinct cytokine profile has been established in murine models.[11] In humans, significant difference in cytokine secretion profiles has been observed in peripheral blood mononuclear cells (PBMCs) from HLA-B8, -DR3-positive individuals.[12] HLA-DR2 has been shown to be associated with susceptibility to pulmonary tuberculosis (PTB) among Indian populations.[13–15] The immune mechanisms behind HLA-DR associated susceptibility to TB remains unclear. Our earlier studies have shown that HLA-DR antigens can influence humoral and cell-mediated immune responses to TB.[16] In the present study, we investigated the influence of HLA-DRB1 alleles on live *M. tuberculosis* and its culture filtrate antigen (CFA) induced cytokine response in PTB patients and healthy controls.

Patients and methods

Study subjects

Forty-eight patients with PTB comprising 31 males (mean age $\pm$ S.D. $= 36.5 \pm 10.9$ yr) and 17 females (mean age $\pm$ S.D. $= 32.8 \pm 10.2$ yr), from Tuberculosis Research Centre, Chennai, were included in the study before anti-TB treatment was started. Diagnosis of TB was made by chest radiography, Ziehl–Neilsen staining of sputum smears and was confirmed by sputum culture. All patients were human immunodeficiency virus (HIV) negative and none was known to present any immunosuppressive condition. Fifty-eight endemic normal healthy subjects (NHS) comprising 38 males (mean age $\pm$ S.D. $= 31.2 \pm 7.7$ yr) and 20 females (mean age $\pm$ S.D. $= 26.8 \pm 4$ yr) were also studied. The study was approved by the institutional ethical committee. Patients and controls were from the same ethnic group of the south Indian population from the state of Tamil Nadu.

Cell preparation and *in vitro* culture

PBMCs were isolated by density gradient centrifugation on Ficoll-Hypaque. PBMCs were suspended at a concentration of 2×10^6 cells/ml in RPMI 1640 (Sigma Aldrich, St. Louis, MO, USA) supplemented with 2 mM glutamine, 0.1 mM sodium bicarbonate and 2% autologous serum and plated in 48 well plate (Costar, Cambridge, MA, USA). Live *M. tuberculosis* H37Rv (multiplicity of infection 1 macrophage: 10 bacilli) or *M. tuberculosis* CFA 10 μg/ml, prepared as described earlier,[17] was added to respective wells. After 72 h of culture at 37 °C and 5% CO_2, supernatants were harvested and frozen at −80 °C. Seventy-two hours has been shown to be the optimal time for cytokine secretion.[18–20]

Measurement of cytokine levels by ELISA

Cytokine levels of IFN-γ, IL-12p40, IL-4, IL-5, IL-6, IL-8, IL-10 and TGF-β in the culture supernatants were measured using ELISA kits (R&D Systems, Minneapolis, MN, USA). The detection limits for these assays were 15–1000 pg/ml for IFN-γ and IL-6; 30–2000 pg/ml for IL-10, IL-8 and TGF-β; 62–4000 pg/ml for IL-12p40; 31.2–2000 pg/ml for IL-4 and 23.4–1500 pg/ml for IL-5.

Genotyping of HLA-DRB1 alleles

Genomic DNA was isolated by the salting-out technique from the granulocytes.[21] HLA-DRB1 typing was done by polymerase chain reaction and dot-blot hybridization with biotinylated sequence specific oligonucleotide probes, followed by detection using chemiluminescence method.[22]

Statistical analysis

Results were analysed using Student's '*t*' test (unpaired, two tailed) for comparison of cytokine levels between DRB1 allele-positive and DRB1 allele-negative group. Data were shown as mean $\pm$ SEM (standard error of mean) and were considered statistically significant when the p value was less than or $= 0.05$.

Results

Cytokine profile in patients and controls

An increase in IL-6, IL-8, TGF-β level and a decrease in IL-10, IFN-γ, IL-12p40 level in response to live *M. tuberculosis* and CFA of *M. tuberculosis* were observed in PTB patients when compared to NHS. In PTB patients and NHS, a significant increase in the cytokine level of IL-6, IL-8, IL-10, IFN-γ,