Correspondence

B Lymphocytes from Patients with Tuberculosis Exhibit Hampered Antigen-Specific Responses with Concomitant Overexpression of Interleukin-8

To the Editor—Tuberculosis due to *Mycobacterium tuberculosis* is 1 of the 3 major killers among infectious diseases. Deciphering the interactions between *M. tuberculosis* and the innate and adaptive immune compartments of the host is critical for understanding the pathogenesis of tuberculosis and for designing effective immunotherapeutic interventions. By use of a juvenile rhesus monkey model, Qiu et al recently demonstrated that severe tuberculosis induces unbalanced up-regulation of the immune gene networks of inflammatory cytokines, chemokines, and their receptors, but low levels of T cell responses specific to purified protein derivative [1]. The authors conclude that the overexpression of immune genes after tuberculosis infection favors inflammation and suppression of antigen-specific T cells.

B lymphocytes, along with T cells, constitute the adaptive immune compartment. In addition to producing antibodies, B cells act as antigen presenting cells and can produce various inflammatory and immunoregulatory cytokines and chemokines [2]. Thus, B cells have a plethora of functions that regulate the course of immune response and inflammation. B cells have been shown to be present at the site of granulomatous reactions during tuberculosis infection in both mice and humans [3]. Therefore, in view of the latest report on decreased tuberculosis antigen–specific T cell responses after severe tuberculosis, we aimed to decipher antigen-specific B cell responses and their inflammatory cytokine profiles in patients with tuberculosis.

Peripheral blood mononuclear cells were isolated from heparinized blood samples obtained from healthy donors and patients with pulmonary tuberculosis. These patients had clinical symptoms of tuberculosis and positive tuberculin skin test results, and the presence of acid-fast bacilli was verified in sputum samples. Blood samples were collected after obtaining written informed consent, using protocols approved by our institutional ethics committee. B lymphocytes were isolated from peripheral blood mononuclear cells by using CD19 beads (Miltenyi Biotech). Purified B cells were stimulated with either the total membrane fraction of *M. tuberculosis* strain H37Rv (Colorado State University [National Institute of Allergy and Infectious Diseases, National Institutes of Health; Tuberculosis Research Materials contract N01-AI-40091]) or with lipopolysaccharide (from *Escherichia coli*).

B cells from healthy donors proliferated in response to stimulation by total membrane antigens of *M. tuberculosis* (figure, panel *A*). Interestingly, similar to the report of low levels of antigen-specific T cell responses, B cells from patients with tuberculosis showed significantly suppressed antigen-specific responses (figure, panel *B*). However, B cells obtained from these patients are not intrinsically defective and are able to respond to mitogenic stimuli (lipopolysaccharide). Together, our results suggest that severe tuberculosis is associated with hampered antigen-specific B cell responses in patients.

We then analyzed the cytokines produced in response to the stimulation of B cells with total membrane fractions of H37Rv. We found that the patients' B cells produce an increased amount of the inflammatory cytokines interleukin (IL)–8 and IL-6 ($P = .09$, by use of the Student's *t* test), with no significant changes in the levels of IL-1β, IL-12 p70, IL-10, or tumor necrosis factor (figure, panel *C*). These observations suggest that severe tuberculosis induces the up-regulation of B cell inflammatory cytokines but low levels of antigen-specific B cell responses. Interestingly, the presence of IL-8 messenger RNA has been demonstrated in *M. tuberculosis*–infected tissues, and IL-8 is one of the important chemokines involved in cellular recruitment to the tuberculosis granuloma [4]. It has also been demonstrated that IL-8 concentrations in plasma are higher in patients who died from tuberculosis than in patients who survive tuberculosis, and hence, it can be concluded that IL-8 is associated with the severity of tuberculosis [5]. Our results suggest that B cells contribute to the to high levels of IL-8 observed in tuberculosis patients.

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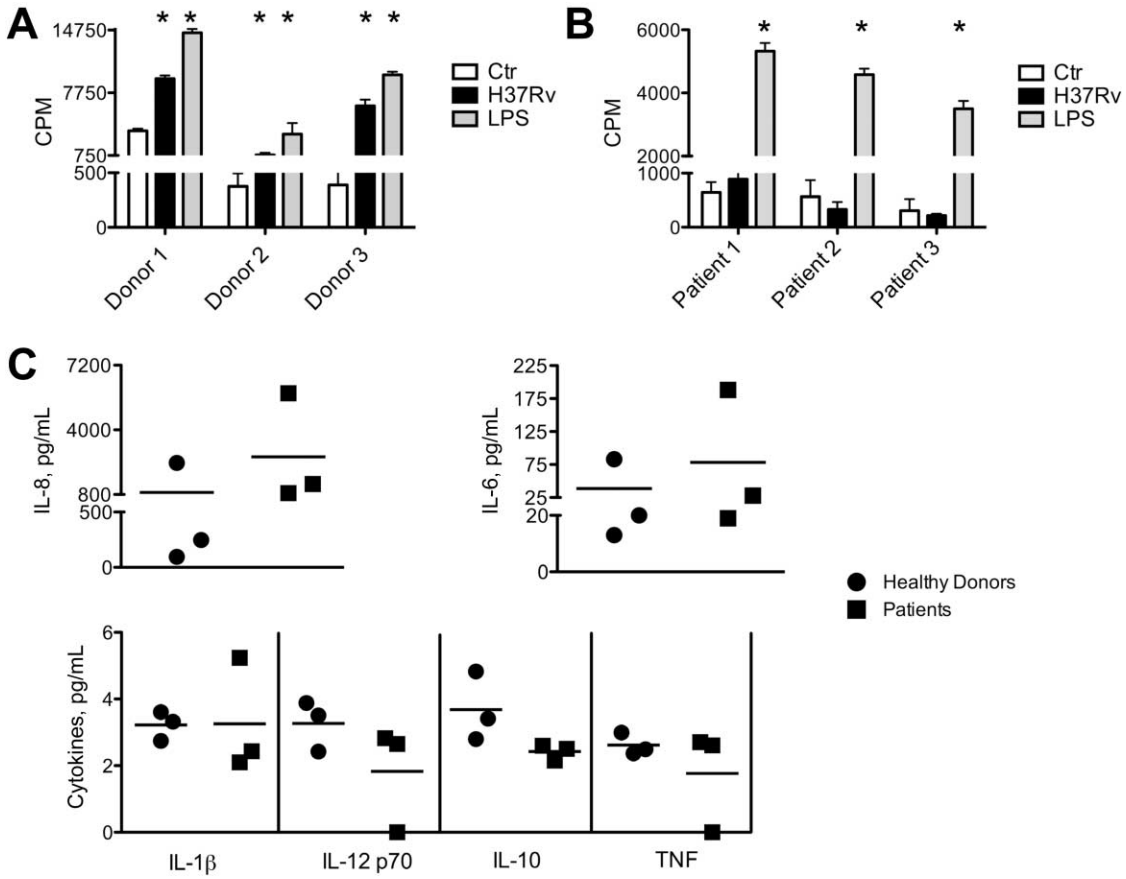

Figure. Lymphoproliferation and cytokine secretion of human B cells in response to total membrane antigens of *Mycobacterium tuberculosis* H37Rv. B cells from 3 purified protein derivative–positive healthy donors *(A)* and 3 patients with tuberculosis *(B)* were assayed for the proliferative response to total membrane fraction of H37Rv. Purified B cells were cultured at a concentration of 2.5×10^4 cells/well/200 μL in the presence of RPMI 1640, 10% fetal calf serum, and 10 μg/mL of $F(ab')_2$ fragments of rabbit anti–human IgM antibodies alone (control [Ctr]), with 5 μg/mL total membrane antigens (H37Rv), or with 1μg/mL lipopolysaccharide (LPS) in 96-well, round-bottomed plates for 5 days. After 4 days, the cells were pulsed for 16 h with 37 GBq of [^{3}H]-thymidine to quantify B cell proliferation. Radioactive incorporation was measured by standard liquid scintillation counting, and the results were expressed as counts per minute (mean $\pm$ standard error of quadruplicate values). Statistical significance for the comparison to Ctr is indicated (*$P < .05$, by use of the Student's *t* test). *C*, The secretion of cytokines (in picograms per milliliter) in cell-free supernatants, as analyzed by cytokine bead array assay (BD Biosciences). Mean values are indicated with a horizontal bars. CPM, counts per minute; IL, interleukin; TNF, tumor necrosis factor.

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