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Down-modulation of mycobacterial-induced IL-1β production in human mononuclear cells by IL-4

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SUMMARY

Tuberculosis is characterized by a cellular immune response mediated by various cytokines, including IL-1β released by stimulated mononuclear cells. It is now well established that IL-1β plays an important role in local and systemic inflammatory response in tuberculosis. Here we have demonstrated, for the first time, that addition of IL-4 to human mononuclear cells obtained from 11 healthy bacille Calmette–Guérin (BCG)-vaccinated donors reduced BCG-induced production of IL-1β by 91·46 $\pm$ 2·2%. This inhibitory effect was found highly significant ($P < 0{\cdot}001$) and was dose-dependent. The effect of IL-4 on the secretion of IL-1β was specific, since a complete reversion was obtained with a neutralizing MoAb to human IL-4. In addition, this inhibitory effect was not attributed to a cytotoxic effect, since trypan blue exclusion studies indicated no loss of cell viability in response to IL-4. Interestingly, the induction of IL-1β was regulated by IL-4, at least in part, by a direct mechanism mediated through the 130 extracellular domain of the IL-4 receptor, as demonstrated by incubation of the mononuclear cells with the neutralizing anti-IL-4 receptor MoAb. Finally, a significant down-regulation of IL-1β secretion was observed in hsp70-stimulated mononuclear cells cultured with IL-4. Further experimental work is needed to establish the relevance of IL-4 in human mycobacterial infection *in vivo*. However, an understanding of the mechanisms that control IL-1β secretion in human mycobacterial infections is essential to understand the pathogenesis of tuberculosis.

Keywords heat shock protein IL-1 IL-4 mycobacteria tuberculosis

INTRODUCTION

Tuberculosis remains a major health problem worldwide, with current estimates indicating eight million new active cases a year, accounting for three million deaths a year [1]. *Mycobacterium tuberculosis* infection requires greater knowledge of the pathogenesis of the disease to understand the mechanisms involved in the effect of mycobacterial constituents on the human immune response. Previous work has demonstrated that this mycobacterial infection is characterized by symptoms such as fever and chest pain that are considered to be mediated by proinflammatory cytokines such as tumour necrosis factor-alpha (TNF-α) and IL-1β [2–4]. However, TNF-α may contribute to the granulomatous reaction that may limit further mycobacterial growth [5–8], and IL-1β plays an important role in the inflammatory response which effectively eliminates the bacilli [9,10]. Therefore it is highly important to understand the immunoregulatory mechanisms involved in the control of proinflammatory cytokine production. Recently, it has been demonstrated that at the site of mycobacterial infection mRNA for Th1 lymphokines predominated in tissue lesions of patients with immunological resistance to mycobacterial antigens, whereas mRNA for Th2 lymphokines was prominent in patients with extensive disease [11,12]. On the basis of these findings, it has been proposed that a shift toward a Th2 type of immune response induced by mycobacterial antigens may explain the chronicity of mycobacterial infection and the specific immunosuppressor activity of IL-4 and/or IL-10 on proinflammatory cytokine production. We have tested directly the importance of IL-4 for the regulation of mycobacterial-induced IL-1β production in human mononuclear cells. Ours is the first demonstration that shows that IL-4 has an inhibitory effect on IL-1β production by human mononuclear cells activated with bacille Calmette–Guérin (BCG) or the 70-kD mycobacterial heat shock protein, and that this inhibitory effect is, at least in part, mediated by a direct mechanism through the 130-kD chain of the IL-4 receptor. A better understanding of the mechanisms that down-regulate IL-1β production is of basic importance to immunomodulate the immune system in *Myco. tuberculosis* infection.

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MATERIALS AND METHODS

Donors

Eleven BCG-vaccinated healthy donors were tested in this study.

All donors were free of inflammatory diseases, and none had received any anti-inflammatory drugs during the previous 3 weeks. None had clinically evident mycobacterial infections.

Cytokines and reagents

Purified human recombinant IL-4 was purchased from Amersham (Aylesbury, UK). MoAb to human IL-4 was purchased from Boehringer (Mannheim, Germany). The neutralizing anti-IL-4 receptor MoAb specific for the extracellular domain of 130-kD IL-4 receptor was kindly provided by Dr O. Djossou (Schering-Plough Research Institute, Dardilly, France).

Mycobacterial antigens

BCG was provided by Dr J. Ruiz-Puente (Instituto Nacional de Higiene, Mexico). The purified recombinant 70-kD heat shock protein (hsp70) of *Myco. tuberculosis* was kindly provided by Dr M. Singh (G.B.F., Braunschweig, Germany).

Stimulation of mononuclear cells with mycobacterial antigens

Peripheral blood mononuclear cells (PBMC) were isolated from healthy donors by centrifugation on Histopaque (Sigma Chemical Co., St Louis, MO) density gradient. For generation of supernatants from PBMC stimulated with mycobacterial antigens, cells (1×10^6/ml) were cultured with increasing concentrations of BCG or hsp70 in complete medium consisting of RPMI 1640 supplemented with L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 μg/ml), and 5% heat-inactivated human serum during 24 h. Supernatants were collected, centrifuged, and stored at –20°C until use.

Measurement of IL-1β production

The amount of IL-1β present in culture supernatants was quantified by commercially available ELISA kits (Amersham, Aylesbury, UK). Supernatants were tested after appropriate dilution. Results are expressed as pg/ml. The detection limit for IL-1β was 0·3 pg/ml.

Statistical analysis

All data are mean ± s.e.m. of the indicated number of experiments. Comparison between groups was determined by Student's *t*-test; $P < 0{\cdot}01$ was considered significant.

RESULTS

IL-4 effects on BCG-stimulated production of IL-1β

In the first set of experiments, we examined the production of IL-1β by human mononuclear cells after stimulation with increasing concentrations of BCG. Figure 1a shows a dose-dependent increase of BCG-induced IL-1β production by mononuclear cells of three individuals immunized by BCG vaccination, and that maximal production was reached with 5 μg/ml of BCG. The addition of polymixin B (10 μg/ml) to cell cultures treated with BCG indicated that BCG was devoid of contaminating lipopolysaccharide (LPS) (data not shown). Therefore, the BCG-induced IL-1β production was not due to possible endotoxin contamination. In the second set of experiments, when used at an optimal concentration of BCG (5 μg/ml) to stimulate PBMC from 11 BCG-vaccinated healthy donors *in vitro*, all supernatants were found to contain significant IL-1β secretion (range 1160–2400 pg/ml) (Fig. 1b). In contrast to IL-1β production by BCG-stimulated PBMC, control cultures (medium alone) showed low IL-1β secretion (range 30–410 pg/ml). This difference was found highly significant ($P < 0{\cdot}005$). In the present study, all healthy donors were vaccinated with BCG in childhood, therefore these results indicate the establishment of a highly responsive and long-lived mycobacterium-specific memory mononuclear cell repertoire.

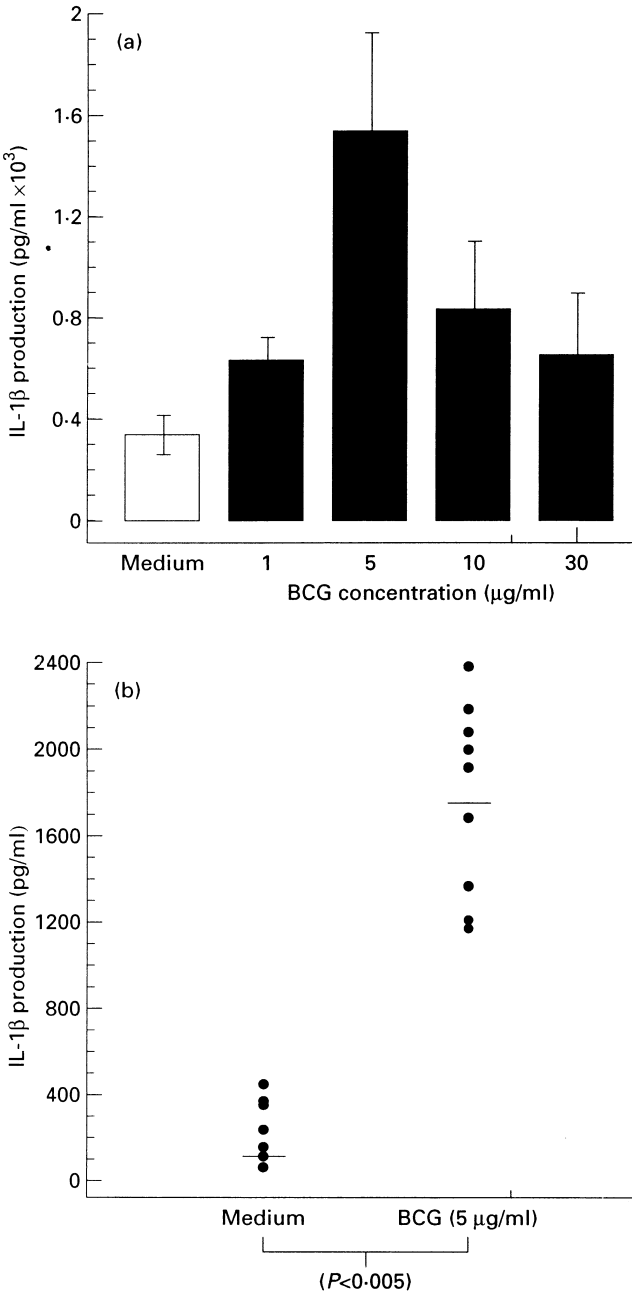

Fig. 1. Induction of IL-1β by bacille Calmette–Guérin (BCG) in human mononuclear cells. (a) Human mononuclear cells (10^6/ml) were cultured with increasing concentrations of BCG or medium alone for 24 h. Cell-free supernatants were assessed for IL-1β by ELISA. Results are the mean ± s.e.m. for three experiments. (b) Peripheral blood mononuclear cells (PBMC) from 11 BCG-vaccinated healthy donors were incubated with the indicated concentration of BCG or medium alone for 24 h. IL-1β production in BCG-activated mononuclear cells was significantly larger than in control cultures ($P < 0{\cdot}005$).

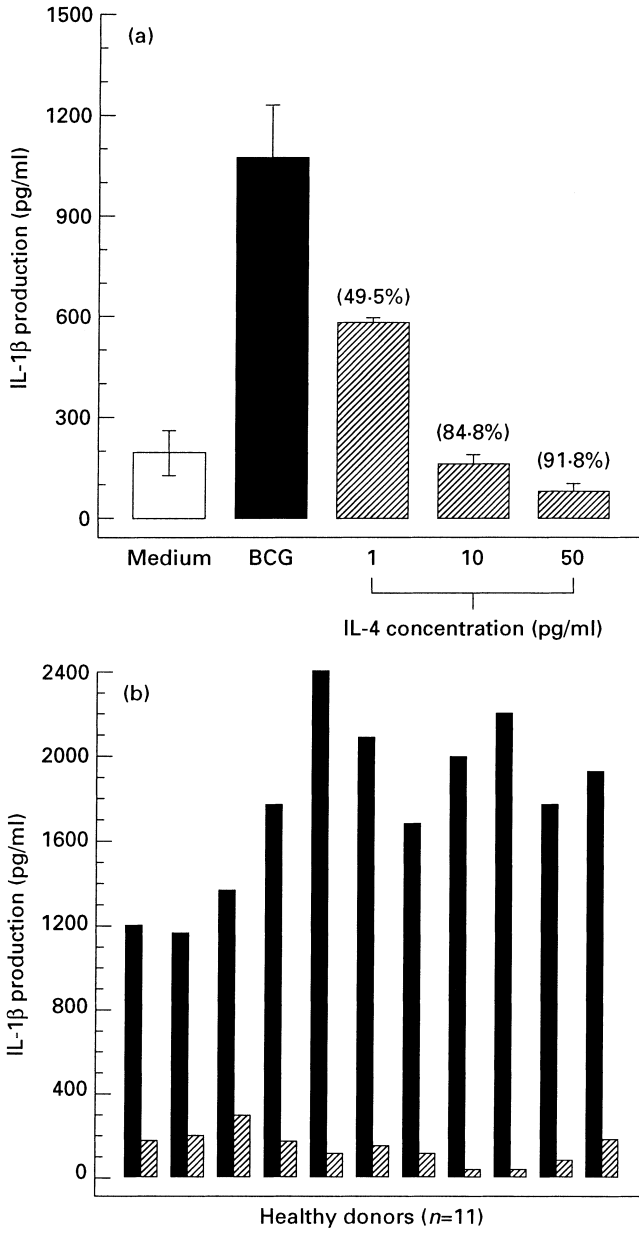

Fig. 2. Effect of IL-4 on IL-1β production by bacille Calmette–Guérin (BCG)-stimulated human mononuclear cells. (a) Human mononuclear cells (10^6/ml) were stimulated for 24 h with BCG (5 μg/ml) in the absence or presence of increasing concentrations of IL-4. Shown are the mean $\pm$ s.e.m. of three independent experiments. Results in parentheses indicate the percentage of inhibition in the presence of each concentration of IL-4, compared with control cultures (BCG) which did not receive IL-4. (b) Addition of IL-4 (50 pg/ml) to human mononuclear cells obtained from 11 healthy BCG-vaccinated donors reduced BCG-induced production of IL-1β by 91·46 $\pm$ 2·2% (▨) compared with BCG-stimulated cultures (■). This inhibitory effect was found highly significant ($P < 0{\cdot}001$).

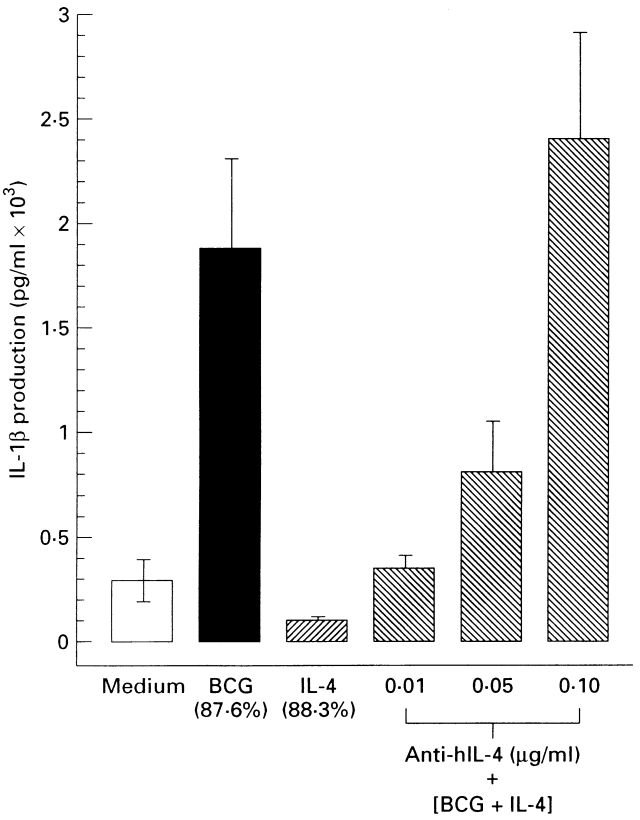

Fig. 3. Blocking of IL-4 by the specific anti-IL-4 antibodies. Mononuclear cells (10^6/ml) were cultured with bacille Calmette–Guérin (BCG; 5 μg/ml) and IL-4 (50 pg/ml) in the presence of increasing concentrations of a neutralizing MoAb against IL-4. Results are the mean $\pm$ s.e.m. of three separate experiments. Values in parentheses indicate the percentage of cell viability in the absence or presence of IL-4.

Since IL-4 has been described to inhibit the synthesis of proinflammatory cytokines by human cells stimulated with mitogen [13,14], we tested the effect of IL-4 on BCG-induced IL-1β production by human mononuclear cells. Of importance, BCG-induced IL-1β secretion by human mononuclear cells from three healthy donors was blocked with a pronounced inhibition of secretion by IL-4, and this inhibitory effect was dose-dependent (Fig. 2a). Maximal inhibition was reached with 50 pg/ml (91·8% of inhibition) and 49·5% of inhibition was obtained with 1 pg/ml. When used at an optimal concentration of IL-4 (50 pg/ml) as determined in experiments described above, addition of IL-4 to human mononuclear cells obtained from 11 healthy BCG-vaccinated donors reduced BCG-induced production of IL-1β by 91·46 $\pm$ 2·2% (range 76·5–99·0%, $n = 11$) (Fig. 2b). Compared with BCG-stimulated cultures, this inhibitory effect was found highly significant ($P < 0{\cdot}001$).

To investigate further the specificity of the inhibitory effect of IL-4, antibodies directed against IL-4 were tested at increasing concentrations for their ability to antagonize the inhibitory effect of IL-4 on BCG-induced IL-1β production by human mononuclear cells cultured with BCG (5 μg/ml) and IL-4 (50 pg/ml). As illustrated in Fig. 3, blocking IL-4 with the neutralizing mouse MoAb to human IL-4 (anti-hIL-4) resulted in an increase in the IL-1β produced by human mononuclear cells in a dose-dependent manner, and a complete reversion was obtained with 0·1 μg/ml of antibody. To evaluate whether the inhibitory effect of IL-4 may be, at least in part, a direct mechanism mediated by the IL-4 receptor, we stimulated mononuclear cells with BCG 5 μg/ml in the presence of IL-4 (50 pg/ml) after a 2-h preincubation with increasing concentrations (0·01–10 μg/ml) of a neutralizing anti-IL-4 receptor MoAb. In this study, the inhibition of IL-4 action

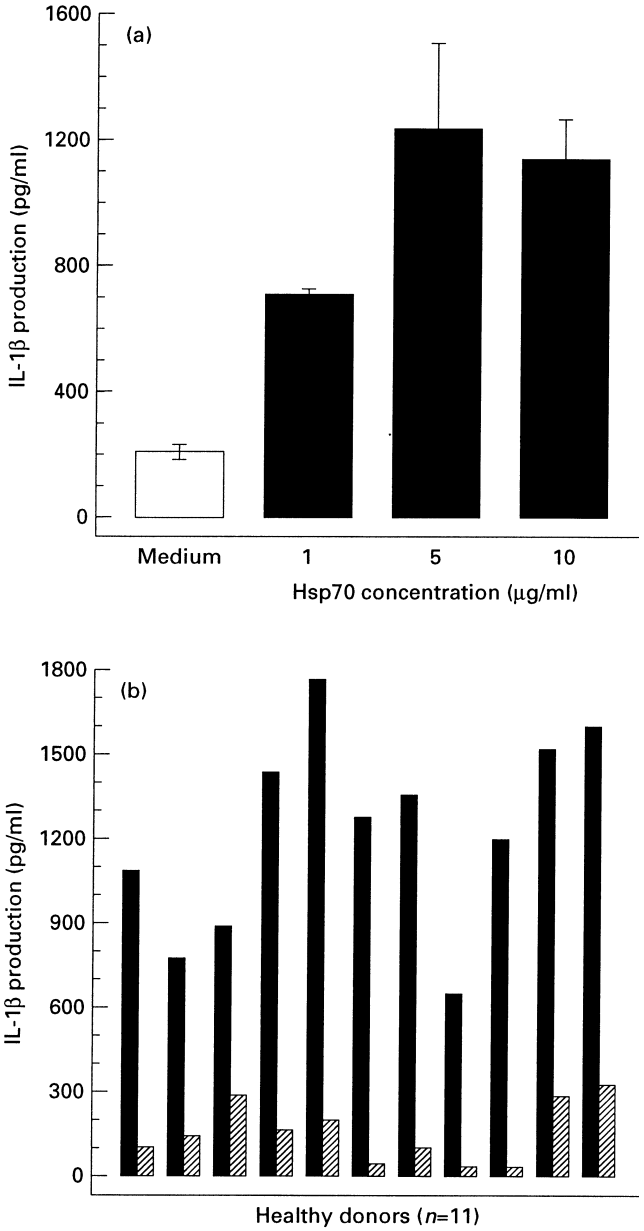

Fig. 4. Inhibition of 70-kD-induced IL-1β production in human mononuclear cells by IL-4. (a) Human mononuclear cells (10^6/ml) were incubated for 24 h at 37°C with different concentrations of hsp 70. Cell-free supernatants were then tested for IL-1β by ELISA. Results are expressed as mean ± s.e.m. of three separate experiments. (b) Addition of IL-4 to human mononuclear cells obtained from 11 healthy bacille Calmette–Guérin (BCG)-vaccinated donors reduced hsp 70-induced production of IL-1β. This inhibition was found highly significant ($P < 0{\cdot}005$). ▨, IL-4; ■, hsp 70.

using a blocking antibody specific for the 130-kD chain of the IL-4 receptor was dose-dependent (data not shown). Furthermore, trypan blue exclusion studies indicated no loss of cell viability in response to IL-4. These results indicate that the inhibitory effect of IL-4 on BCG-induced IL-1β production was not due to a cytotoxic effect (Fig. 3). Simultaneously, we examined the production of IL-4 by human mononuclear cells after stimulation with BCG. We were unable to detect significant IL-4 production (data not shown).

Modulation of hsp70-induced IL-1β secretion by IL-4

Since mononuclear cells from healthy individuals were activated by whole mycobacteria, thus presumably able to recognize several different mycobacterial antigens, we next addressed the question, whether IL-1β production by human mononuclear cells stimulated with a mycobacterial heat shock protein of *Myco. tuberculosis* (70 kD) could be blocked by IL-4. Results illustrated in Fig. 4a show that IL-1β secretion was significantly generated by hsp70 in a dose-dependent manner, and that maximal production was reached with 5 μg/ml hsp70. On the other hand, down-regulation of IL-1β secretion at 24 h was observed in hsp70-stimulated mononuclear cells cultured with IL-4 (30 pg/ml) compared with cell cultures in the absence of IL-4 ($83{\cdot}05 \pm 4{\cdot}3$% of inhibition (mean ± s.e.m.); range 80·0–97·5%; $n = 11$) (Fig. 4b). This inhibition was found highly significant ($P < 0{\cdot}005$).

DISCUSSION

Because IL-1β has been implicated as a potential mediator of the local and systemic inflammatory process seen in tuberculosis, it is important to determine the means to control its production. Our results indicate that IL-4 inhibits secretion of IL-1β induced by BCG or the 70-kD hsp of *Myco. tuberculosis*. Such an inhibition is in agreement with the concept that IL-4 deactivates macrophages [15–19].

The precise mechanim(s) of IL-4-induced inhibition are not well understood. To determine how IL-4 inhibited IL-1β secretion, we investigated whether this inhibitory effect could be modulated by using a MoAb directed against the IL-4 receptor. Our data provide evidence that IL-4 could act on IL-1β production, at least in part, in a direct fashion through the 130-kD chain of the IL-4 receptor. However, it is possible that the anti-IL-1β-mediated down-regulation of IL-4 may be the indirect result of an inhibitory effect on TNF-α production, which it has been reported induces IL-1β production. On the other hand, Donnelly *et al*. have demonstrated that LPS-induced IL-1 could be inhibited by IL-4, at least in part, by repressing IL-1 gene transcription [20]. Therefore, it is possible that mycobacterial-induced IL-1β might be regulated at the transcriptional level. To further investigate the intracellular second messenger pathways involved in the mechanism of IL-1β gene regulation, studies on signal transduction using a strategy of various specific protein kinase inhibitors are in progress.

On the other hand, it is noteworthy that this inhibitory effect was highly efficient with a very low concentration of IL-4. Based on this observation we expected that factors other than IL-4 may be involved in down-regulation of cellular response to mycobacterial antigens. In fact, IL-10 is a cytokine that is known to down-regulate human cellular responses in mice as in humans [21–27]. Recently, the production of IL-10 in pleural fluid has been demonstrated in response to high concentrations of mycobacterial antigens such as lipoarabinomannan [28]. Therefore, it is possible that production of IL-10 by human mononuclear cells in response to mycobacterial antigens may down-regulate IL-1β secretion in culture. Unfortunatelly, this anti-inflammatory cytokine was not available when we started these experiments.

In this study we put forward the hypothesis that IL-4, an anti-inflammatory cytokine, contributes to the immunopathology of mycobacterial infection through down-regulation of the production of IL-1β. In support of this hypothesis, we were unable to detect significant IL-4 production among mononuclear cells obtained from healthy donors upon stimulation with mycobacterial antigens.

These results are in agreement with published data on cytokine production in the human immune response, which indicate that the amounts of IL-4 produced in supernatants from polyclonally activated PBMC derived from healthy donors were below the detection limit [29]. Furthermore, it has been shown that T cell clones from healthy donors specific for purified protein derivative (PPD) or the mycobacterial 65-kD protein produce Th1 lymphokines [30,31]. It has been demonstrated that transforming growth factor-beta (TGF-β) differentially regulates the synthesis of Th1 or Th2 cell lymphokines by human T lymphocytes, and it has been proposed that TGF-β inhibits the production of IL-4 by blocking the response of T cells to IL-2 [29,32]. More importantly, it is now becoming clear that Th1 and Th2 cells down-regulate each other by cytokines such as IL-4 and IL-12 [33,34]. IL-12 has been identified as an inducer of Th1 cell responses, whereas IL-4 has been identified as an inducer of Th2 cell responses [35,36]. Thus, IL-12 may be critical as a natural initiator of the development of Th1 responses. Most striking is the requirement for IL-4 that mediates the subsequent development of Th2 cells.

In conclusion, data presented in this study demonstrate that IL-4 may be responsible, at least in part, for the down-regulation of mycobacterial protein-induced IL-1β in human mononuclear cells. Further studies are needed to establish the relevance of IL-4 in the pathogenesis of disease due to *Myco. tuberculosis*, especially when mycobacteria induces a predominantly Th2 cytokine profile which could aggravate the mycobacterial infection. However, these data may represent an important mechanism for the down-regulation of mycobacterial-induced IL-1β production.

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