Inhibition of *Mycobacterium bovis* BCG-Induced Tumor Necrosis Factor Alpha Secretion in Human Cells by Transforming Growth Factor β

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The effect of exogenous transforming growth factor β (TGF-β) on *Mycobacterium bovis* BCG-induced tumor necrosis factor alpha (TNF-α) production by human mononuclear cells was studied. It was found that TNF-α production by human cells stimulated with BCG was significantly inhibited by TGF-β. The specificity of the observed inhibition was demonstrated, since the addition of an anti-TGF-β neutralizing monoclonal antibody completely reversed the inhibitory effect. Furthermore, the suppressive effect of TGF-β on TNF-α secretion in this system was not due to a direct cytotoxic effect, since cell viability was comparable in the presence or absence of TGF-β. Interestingly, our results demonstrated comparative suppressive effects of TGF-β and interleukin-10 on BCG-induced TNF-α secretion. Together, the data demonstrate, for the first time, that TGF-β inhibits BCG-induced TNF-α secretion by human cells.
It is important to determine whether TGF-β (56.9% inhibition) was neutralized with 10-μg/ml antibody (10.5% inhibition). It is important to note that BCG-induced TNF-α secretion did not differ significantly between cell cultures that had received BCG alone or in combination with TGF-β in the presence of 10-μg/ml neutralizing TGF-β (P = 0.07) (Fig. 3). In contrast, an isotype-matched control IgG1 antibody was without effect (60.3% inhibition). These results indicate the specificity of the inhibitory effect of TGF-β. In addition, TGF-β alone or in the presence of BCG did not affect the viability of the cells, as determined by their ability to exclude trypan blue (Table 1). Together, these results suggest that the inhibitory effect of TGF-β on BCG-induced TNF-α secretion is not accompanied by a cytotoxic effect.

Because IL-10 is a potent downregulator of the immune response implicated in mycobacterial infections (18), we next compared the inhibitory effects of TGF-β and IL-10 on BCG-induced TNF-α secretion. Human mononuclear cells were stimulated with BCG in the presence of IL-10 (10 ng/ml) as reported previously (16). As shown in Fig. 4, the addition of IL-10 resulted in a significant decrease in BCG-induced TNF-α levels, from 1,190 ± 218.8 (mean ± the standard error of the mean [SEM]) to 217 ± 41.7 pg/ml (P < 0.01 versus BCG alone). The effect of IL-10 was dose dependent (data not shown). Although the inhibition was more pronounced, in cultures incubated with BCG in the presence of IL-10, a significant difference was not achieved (P = 0.31) (Fig. 4). These results demonstrate that TGF-β, like IL-10, is critical for con-

![FIG. 1. TNF-α secretion by BCG-activated human cells.](image1)

Mononuclear cells from seven BCG-vaccinated, healthy donors were incubated at 10^6 cells/ml for 18 h with various concentrations of BCG. Culture supernatants were assayed for TNF-α activity by ELISA. Results are expressed as means ± SEMs.

![FIG. 2. Effect of TGF-β on BCG-induced TNF-α production.](image2)

Cells at 10^6/ml were pretreated with various concentrations of TGF-β for 2 h prior to the addition of BCG (10 μg/ml) and incubated for 20 h at 37°C. Simultaneously, cells were incubated for 20 h with TGF-β (10 ng/ml) alone. Cell-free supernatants were assessed for TNF-α activity by ELISA. The values are the means ± the SEMs for seven different individuals. The concentration of TNF-α in cultures containing medium alone was 84 ± 28 pg/ml (mean ± SEM). The values in parentheses indicate percent inhibition by TGF-β with respect to BCG cultures. *, significant difference (P < 0.01) from BCG cultures.
trolling the production of TNF-α by mycobacterium-activated human cells.

Although the efficacy of the BCG vaccine for preventing tuberculosis has been found to vary considerably, BCG is the only currently available vaccine against M. tuberculosis and is still given routinely to millions of children in Mexico. It has been suggested that the lack of an effective means for preventing resistance to M. tuberculosis infection can be due to down-regulation of the immune response (3). Previous studies have shown that TGF-β may be an important mechanism by which mycobacteria evade the host’s immune response (2). Since TNF-α can stimulate human cells to inhibit intracellular growth of mycobacteria and is an important cytokine required in the development of BCG-induced bactericidal granulomas, in this study, we examined whether TNF-α secretion induced by BCG is downregulated by TGF-β. Our results demonstrate a suppressor effect of exogenous TGF-β on BCG-induced TNF-α secretion. The suppressor effect of TGF-β on BCG-induced TNF-α secretion was, indeed, due to TGF-β, since a significant reversion was obtained with a neutralizing monoclonal antibody to TGF-β. Such an inhibition is in agreement with the finding that TGF-β inhibits the activation of macrophages (22) and the generation of cytokines, including IL-2, TNF-α, and gamma interferon (7). In contrast, a recent study (17) showed that TGF-β promotes the generation of Th1 cells, probably enhancing gamma interferon production. In the present study, however, the addition of TGF-β resulted in a decrease in cytokine production. These differences may reflect differences in the antigen recognition of a superantigen and mycobacteria.

It is well known that control of tuberculous infection occurs in a granuloma. It is also known that TNF-α is an important immunomodulator required in the development of BCG-induced bactericidal granulomas. Therefore, the effect of TGF-β on endogenous TNF-α production induced by BCG may provide an important mechanism in determining the immune responses of susceptibility or resistance in humans infected with M. tuberculosis.

The mechanism(s) involved in the suppressive effect of TGF-β on BCG-induced TNF-α secretion is not well understood. Recently, Chantry et al. have demonstrated that TGF-β may inhibit translation of the TNF-α mRNA (2). Therefore, it is possible that the suppressive effect of TGF-β on TNF-α secretion by cells stimulated with BCG may result from a direct

| TGF-β concn (ng/ml) | BCG | Cell viability (%) |
|---------------------|-----|--------------------|
| −                   | +   | 90.51              |
| 0.1                 | +   | 100.00             |
| 1.0                 | +   | 84.30              |
| 10.0                | +   | 97.20              |
| +                   | −   | 95.20              |

a Mononuclear cells were pretreated either in the absence of TGF-β or in the presence of increasing concentrations of TGF-β for 2 h and then incubated with BCG (10 µg/ml) for 18 h.

b Cell viability was determined by trypan blue exclusion. The results represent the means for seven donors.
effect at the level of translation. Alternatively, the anti-TGF-β antibody-mediated downregulation of TNF-α may be the indirect result of interfering IL-2-mediated pathways of signal transduction (8, 10, 12) and/or decreased antigen presentation by modulation of the expression of HLA class II molecules on antigen-presenting cells (4). The present experimental system is being extended to determine the effect of TGF-β on the expression of HLA-DR molecules (by examining expression on monocytes by flow cytometry).

In conclusion, data presented in this study demonstrate the effect of TGF-β on BCG-induced TNF-α secretion and, at the same time, suggest that TGF-β might be an important regulatory cytokine for control of the host’s immune response to mycobacterial infection. Further studies are necessary to determine whether the inhibitory effect of TGF-β, indeed, suppress a human protective immune response in vivo.

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