Cytokine Production by Bronchoalveolar Lavage Cells in Chronic Beryllium Disease

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Chronic beryllium disease (CBD) begins as a sensitizing cell-mediated immune response to beryllium antigen that progresses to granulomatous lung disease. Previous studies demonstrated the involvement of proinflammatory cytokines in the disease process, but the pattern and regulation of cytokine release is unknown. Using bronchoalveolar lavage (BAL) cells from CBD patients in short-term tissue culture, we evaluated cytokine protein levels by enzyme-linked immunosorbent assay and T-lymphocyte proliferation by tritiated thymidine incorporation. We observed the beryllium-stimulated release of tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interleukin-2 (IL-2), and interferon-gamma (IFN-γ) but not interleukin-4 (IL-4). Beryllium-stimulated IFN-γ release was sustained to 168 hr in culture, whereas IL-2 concentrations returned to baseline after 24 hr. Neutralization of IL-2 decreased beryllium-stimulated T-lymphocyte proliferation, but the level of proliferation remained elevated in comparison to unstimulated BAL cells. These data suggest that T helper 1 (Th1) lymphocytes participate in the beryllium disease process; that IFN-γ levels remain elevated after IL-2 levels return to baseline; and that IL-2 participates directly in beryllium-stimulated T-cell proliferation, but other T-lymphocyte mitogenic cytokines may be involved. — Environ Health Perspect 104(Suppl 5):969–971 (1996)

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Introduction

Chronic beryllium disease (CBD) is an occupationally acquired lung disease with clinical findings similar to those of other granulomatous diseases such as sarcoidosis, schistosomiasis, and tuberculosis. The disease process begins as a sensitizing cell-mediated immune response to beryllium antigen that, over time, results in the development of noncaseating granulomas (J). An understanding of the cellular and molecular events that underlie the transition from antigen sensitization to disease may provide improved markers of disease progression and earlier opportunities for therapeutic intervention.

The antigen-specific inflammatory response is a cell-mediated process orchestrated by cytokines. These intracellular signaling molecules form the feed-forward and feed-back loops that initiate and then attenuate the cell-mediated immune response to antigen (Figure 1). In CBD, an antigen-presenting cell (APC) presents beryllium, most probably in conjunction with a peptide, to a T lymphocyte in a major histocompatibility complex (MHC) class II-restricted process that results in T-lymphocyte proliferation (2,3). Alveolar macrophages and T lymphocytes, the predominant cells found in the lungs of beryllium-diseased individuals, are thought to mediate the beryllium-specific immune response (J). Previous studies of cytokines in beryllium disease showed that alveolar macrophages express elevated levels of mRNA for tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) but not for interleukin-1 beta (IL-1β) (4) and that T-lymphocyte proliferation involves increased levels of interleukin-2 (IL-2) and IL-2 receptor (1,3). These studies confirm the role of cytokines in the disorder but not the specific mechanism by which cytokines participate in the disease process.

We hypothesize that the addition of beryllium salts to CBD-derived bronchoalveolar (BAL) cells in vitro will stimulate the release of proinflammatory cytokines that drive granuloma formation and disease. For this study, we established an ex vivo BAL cell system to evaluate beryllium-stimulated release of TNF-α and IL-6; the T-helper 1 (Th1) cytokines, IL-2 and IFN-γ, and the T helper 2 (Th2) cytokine, IL-4. To examine the interdependence of these cytokines, we evaluated the effect of IL-2 neutralizing antibody on T-lymphocyte proliferation and on release of TNF-α, IL-6, and IFN-γ.

Methods

In this study, we obtained BAL cells from a nonexposed population (n = 4) and from patients with CBD (n = 5) by standard lavage methods previously reported (5). Informed consent was secured from all participants prior to the procedure. Cells were cultured at 1 × 10⁶ cells/ml for up to 7 days under standard mammalian tissue culture conditions in the presence or absence of 100 μM BeSO₄ and in the presence and absence of titrated amounts of antihuman IL-2 neutralizing antibody (R&D Systems, Minneapolis, MN).
concentration of cytokines was measured by enzyme-linked immunosorbent assay (ELISA) (R&D Systems) and T-lymphocyte proliferation was measured by tritiated thymidine incorporation.

Results

Unstimulated cells and beryllium-stimulated cells from nonexposed individuals released low levels of the cytokines TNF-α and IL-6 but not the T-lymphocyte-associated cytokines IL-2, IL-4, and IFN-γ (Figure 2). In the absence of beryllium salts, BAL cells from patients with CBD demonstrated cytokine profiles similar to those observed in the nonexposed population. However, beryllium salts increased release of TNF-α and IL-6 from BAL cells of beryllium-diseased individuals. Supernatant concentrations of IL-2 and IFN-γ also increased, but no spontaneous or beryllium-stimulated release of IL-4 was observed. IFN-γ levels remained elevated to 168 hr, whereas IL-2 levels peaked between 6 and 24 hr after the addition of beryllium salts and returned to baseline by 48 hr.

Neutralization of IL-2 decreased T-lymphocyte proliferation to a dose-dependent manner (Figure 3). However, complete neutralization of IL-2 did not return the level of beryllium-stimulated T-lymphocyte proliferation to the levels observed for unstimulated BAL cells in four of five patients studied. Neutralization of IL-2 did not alter TNF-α and IL-6 release, but it did cause a decrease in the concentration of IFN-γ (Figure 4).

Discussion

These studies suggest that this ex vivo cell system allows us to evaluate the role of cytokines in the beryllium-stimulated, cell-mediated immune response through the sequential and temporal manipulation of the cytokine network. In CBD, there is beryllium-stimulated release of TNF-α, IL-6, IL-2, and IFN-γ. The T cell-associated release of IL-2 and IFN-γ but not IL-4 is consistent with the Th1 phenotype. And T-cell proliferation in the presence of IL-2 neutralizing antibody suggests a role for other T-lymphocyte mitogenic cytokines.

The partial IL-2 dependence of IFN-γ release observed in this study is also found in other cell systems (6,7). However, most studies describe a parallel time course for IL-2 and IFN-γ in contrast to the sustained release of IFN-γ but not IL-2 observed in our beryllium-stimulated BAL cell system. Previous research on IFN-γ indicates that following mitogen stimulation in vitro, both IL-2 and IFN-γ protein levels peak at about 20 to 24 hr and then return to baseline (8,9) or, in antigen-driven diseases such as schistosomiasis and leishmaniasis, both IL-2 and IFN-γ levels remain elevated for 3 to 18 days (10–12). It is intriguing to consider that IFN-γ drives the transition from cell-mediated immune response to granuloma formation and disease (Figure 1). IFN-γ upregulates macrophage-derived cytokine production (13), increases expression of the MHC class II molecules (14), and stimulates expansion of the Th1 lymphocyte subsets (15)—all components of the normal cell-mediated immune response and the beryllium disease process. Studies on the interdependence of CBD-associated cytokines are continuing, and extension of these studies to patients with beryllium sensitization is planned.

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