Anti-inflammatory effects of theophylline: modulation of cytokine production

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Background: The basis for the efficacy of theophylline in the treatment of asthma remains enigmatic. Although commonly classified as a bronchodilator, its ability to dilate smooth muscle is considered fairly poor and clinical responses are often independent of bronchodilatation. Recent studies have suggested that immunomodulatory activities may contribute to the therapeutic benefit mediated by theophylline.

Objective: We performed these preliminary studies to determine whether theophylline modulates cytokine production by peripheral blood mononuclear cells.

Methods: Peripheral blood mononuclear cells were obtained from 24 asthmatic subjects and were left in a resting state or stimulated with either mitogens (phytohemagglutinin, lipopolysaccharide) or antigen (tetanus, cat) with or without the additional presence of theophylline (15 μg/dL). Supernatants were collected and evaluated for cytokine concentration by ELISA.

Results: Theophylline neither inhibited production of allergenic cytokines such as IL-4 nor modulated the repertoire of cytokines produced by T<sub>h</sub> cells. A statistically significant inhibition of spontaneous interferon-γ synthesis was observed (24.5 ± 8.6 to 13.4 ± 4.2; P < .05). Theophylline did have anti-inflammatory effects on cytokines primarily produced by mononuclear phagocytic cells. Theophylline mediated a slight inhibition of TNF-α production (0.26 ± 0.08 to 0.21 ± 0.06; P < .05). Theophylline was also associated with a 2.8-fold increase in spontaneous production of the anti-inflammatory cytokine IL-10 (0.35 ± 0.08 to 0.98 ± 0.16 ng; P < .01).

Conclusions: A relative absence of IL-10 characterizes the asthmatic airways and may contribute to the development and severity of allergic inflammation. Induction of IL-10 production by theophylline may therefore mitigate inflammation and contribute to the clinical efficacy of this class of medications.

INTRODUCTION

Although used clinically in the treatment of asthma for more than 50 years, the mechanism of action for theophylline remains an enigma. Theophylline is commonly considered to be a bronchodilator, however, its ability to dilate the smooth muscle of the bronchi is fairly poor and clinical responses are often independent of objective evidence for bronchodilation. Asthma is now recognized as being an autoimmune inflammatory disease of the airways with airway obstruction developing secondary to the effects of airway occlusion with mucus, inflammatory cells, and desquamated epithelium as well as edema and—possibly only to a lesser extent—bronchospasm. We reasoned that theophylline may therefore function, at least in part, to lessen airway inflammation. The generation of pro-inflammatory cytokines in the asthmatic patient’s airways is an important mediator of this autoimmune inflammation. We have performed these preliminary studies to determine whether theophylline modulates cytokine production by peripheral blood mononuclear cells.

MATERIALS AND METHODS

Subjects

Subjects with asthma were drawn from patients at the National Jewish Center for Immunology and Respiratory Medicine. Our study group consisted of 24 asthmatic subjects of mild (n = 15) to moderate severity. Their age range was 17 to 49 years (mean 36 ± 8), with 14 males and 10 females. The presence of asthma was documented by history, physical examination, and the presence of reversible airway disease on pulmonary function testing (≥10% improvement in FEV<sub>1</sub>). Subjects were chosen who had previously undergone skin testing as part of their diagnostic evaluation and were documented to be allergic to cat (Fel d 1) as demonstrated by a wheal ≥3 mm via prick testing. Subjects were excluded if they required systemic corticosteroids or theophylline. Topical corticosteroids at the usual recommended doses (eg, beclomethasone 4 inhalations bid) did not preclude participation and were being used by ten subjects at the time of the study. None of the subjects was using either cromolyn or nedocromil. Full informed consent was obtained and our protocol was approved by the Investigational Review Board.

Mononuclear Cell Activation

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood via ficoll-hypaque centrifugation. Peripheral blood mononuclear cells were resuspended in complete RPMI-1640 medium supplemented with 10% autologous serum. Cells were either left in a resting state or stimulated with a panel of mitogens and antigens with and

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without the additional presence of theophylline (15 μg/dL; Sigma, St. Louis, MO). Phytohemagglutinin (PHA) and antigens were used as a concentration demonstrated to produce suboptimal proliferation of PBMCs in preliminary studies. Mitogenic stimulation was performed with phytohemagglutinin (0.25 μg/mL; Wellcome Diagnostics, England) for two days. Tetanus extract was dialyzed and filtered prior to use.

ELISAs

Cytokine secretion was assessed by ELISA using commercially available kits (TNF-α—Cistron Biotechnology, Pine Brook, NJ; GM-CSF and IL-4—Genzyme, Boston, MA, and IFN-γ—Biosource International, Camarillo, CA). Our IL-10 ELISA uses a sandwich technique with two rat monoclonal antibodies (Pharmingen, San Diego, CA), one of which is biotinylated.4 Briefly, flat-bottomed microtiter plates were provided either precoated or subsequently coated with anti-cytokine capture antibodies. Authentic cytokine standards and samples (neat—1:100 dilution) were applied in duplicate and allowed to bind overnight at 4°C. After washing, enzyme-linked anti-cytokine capture antibodies were applied. Finally, substrate was introduced and color change determined.

Data are expressed as the concentration of cytokine produced/mL and analyzed between the paired control and theophylline-containing samples. Sensitivity for the IL-10 ELISA was 100 pg/mL. The other ELISAs (IL-4, GM-CSF, IFN-γ, IL-1, and TNF-α) were sensitive to ~10 pg/mL except for GM-CSF which was sensitive down to 2.5 pg/mL.

Statistical Analyses

For the ELISAs, we performed paired t-tests obtained for each subject (control and with theophylline) for each of the cytokines using JMP 3.1 software on a MacIntosh Illcx computer.

RESULTS

T Cell—Derived Cytokines

Peripheral blood mononuclear cells were stimulated with the mitogen phytohemagglutinin for two days and for eight days with an allergenic (Fel d 1) and non-allergic (tetanus) antigen. Data are displayed in Tables 1 and 2. No differences in cytokine production were observed in the presence of theophylline for IL-4 or GM-CSF. Theophylline was associated with a statistically significant inhibition of spontaneous IFN-γ production (24.5 ± 8.6 pg/mL to 13.4 ± 4.2; P < .05). No differences were observed in IFN-γ synthesis stimulated by phytohemagglutinin, tetanus, or Fel d 1.

Mononuclear Phagocytic Cell—Derived Cytokines

Peripheral blood mononuclear cells were stimulated with a suboptimal concentration of lipopolysaccharide for 48 hours and supernatants assayed for IL-1, TNF-α, and IL-10 (Table 3). Interleukin-1 was readily generated by resting mononuclear cells and no significant differences were seen either with further stimulation with lipopolysaccharide or in the additional presence of theophylline. The presence of theophylline was associated with a small (0.26 ± 0.08 ng/mL to 0.21 ± 0.06) but statistically significant decrease in spontaneous TNF-α production (P < .05). No differences were observed in the lipopolysaccharide-stimulated sample; however, theophylline was associated with a 2.8-fold increase in unstimulated IL-10 production (0.35 ± 0.08 ng/mL to 0.98 ± 0.16 ng; P < .01). No further increase in IL-10 production was observed in the lipopolysaccharide-stimulated samples.

DISCUSSION

Although available for more than 50 years, the mechanism of action of theophylline remains unknown.1 Theophylline, although classified as such, is a poor bronchodilator. While theophylline improves airflow in a dose-dependent fashion, bronchodilation is poor and occurs at concentrations greater than those required for therapeutic improvement. There is a paradox that therapeutic concentrations of theophylline minimally inhibit the immediate—bronchospastic—response to an allergen challenge, whereas it effectively inhibits the late—inflammatory—response.5,8 Similarly, in a guinea-pig model of asthma, theophylline was required at 16 mg/kg to inhibit the bronchospasm induced by histamine inhalation, whereas 0.5 mg/kg was sufficient to prevent the airways obstruction induced in sensitized guinea pigs to ovalbumin challenge.9 Numerous other studies have also suggested an anti-inflammatory basis for theophylline’s mode of action. For example, in vitro, theophylline mitigates the neutrophil respiratory burst and leukotriene metabolism.10,11 Theophylline also mitigates the respiratory burst and killing mediated by bronchoalveolar lavage-derived alveolar macrophages.12 Similarly, in an in vivo animal model, therapeutic serum concentrations of theophylline are associated

Table 1. Effects of Theophylline on Mitogen Driven T Lymphocyte-Derived Cytokines

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Resting</th>
<th>Resting + Theophylline</th>
<th>PHA†-Stimulated</th>
<th>PHA†+Theophylline</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4</td>
<td>14 ± 4</td>
<td>16 ± 3</td>
<td>27 ± 4</td>
<td>36 ± 6</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>1.66 ± 0.44</td>
<td>2.45 ± 0.68</td>
<td>13.76 ± 1.94</td>
<td>15.57 ± 2.08</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>24.5 ± 8.6</td>
<td>13.4 ± 4.2†</td>
<td>429.3 ± 47.1</td>
<td>449.7 ± 53.8</td>
</tr>
</tbody>
</table>

* Concentrations of IL-4, GM-CSF, and IFN-γ in pg/mL supernatant.
† phytohemagglutinin.
‡ P < .05 compared with resting PBMC.
with a diminished leukocyte influx into a bacterial-challenged lung and diminished bactericidal activity.

While suggestive of an anti-inflammatory effect, none of these studies is satisfactory in establishing such a role. These studies have either been dependent on studies of peripheral blood cells (the several neutrophil studies), do not propose an anti-inflammatory mechanism likely to be relevant to asthma control (diminished bacterial killing), or represent animal models. Animal models of asthma may not accurately reflect human asthma while effects on the neutrophil may not have direct relevance to asthma.

Recent advances have suggested a role for the T lymphocyte and inflammatory cell-derived cytokines in the initiation of asthmatic inflammation. While not a prominent feature of BAL cell pellets, bronchial biopsies of asthmatics are replete with CD4+ T lymphocytes. Furthermore, these T cells can be shown to be in an activated state via their expression of high affinity IL-2 receptors (CD25) and MHC Class II molecules. In allergic asthmatics these T lymphocytes are of the phenotype of Th2-like cells, expressing mRNA transcripts for interleukins (IL)-3, IL-4, IL-5, IL-9, IL-13, and GM-CSF. In addition to T lymphocytes, numerous other cells including alveolar macrophages, airway epithelium, eosinophils, and mast cells may also be sources for these and other pro-inflammatory cytokines in asthma. These cytokines are responsible for the clinical-pathologic features of asthma including the immunoglobulin isotype switch to IgE (IL-4 and IL-13), eosinophil differentiation, proliferation, and activation (IL-5, GM-CSF, and IL-3) and mast cell proliferation (IL-9 and IL-3); thus, the secretion of these cytokines leads to the pathologic findings observed in asthma.

Several studies have suggested an effect of theophylline on T lymphocyte function. In 1987, Fink et al reported that theophylline therapy is associated with increased numbers of "suppressor" cells. Similarly, Ward et al recently reported an increase in CD4+ T lymphocytes 48 hours after an allergen challenge which was no longer apparent after treatment with theophylline. These studies must be interpreted with caution as changes in peripheral blood may represent a reciprocal subset of T lymphocytes to those which are responding to the airways inflammation. Evidence for an effect of theophylline on cytokine production is also mostly circumstantial. Our studies have shown that asthma is characterized by the presence of "primed" leukocytes and that this priming is lessened in the presence of theophylline. Priming refers to the phenomenon that neutrophils are normally refractory to activating stimuli such as f-met-leu-phe, unless they have received a co-activation signal. These priming co-stimuli are typically lymphocyte-derived cytokines and include IL-4, IL-6, TNF, and GM- and G-CSF. The mitigation of priming in subjects on theophylline suggests the possibility that neutrophils have not been exposed to these cytokines in vivo. More suggestive is the recognition that several of the biochemical pathways proposed for theophylline may directly inhibit cytokine gene transcription; thus, theophylline—at therapeutic concentrations—does increase cellular cAMP concentrations and inhibits Ca++ influxes into the cell. While inhibiting only approximately 10% to 20% of total phosphodiesterase activity at normal serum concentrations, theophylline does synergize with activators of adenylyl cyclase such as β-agonists to raise intracellular cAMP concentrations. Cytokine gene promoters are associated with cAMP responsive elements (CREs) and transcription of the protein which binds to CRE (CREB) is itself cAMP-dependent. In addition, NFAT (nuclear factor of activated T cells) is a determinant of IL-2 and IL-4 gene transcription and its activation is Ca++ dependent. Pentoxifylline, a methylxanthine similar to theophylline is a relatively weak, nonspecific phosphodiesterase inhibitor, mitigates TNF-α production. In addition to transcription rate, cytokine gene production is tightly regulated through specific RNAses that specifically control metabolism of these gene transcripts. Modulation of these mechanisms represents an additional potential target for cytokine gene regulation.

We hypothesized that the presence of theophylline would lead to inhibition of cytokine gene transcription and secretion. Decreased production of both nonspecific inflammatory cytokines (IL-1, IL-6, and TNF-α) and cytokines more specific for allergic inflammation (IL-4 and GM-CSF) were

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Resting</th>
<th>Resting + Theophylline</th>
<th>LPS† Stimulated</th>
<th>LPS† + Theophylline</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1</td>
<td>3.81 ± 0.94</td>
<td>4.00 ± 0.98</td>
<td>3.41 ± 0.64</td>
<td>3.30 ± 0.61</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.26 ± 0.08</td>
<td>0.21 ± 0.06‡</td>
<td>2.27 ± 0.28</td>
<td>2.43 ± 0.33</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.35 ± 0.08</td>
<td>0.98 ± 0.16§</td>
<td>2.82 ± 0.37</td>
<td>3.01 ± 0.40</td>
</tr>
</tbody>
</table>

* Concentration of IL-1, TNF-α, and IL-10 in ng/mL.
† Lipopolysaccharide.
‡ P < .05 compared with resting PBMC.
§ P < .01 compared with resting PBMC.
evaluated. It was expected from previous studies\(^2^{24-26}\) that stimulation with the nonallergen (tetanus) should produce a TH1-like pattern of cytokine synthesis (IFN-\(\gamma\)) whereas stimulation with allergen should produce a TH2-like pattern (IL-4, IL-5). Since the major determinant of TH phenotype is the cytokine milieu itself, small changes in cytokine secretion produce major shifts in the repertoire of cytokines observed after antigenic stimulation (eg, from TH\(2^{2}\) to TH\(1^{2}_{1}\)). Our findings do not support a role for theophylline in modulating either qualitative differences in T cell cytokine production or shifts in T cell repertoire. We did, however, find evidence for an anti-inflammatory effect of theophylline through the inhibition of TNF-\(\alpha\) and IFN-\(\gamma\) synthesis (Fig 1).

In addition to mitigation of pro-inflammatory cytokine production, anti-inflammatory effects may also be mediated through increased production of the anti-inflammatory cytokine IL-10. Human IL-10 is an anti-inflammatory cytokine that in the lung is predominantly produced by alveolar macrophages (unpublished observations) and that inhibits IL-2 and IFN-\(\gamma\) production by TH\(1^{2}_{1}\) cells,\(^2,28\) IL-4 and IL-5 by TH\(2^{2}\) lymphocytes,\(^28\) IFN-\(\gamma\) and TNF-\(\alpha\) by NK cells\(^29\) and IL-1\(\beta\), IL-6, IL-8, IL-12, and TNF-\(\alpha\) by mononuclear phagocytes.\(^30-33\) Mitigation of allergic inflammation by IL-10 is supported by its ability to inhibit IgE production,\(^34\) shorten eosinophil survival,\(^35\) and induce allergen-specific T cell nonresponsiveness.\(^36\) In summary, these observations support the concept that IL-10 may function as an anti-inflammatory cytokine and may inhibit allergic inflammatory reactions.\(^37\) We have previously reported that asthma is characterized by diminished spontaneous and stimulated production of IL-10.\(^37\) Our studies demonstrate increased spontaneous production of IL-10 in the presence of theophylline (Table 3).

In summary, these studies support an anti-inflammatory role for theophylline in asthma through induction of IL-10 synthesis. Asthma is characterized by diminished IL-10 production in the lung. Induction of IL-10 production could be expected to be associated with decreased production of pro-inflammatory cytokines including IL-1\(\beta\), TNF-\(\alpha\), IL-6, IL-12, and IFN-\(\gamma\), diminished IgE production, shortened eosinophil survival, and induction of allergen-specific T cell nonresponsiveness. The diminished production of IFN-\(\gamma\) and TNF-\(\alpha\) observed in this study may have reflected, in part, increased production of IL-10. Mitigation of production of other cytokines may require prolonged incubation of the antigen-presenting cells prior to their exposure to antigen. The relevance of these observations can only be determined after confirmation of an ability of theophylline to reverse in vivo the down-regulation of IL-10 synthesis.

REFERENCES


