Functional Differences of the Lymphocytes in Nasal Polyps between Allergic and Non-Allergic Patients

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ABSTRACT
Lymphocytes can produce various kinds of cytokines which are responsible for the development of the infectious and allergic inflammation. We focused on the role of the lymphocyte in the pathogenesis of the nasal polyp. This study was designed to evaluate the functional differences of the lymphocytes between allergic and non-allergic polyp. Lymphocytes were obtained from peripheral blood and tissues of polyp in 12 patients with non-allergic polyp, 7 patients with allergic polyp and 5 normal subjects as control. Cytokines were measured with ELISA from each group of lymphocytes after stimulation with Concanavalin A (Con A). We compared the production of interleukin (IL)-2, IL-4, tumor necrosis factor (TNF)-α and interferon (IFN)-γ between the non-allergic and allergic groups. The levels of IL-4 and IL-6 from polyp tissue lymphocytes were higher in allergic group, while those of IL-2 and IFN-γ were higher in non-allergic group. The levels of IL-4, IL-6 and TNF-α from peripheral blood lymphocytes were higher in allergic group, and IFN-γ was higher in non-allergic group. These results suggest that cytokine productivity of the polyp tissue lymphocytes appear to be parallel to that of the peripheral blood lymphocytes in each group and shows distinct pattern of cytokine production between two groups.

KEY WORDS Nasal polyp · Allergy · Lymphocyte · Cytokine · Concanavalin A.

INTRODUCTION
Numerous studies have been conducted on the pathogenesis of nasal polypsis. However the relationship of the polyp and allergy has not been proved yet. Nasal polyps are associated with numerous upper respiratory tract diseases including allergic rhinitis, recurrent sinusitis, asthma, aspirin intolerance, and cystic fibrosis. Allergy and bacterial infection have generally been accepted as important etiologic factors in the formation of nasal polyps.1)

Nasal polyps have respiratory epithelium with infiltration of eosinophils, T and B lymphocytes, polymorphonuclear cells (PMNs) and metachromatic cells. A number of studies have demonstrated that Th2-type T lymphocytes at sites of allergic inflammation produce granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin (IL)-3, IL-4 and IL-5.2-4) IL-4 is required for the production of IgE by inducing isotype switch in B cells.5) And IL-3, IL-5 and GM-CSF stimulate the growth and differentiation of eosinophils and increase the survival of eosinophils.

It has been suggested that the development and maintenance of the allergic inflammation may be due to T-lymphocyte activation with predominant production of the cytokines IL-4 and IL-5. However, the reports are not consistent with regard to the profile of cytokines in nasal polyp of allergic patients.6-8) So we evaluated the functional differences of lymphocytes in nasal polyp and peripheral blood between allergic and non-allergic patients by measuring cytokines after stimulation with Con A.

MATERIALS AND METHODS
Subjects
Nineteen patients with nasal polyp and chronic sinusitis were involved in this study. Among them, 7 patients had allergy to house dust mite, which was confirmed by positive skin test and/or RAST. The remaining 12 patients had no history of allergic diseases and showed negative skin prick test responses, and their serum IgE levels were less than 80 IU/ml. Five healthy volunteers were also included in this study as controls. None of study subjects had taken oral corticosteroids within 6 weeks before the study. Informed consent were obtained from all subjects.
Isolation of lymphocytes

Lymphocytes were isolated from nasal polyp specimens and peripheral blood of all patients. And peripheral blood lymphocytes were isolated from control subjects. Polyp tissue was digested with trypsin and lymphocytes were isolated by using Ficoll-Conray density centrifugation. Peripheral blood lymphocytes of patient and control groups were also isolated in a similar fashion by a Ficoll-Conray gradient. Isolated lymphocytes at a concentration of $1 \times 10^6$ cells/ml were stimulated with Con A ($10 \mu\text{g}/\text{ml}$) for 48 hours. Cultured supernatants were kept at $20^\circ\text{C}$ until cytokine analysis.

Measurement of cytokines

Cytokine levels in cultured supernatants were analyzed by ELISA for IL-2, IL-4, IL-6, TNF-$\alpha$ and IFN-$\gamma$.

Statistical analyses

Statistical analyses were performed using unpaired t-test. Values of $p$ less than 0.01 were accepted as being statistically significant.

RESULTS

Cytokine production from polyp lymphocytes

Con A stimulated secretion of cytokine by lymphocytes isolated from polyp of allergic patients were compared with those from polyp of non-allergic patients. IL-4 and IL-6 were significantly higher in allergic group. And the levels of IL-2 and IFN-$\gamma$ were significantly higher in non-allergic group. But TNF-$\alpha$ was not significantly different between two groups (Fig. 1).

Cytokine production from peripheral blood lymphocytes

Cytokine levels of peripheral blood lymphocytes were compared between allergic and non-allergic patients. The levels of IL-4, IL-6, and TNF-$\alpha$ were significantly higher in allergic group. In contrast, the levels of IFN-$\gamma$ were significantly higher in non-allergic group. But IL-2 was not significantly different between two groups.

Compared to control group, IL-4 was significantly higher in allergic group, whereas it was lower in non-allergic group. IL-6 and TNF-$\alpha$ were significantly higher in allergic group than in control group, but not in non-allergic group. In contrast, IFN-$\gamma$ was significantly higher in non-allergic group than in control group.
control group, but not in allergic group (Fig. 2).

**DISCUSSION**

Some immunohistochemical studies about lymphocyte subpopulations in the nasal polyp revealed significantly more CD8\(^+\) than CD4\(^+\) cells.\(^{9,10}\) They suggested that cellular immunity of T lymphocytes rather than allergy may be involved in the formation of nasal polyp. We used Con A to stimulate T lymphocytes and this mitogen does not require the presence of accessory cells to activate T cells and dose not induce cytokine production from other cells.\(^{11}\) The activated T cells include subpopulations of both CD4\(^+\) and CD8\(^+\) cells. CD4\(^+\) T cell is divided into Th1 and Th2 subsets according to their cytokine profiles in tissue and peripheral blood of different disease. Th1 subset secretes IFN-\(\gamma\) and IL-2. IFN-\(\gamma\) is involved in delayed hypersensitivity, macrophage attraction and suppression of IgE secretion. Th2 type cytokines are IL-4, IL-5, IL-6, IL-10, and IL-13.

CD8\(^+\) cells are considered to have suppressor/cytotoxic function. But recently, distinct cytokine-secreting subsets of CD8\(^+\) T cell (Tc), similar to their CD4\(^+\) Th1 and Th2-type counterparts, have been identified. Tc1 cells secrete predominantly IL-2 and IFN-\(\gamma\), and Tc2 cells secrete IL-4 and IL-5.\(^{12-14}\) As with CD4\(^+\) T cells, the presence of IL-4 in vitro favors the differentiation of CD8\(^+\) T cells toward Tc2-type immune response, resulting in production of IL-4 and IL-5, suppression of IFN-\(\gamma\) production, and facilitation in IgE synthesis.\(^{15,16}\) The presence of IL-12 and IFN-\(\gamma\) is reported to favor the generation of Th1 cells in CD4\(^+\) T cells. And the effect of these cytokines on CD8\(^+\) T cells exhibits strong similarities with that on CD4\(^+\) T cell.\(^{13,16}\)

IL-4 induces the production of IgE and also stimulates the expression of VCAM-1 on endothelial cells, resulting in increased binding of lymphocytes, monocytes, and especially eosinophils.\(^{17}\) IL-6 helps the production of IgE. IL-5, which was not evaluated in this study, is the most specific eosinophil growth and differentiation factor known.\(^{18}\)

Walker et al.\(^{19}\) compared patients with intrinsic asthma versus patients with extrinsic asthma. Patient with intrinsic asthma had normal levels of IgE and negative results of skin tests for Aeroallergens. Extrinsic asthma shows increased levels of IL-4 and IL-5 and low levels of IFN-\(\gamma\) and IL-2. However intrinsic asthma have elevated levels of IL-2, IFN-\(\gamma\) and IL-5 and low levels of IL-4.

Nasal polyp of non-allergic patients shows high levels of IFN-\(\gamma\), IL-2 and tissue eosinophilia similar to intrinsic asthma. If the level of IL-5 is high in nasal polyp of non-allergic patient, it would indicate a similar immunologic picture to intrinsic asthma.\(^{19}\) But there are controversies about IL-5 levels in nasal polyp.\(^{67}\)

Localized nasal allergy could be an etiologic factor for the development of nasal polyp. Cho et al.\(^{20}\) demonstrated local production of specific IgE in nasal polyp tissues. We found that cytokine productivity of lymphocytes in nasal polyp were parallel to that of peripheral blood lymphocytes. So it is likely that a certain systemic factor such as allergy or infection could be a cause of nasal polyp. In allergic patients, nasal polyp lymphocytes were predominantly Th2 type, whereas in non-allergic patients they were predominantly Th1 type in our study. But this cannot be the direct evidence that Th2 type lymphocytes in nasal polyp play a major role in the formation of nasal polyp in allergic patients.

**REFERENCES**


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