ORIGINAL ARTICLE

T Helper Cell Population and Eosinophilia in Nasal Polyps

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■ Abstract

Objective: To analyze the immunological pattern of nasal polyposis in patients with and without allergy, the percentages of CD4$^+$ cells expressing intracellular interferon-γ and interleukin-4 (T helper [T_H] type 1 and 2 cells) were measured by flow cytometry in samples from patients with nasal polyps.

Methods: Samples from 32 patients (16 atopic, 16 nonatopic) were studied. The fresh nasal polyp samples were prepared in single cell suspension for flow cytometry. Eosinophils were counted in hematoxylin-and-eosin–stained sections of all the samples.

Results: T_H1 cells were predominant in all the nasal polyps, with no significant differences in the mean (±SD) percentages of T_H1 cells between the 2 groups (46.28% ± 14.95% vs 38.25% ± 9.16%, $P >$ .05). The mean percentage of T_H2 cells in the polyps from the atopic patient group was significantly greater than in polyps from nonatopic group (7.34% ± 2.54% vs 0.63% ± 0.31%, respectively; $P <$ .01); the eosinophil count was significantly higher in atopic patient polyp samples (54.5 ± 15.76 eosinophils/HPF) than in nonatopic ones (14.38 ± 5.6 eosinophils/HPF, $P <$ .01). The mean percentage of T_H1 cell correlated with eosinophil count in the polyp samples overall (r = 0.80, $P <$ .01).

Conclusions: T_H1 cells were predominant in nasal polyp tissue. Polyps from atopic patients had more T_H2 cells and eosinophils than nonatopic patients' polyps did. Eosinophil recruitment in nasal polyposis is probably associated with T_H2 cell infiltration. Nonatopic and atopic patients' polyps have different immunological patterns.

Key words: Nasal polyps. T helper cells: T_H1, T_H2. Flow cytometry. Eosinophilia.

■ Resumen

Objetivo: Para analizar el patrón inmunológico de la poliposis nasal en pacientes con y sin alergia, se cuantificaron los porcentajes de células CD4$^+$ que expresaban el interferón-γ intracelular e interleucina 4 (linfocitos T cooperadores de tipo T_H1 de tipo T_H2) mediante citometría de flujo en muestras procedentes de pacientes con pólipos nasales.

Métodos: Se estudiaron muestras de 32 pacientes (16 atópicos y 16 no atópicos). Las muestras frescas de pólipos nasales se prepararon en una suspensión unicelular para la citometría de flujo. Se efectuó un recuento de los eosinófilos en secciones con tinción de eosina y hematoxilina de todas las muestras.

Resultados: Los linfocitos T_H1 predominaban en todos los pólipos nasales sin diferencias significativas en la media (± Desviación estándar) de porcentajes de los linfocitos T_H1 entre los dos grupos (46,28% ± 14,95% frente a 38,25% ± 9,16%, $P >$ 0,05). El porcentaje medio de linfocitos T_H2 en los pólipos de pacientes atópicos fue significativamente superior al porcentaje del grupo no atópico (7,34% ± 2,54% frente a 0,63% ± 0,31%, respectivamente; $P <$ 0,01); el recuento de eosinófilos fue significativamente superior en las muestras de pólipos de pacientes atópicos (54,5 ± 15,76 eosinófilos/campo de alta potencia) comparado con los no atópicos (14,38 ± 5,6 eosinófilos/campo de alta potencia, $P <$ 0,01). El porcentaje medio de linfocitos T_H2 se correlacionó con el recuento de eosinófilos de las muestras de pólipos en general (r = 0,80, $P <$ 0,01).

Conclusiones: Los linfocitos T_H1 predominaban en el tejido de los pólipos nasales. Los pólipos procedentes de pacientes atópicos tenían más linfocitos T_H2 y eosinófilos que los pólipos de no atópicos. El reclutamiento de eosinófilos en la poliposis nasal está asociado probablemente con la infiltración de linfocitos T_H2. Los pólipos de pacientes atópicos y de los no atópicos tienen distintos patrones inmunológicos.

Palabras clave: Pólipos nasales. Linfocitos T cooperadores: T_H1, T_H2. Citometría de flujo. Eosinofilia.

Introduction

Nasal polyposis remains a common clinical entity that originates in chronic inflammation of the lateral wall of the nose [1]. Inflammation triggers include bacterial, fungal and viral infections, allergy, and environmental pollution [1]. The large numbers of eosinophils and lymphocytes found in the lamina propria of nasal polyps may play a part in their pathogenesis [2]. Also relevant are T helper (T$_H$) type 1 and type 2 cells, which have a crucial role in balancing the immune response: T$_H$1 cells produce interferon-γ (IFN-γ), promoting cell-mediated immunity and control of intracellular pathogens, while T$_H$2 cells produce interleukin (IL) 4, which promotes allergic responses [3,4]. Studies of T cells in nasal polyps have been thus far limited to subpopulations, however, and have not looked at them in depth. There has been even less study of differential eosinophilic infiltration in nasal polyps in allergic and nonallergic patients.

To contribute data that might support pathologic classifications that could guide treatment of nasal polyposis, we used flow cytometry to measure the percentages of CD4$^+$ cells expressing intracellular IFN-γ and IL-4 (to reflect T$_H$1 cells and T$_H$2 cell populations, respectively) in tissue from 32 nasal polyposis patients with and without allergy.

Materials and Methods

Patients

Thirty-two patients (20 males, 12 females; 16 atopic, 16 nonatopic) between 23 and 82 years of age (49.33 ± 14.77 years) were included. The diagnosis of nasal polyposis was based on anterior rhinoscopy, nasal endoscopy, and paranasal sinus computed tomography. None of the patients had significant septal deviation. Classification according to atopic or nonatopic status was based on a medical history of allergy and skin testing. The atopic patients with polyps had positive skin prick tests to common aeroallergens (positive to at least 1 aeroallergen) and they had clinical signs consistent with allergic rhinitis. The nonatopic patients with polyps had negative skin prick tests to common aeroallergens and had no medical history or clinical signs of allergic rhinitis.

Tissue Preparation

Each polyp sample was divided into 2 parts. The first was used fresh for flow cytometry, suspended in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS), minced with surgical scissors, and strained through a nylon mesh (59 mm) in phosphate-buffered saline (PBS) (all chemicals, Sigma, St Louis, Missouri, USA). The cells were collected and centrifuged at 1500 rpm for 5 minutes and resuspended with 2 mL of DMEM. The second part of each sample was stained with hematoxylin and eosin (H/E) for eosinophil counting, the samples were fixed immediately in 4% paraformaldehyde in a 0.1-mol PBS solution, pH 7.4 at 4°C overnight, and embedded in paraffin. Sections were serially cut to 6 μm, mounted on slides and dried in an oven at 37°C overnight. After deparaffinization in xylene, the sections were hydrated through graded ethanol and standard H/E staining was carried out.

Flow Cytometry

Three-color flow cytometry for CD4, IL-4 and IFN-γ was performed with the fluorescent activated cell sorter flow cytometer (FACS Calibur, Becton Dickinson & Co, Franklin Lakes, New Jersey, USA) and CellQuest Software (Becton Dickinson). The T$_H$1 cells were defined by CD4$^+$ lymphocytes with intracellular IFN-γ but without intracellular IL-4. The T$_H$2 cells were defined by CD4$^+$ lymphocytes with intracellular IL-4 but not IFN-γ. Flow cytometry samples were stained according to a described technique, with slight modifications [5,6]. The cells in 2 mL of DMEM were stimulated with a combination of 25 ng/mL of phorbol myristate acetate and 1 mg/mL of ionomycin (Sigma) the presence of monensin (Sigma) and cultured for 4 hours at 37°C in a humidified 5% carbon dioxide incubator; activated cultures of samples were centrifuged at 1500 rpm for 5 minutes, washed with PBS, adjusted to 5×10^5 cells per test. The cell suspensions were divided into 2 parts. One part of each sample was stained with Cy-labeled anti-human CD4 monoclonal antibody (BD Pharmingen, Franklin Lakes, New Jersey, USA) and the other (control) part was stained with Cy-labeled mouse immunoglobulin (Ig) G monoclonal antibody (BD Pharmingen), then incubated for 30 minutes at room temperature in the dark. The cells were washed and fixed with 2% phosphate buffered paraformaldehyde for 20 minutes at room temperature. They were then washed and subsequently incubated with the fluorescence activated cell sorter permeabilizing solution, 1 mL of 0.1% saponin PBS for 10 minutes at room temperature in the dark. The samples were washed with PBS, and intracellular cytokines were stained with fluorescein isothiocyanate (FITC)-labeled anti-human IFN-γ monoclonal antibodies and phycoerythrin (PE)-labeled anti-human IL-4 monoclonal antibodies (Becton Dickinson). Controls were stained with FITC-labeled mouse IgG monoclonal antibody and PE-labeled mice IgG monoclonal antibody (Becton Dickinson); After 30 minutes' incubation, samples were centrifuged and washed with PBS. The cells were resuspended in 300 μL 1% paraformaldehyde passed through a nylon mesh (59 mm), and analyzed with the FACS Calibur. Nonspecific staining with the isotype-matched control monoclonal antibody was <1%.

Quantitative Assessment of Eosinophils

Sections stained with H/E were examined under a light microscope under low-power magnification ($\times 40$) to obtain a general impression of the histopathologic features of the examined specimens. Eosinophils in the subepithelial connective tissue were counted according to a modification of the method of Ruffoli et al [7]. The counting was carried out at $\times 400$ magnification. The visual field was oriented along the whole length of the epithelium basement membrane. To yield the mean number of eosinophils per high power field (HPF) of subepithelial connective tissue, 10 randomly chosen HPFs of a single section were examined. The eosinophils in each

section were counted, and the average number of eosinophils per HPF was calculated.

Statistical Analysis

The mean percentages of T_H1 and T_H2 cells, and the average number of eosinophils were expressed as mean ± SD. Using SPSS for Windows, Ver 11.5 (Chicago, Illinois, USA), the mean percentages of T_H1 and T_H2 cells, and the average numbers of eosinophils in the 2 patient groups were compared with a t test for independent samples. The correlation between the mean percentages of the T_H2 cells and average number of eosinophils overall were analyzed by bivariate correlations (Pearson correlation coefficients). A value of P less than .05 was considered statistically significant.

Results

Mean Percentages of Cytokine-Producing T_H Cells in Polyps

The nasal polyps possessed both T_H1 and T_H2 cells, with a predominance of T_H1 cells in all polyps. Statistical analysis demonstrated that there were no significant differences in the mean percentages of IFN-γ–producing T_H1 cells between the 2 groups (46.28% ± 14.95% vs 38.25% ± 9.16%, respectively; $P > .05$). The mean percentages of IL-4–producing T_H2 cells were significantly higher in atopic patients' polyps than in nonatopic ones (7.34% ± 2.54% vs 0.63% ± 0.31%, respectively; $P < .01$) (Figure 1). In nonatopic patients' polyps, a T_H1-polarized phenotype was more apparent (Figure 2b), whereas in atopic polyps, both T_H1 and T_H2 cell percentages were high (Figure 2c). In general, CD4+ cells produced more IFN-γ than IL-4 in nonatopic patients, whereas CD4+ cells produced both IFN-γ and IL-4 in atopic patients.

Eosinophil Counts

Examination of H/E-stained sections of the nasal polyps revealed that eosinophil counts were significantly higher in atopic patients' polyps compared with nonatopic ones (54.5 ± 15.76 vs 14.38 ± 5.6 eosinophils/HPF,

respectively; $P < .01$) (Figure 3). In atopic patients' polyps, the subepithelial connective tissue was infiltrated by numerous eosinophils, chiefly located around the blood vessels (Figure 4). In nonatopic patients' polyps, the eosinophil infiltration was scarce (Figure 5). The mean percentages of IL-4-producing T_H2 cells correlated with the eosinophil count ($r = 0.80$, $P < .01$).

Discussion

Staphylococcus aureus and fungi are the most common organisms isolated from the mucus adjacent to massive nasal polyps [8], but allergy is also generally believed to be an underlying cause of their development [9] in a process that will involve the T_H2 cells as well as eosinophils. Using flow cytometry to detect T_H1 and T_H2 cell populations in atopic and nonatopic patients' nasal polyps, we found that T_H1 cells were abundant in all the samples and that atopic patients' polyps had more T_H2 cells than the nonatopic patients' did. This pattern is consistent with a study of chemokine receptors in mucosa from ethmoidal sinuses by Elhini et al [10] in which samples from atopic patients showed high expression of CCR4+ T_H2 cells. These findings suggest that atopic and nonatopic patients' polyps have certain differences in immune response and pathogenesis. While T_H1 cells seem to play a role in the pathogenesis of the nonatopic polyps, both types of T_H cell apparently play a role in the pathogenesis of the atopic polyps, involving a more complicated immune process. In distinguishing atopic from nonatopic polyposis, it should therefore be possible to study the T_H cell populations, which will reflect the genuine status of immune response. For example, Bernstein et al [11] found that there were significant differences between lymphocyte subpopulations in nasal polyps and peripheral blood in these different patients, consistent with our hypothesis. The nasal polyp lymphocyte subpopulation may be derived from both the local mucosal immune system and the peripheral blood through migration of lymphocytes.

Our findings also showed that eosinophils were significantly more abundant in atopic patients' polyps, consistent with the study of Gosepath et al [12]. Eosinophilic granulocytes are known to carry a variety of cytotoxic proteins, which can be released by degranulation. These proteins include the
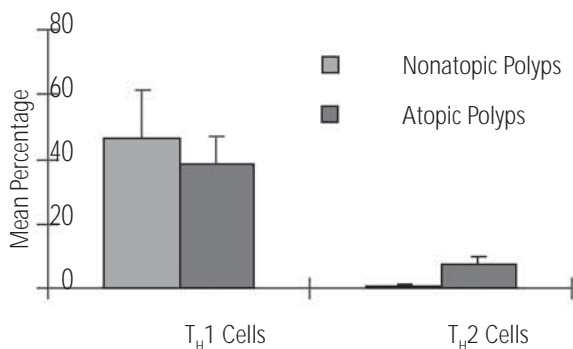
Figure 1. The mean percentage of T helper type 1 (T_H1) cells was similar in the 2 patient groups ($P > .05$). The mean percentage of T_H2 cells was significantly higher in atopic patients' polyps than that in nonatopic ones ($P < .01$).
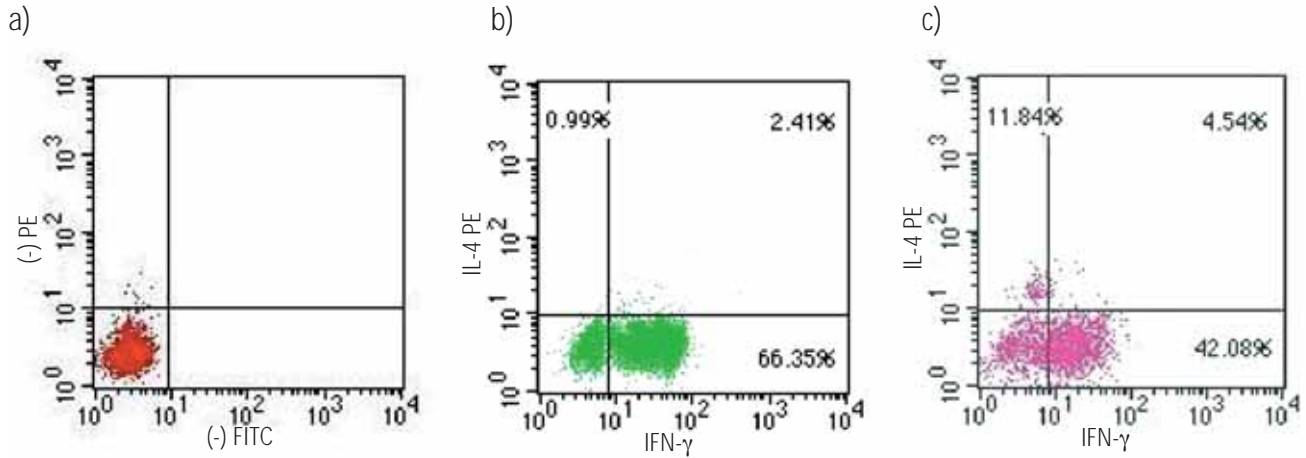
Figure 2. Flow cytometry graph of T helper (T$_H$) cells in nasal polyps. a) In the part of the cell suspension used as the control there are no T$_H$1 or T$_H$2 cells; b) in nonatopic patients' polyps, the T$_H$1-polarized phenotype is more apparent: the percentage of T$_H$1 cells is very high and the percentage of T$_H$2 cells very low (often less than 1%); c) in atopic polyps, T$_H$1 and T$_H$2 polarization is not apparent: T$_H$1 cells are abundant but the percentage of T$_H$2 cells is also high. FITC indicates fluorescein-isothiocyanate; IFN, interferon.
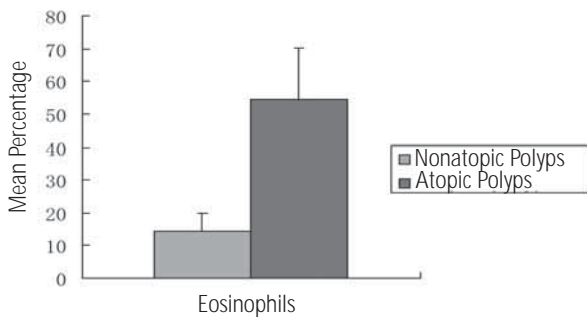

Figure 3. The eosinophil count was significantly higher in the polyp tissue samples from atopic patients than in samples from nonatopic ones ($P < .01$).
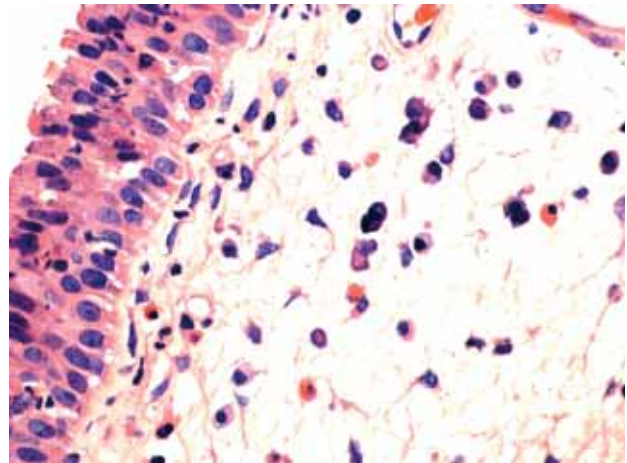

Figure 5. In nonatopic patients' polyps, eosinophil infiltration was scarce. Hematoxylin and eosin staining, $\times$ 400 magnification.
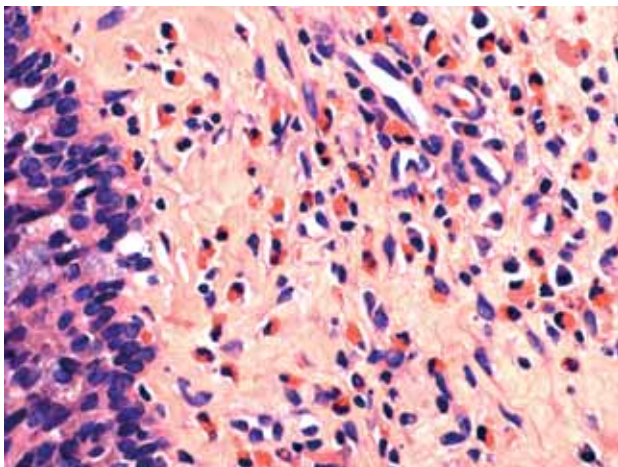

Figure 4. In atopic patients' polyps, the subepithelial connective tissue was infiltrated by numerous eosinophils. Hematoxylin and eosin staining, $\times$ 400 magnification.

eosinophilic cationic protein, major basic protein, eosinophilic peroxidase and eosinophil-derived neurotoxin. These findings indicate that the cytotoxicity of the eosinophils may be a crucial element of an immunological reaction against allergen and fungi present in the nasal mucosa.

The mean percentages of T$_H$2 cells correlated with eosinophilia in the nasal polys we studied, suggesting that eosinophilia is probably associated with these IL-4–producing cells in the pathogenic process. When T$_H$2 cells are stimulated to produce cytokines, the result is migration of eosinophils from blood to tissue, causing the upregulation and increased survival of eosinophils in the nasal polyps.

In summary, T$_H$1 cells were abundant in all the nasal polyps we studied, but atopic patients' polyps had more T$_H$2 cells and eosinophils than the nonatopic patients' polyps did. Eosinophil

recruitment in nasal polyposis is probably associated with T_H2 cell infiltration. Nonatopic and atopic patients have polyps arising from different immunological patterns.

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