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Leukocyte compartments in the nasal secretion medium*

R. Jankowski[1], L. Coffinet[1], H. Audouy[1], B. Foliguet[2]

[1] ENT Department, Central Hospital, Henri Poincare University, Nancy, France
[2] Laboratoire de Biologie Sexuelle, Maternité Régionale, Nancy, France

SUMMARY

Nasal secretions are known to play a role in respiratory tract and host defense. Besides the mucociliary transport and biochemical properties of the mucus, we hypothesize the role of a secretory leukocyte compartment. We designed a time-series study to count leukocytes in baseline, physically- and pharmacologically-induced secretions. Twenty-three healthy volunteers and 29 patients participated in the study. In healthy subjects, secretion weights significantly increased from 24±5 mg (mean±SEM) at baseline to 35±6 mg and 115±12 mg, respectively, in physically and methacholine-induced secretions ($p < 0.05$). The leukocyte count did not change between baseline (14,445±5,010) and physically induced secretions (13,396±6,8401), but significantly increased after methacholine (28,140±11,411; $p=0.02$). Leukocyte differential counts showed, moreover, an increase of lymphocytes in the methacholine-induced compartment. In patients, secretion weights significantly increased from 70±12 mg at baseline to 117±22 mg and 223±28 mg, respectively. The leukocyte count significantly increased between baseline (172,109±95,890) and physically induced secretions (410,503±318,224; $p=0.02$), but decreased after methacholine (112,774±54,860). These data argue for the existence of a secretory leukocyte compartment, with a subcompartment of surface and an intraglandular subcompartment of reserve, the kinetics of which are different in patients and control subjects.

Key words: nasal secretion, cytology, leukocytes, respiratory tract defense

INTRODUCTION

Secretions that physiologically cover the nasal epithelium represent the first line of defense of the nose (Kaliner, 1991). The mucociliary system has a high capacity to remove inhaled micro-organisms, allergens and environmental particles that are trapped into the superficial gel layer.

Biochemical characteristics of the nasal mucus have been well studied, high-lighting for instance the role of secretory IgA and interferon in protection against micro-organisms. Surprisingly, only few studies have focussed on cellular mechanisms that could improve the efficiency of this first line of defense. Recent attention has been paid to possible inflammatory (Churchill and Proud, 1990) or immune (Hellquist et al., 1991) functions of epithelial cells. Although leukocytes are known to be present in small number in healthy secretions, no description of this leukocyte compartment is found in the literature.

Aims of this study were: (1) to collect baseline secretions in healthy volunteers for numeration and differential leukocyte counts; (2) to increase available secretions by spraying into the nose a glandular cholinoceptor agonist (methacholine); and (3) to observe the modifications in the leukocyte compartment.

We found that methacholine-induced leukocyte compartment seems to be quantitatively and qualitatively different from baseline compartment. The same protocol applied to patients with chronic nose complaints showed, moreover, a completely different pattern.

PATIENTS AND METHODS

Study design

The following criteria were used to select healthy volunteers: (1) no past history of medical or surgical disease of the nose; (2) no acute or chronic nose symptoms; (3) no allergic symptoms or past history (hay fever or periodic rhinitis, asthma, eczema, urticaria, food allergy); (4) no use of local drugs; (5) no intake of steroids or anti-inflammatories; (6) smoking habits less than 10 cigarettes per day.

Between May and July 1993, 23 healthy volunteers participated in the study (14 females/9 males; mean age: 28 years, range:

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22-43 years). During the same period, we also selected for the nasal secretion study 29 patients: 15 with nasal polyposis and 14 with chronic rhinosinusitis (10 females/19 males; mean age: 45 years, range: 19-73 years). All subjects gave informed consent before entry, and the study was approved by the Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale de Nancy.

The study was a time-series design. The experiment was performed unilaterally. Baseline secretions were collected by gently suctioning the nasal mucosa for 30 s. Ten minutes later placebo was sprayed, and after 3 min post-placebo secretions were suctioned. Ten minutes later methacholine was sprayed, and after 3 min post-methacholine secretions were suctioned out. Methacholine (Laboratoires Aldrich, St. Quentin Falavier, France) at a concentration of 15 mg/ml, and placebo (normal saline solution used as diluent for the preparation of methacholine solution) were delivered by a metered-pump spray (VP3 pump, Laboratoire Valois, Le Neubourg, France). Two doses of 0.1 ml each were sprayed to deliver a total dose of 1.5 mg methacholine. In healthy subjects, a 10-min period was long enough for reflex hypersecretion following the suctioning of the leukocyte compartments in the nasal secretion medium mucosa, considered as a physical stimulus, to come back to baseline level. The methacholine-induced secretion peak was observed at 3 min (preliminary data).

Nasal secretion collection

Subjects were first asked to blow their nose and to take a semi-seated position on an examination table. Four to 5 cm of a suctioning glass canula (Ets Descharmes, Nancy, France) were gently introduced under direct vision into the nose, along the nasal septum onto the floor, and then swept across the inferior and middle meatus as it was brought out. The suctioning depression was maintained at -100 Pa via a manometer. The suctioning time was 30 s. Any blood contamination led to stop the experiment. Each canula was preweighed (Precisa 160M, Pag Oberlikon AG, Switzerland). The weight of secretions was calculated by subtracting the pre- from the post-collection weights.

The canula was then sealed with a silicone plug at one end and filled with 2 ml of saline, in which a small amount of a mucolytic agent, bromelian (Sigma Chemical, St Quentin Falavier, France), was dissolved. After sealing, the canula was refrigerated until processing within 1-24 h of collection.

Cell processing

The sample was transferred to a tube, followed by a 2-ml saline rinse of the canula, and vigorously shaken to obtain a homogenized suspension. One millilitre of this suspension was transferred to a centrifuge (2,000*g* for 30 min). Supernatant (900 µl) was discarded. The cell pellet was resuspended in the remaining 100 µl.

For the cell count, 10 µl of the final suspension were placed in a Thoma hemocytometer. After 5 min, the number of leukocytes was counted. This number, multiplied by the dilution factor, gave the total number of leukocytes in the starting suspension, i.e., in the amount of collected secretions. The instrumental reproducibility of this measure, tested on 11 samples, showed a coefficient of variation of 28%. Differential counts were performed on cytospin slides. On the basis of total cell count, 200-500 µl of the starting suspension were centrifuged at 1,250*g* for 30 min. Slides were stained with May-Grünwald-Giemsa. We counted 100 leukocytes on each slide and categorized them as neutrophils, eosinophils, basophils, lymphocytes and monocytes, a category that included true monocytes and mononuclear cells of unclear origin. Results were expressed as percentages. Epithelial cells were not counted. The differential count was considered valid only if more than 500 leukocytes were counted in the secretions and when cells morphology did not lead to misidentification. The reader was blinded as to whether the samples were pre- or post-methacholine or diluent. Intra- and inter-observer reproducibility were studied on 17 slides, after blinding and randomization, and showed excellent correlations (r^2 >85% for neutrophil, eosinophil and lymphocyte identification with slopes between 0.98 and 1.1 and intercepts between -4.2 and 1.6 on a y-scale of 100%).

Statistical analysis

Data are presented as mean±standard error of the mean (SEM). Non-parametric statistics were applied. Friedman two-way analysis of variance was performed to test the within-group increase of either secretion weights or leukocyte counts. Post-hoc analysis employed the Wilcoxon signed-rank sum test for paired data. Comparisons between healthy volunteers and patients data were performed using the Kruskal-Wallis one-way analysis of variance and the Mann-Whitney U-test for independent groups.

RESULTS

Healthy subjects

The mean secretion weight at baseline was 24±5 mg. Post-diluent and post-methacholine secretion weights significantly increased to 35±6 mg (p=0.04) and 115±12 mg (p=0.001), respectively, that is 1.5- and 4.8 times, respectively, the baseline weights (Figure 1A).

The mean number of leukocytes counted in baseline secretions was 14,445±5,010. No changes were found after diluent (13,396±6,840 leukocytes). The number of leukocytes significantly increased to 28,140±11,411 after methacholine (p=0.02 over diluent, p=0.08 over baseline; Figure 1B).

The differential count was considered valid on only 10/23 baseline, 10/23 post-diluent, but on 20/23 post-methacholine samples. Baseline counts showed 99.6±0.2% neutrophils, 0.3±0.2% eosinophils, no basophils, no lymphocytes, and 0.1±0.1% monocytes. No significant changes were observed after diluent. Counts of the methacholine-induced compartment appeared, however, different. Lymphocytes increased to 3.6±1.7%. Neutrophils decreased to 92±3.7%, but no changes were observed in eosinophils (4±3.3%), basophils (0%) and monocytes (0.6±0.4%). It is to be noted than one subject dramatically increased his eosinophils from 0% at baseline to 91% after diluent and 66% after methacholine, while none of the 22 other subjects showed values above 2% after diluent and above 11% after methacholine. By comparing only the seven patients who had a complete count for each step of the experiment we found that the lym-

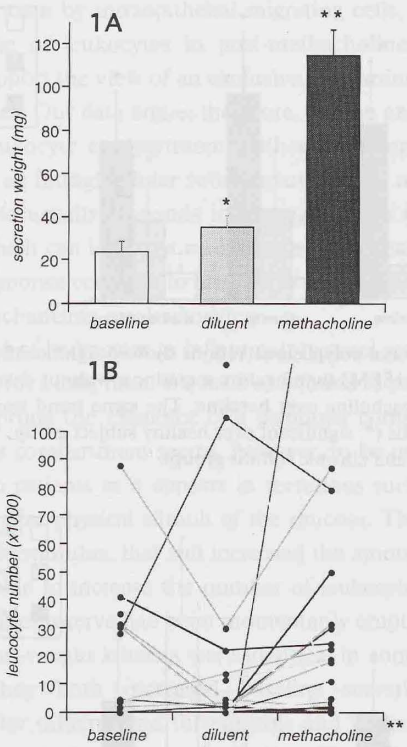

Figure 1. Methacholine significantly increased secretion weights (1A, mean±SEM) and leukocyte numbers (1B, individual leukocyte numbers) in healthy volunteers (n=23; *: significant ($p < 0.05$) over baseline; ** significant over diluent).

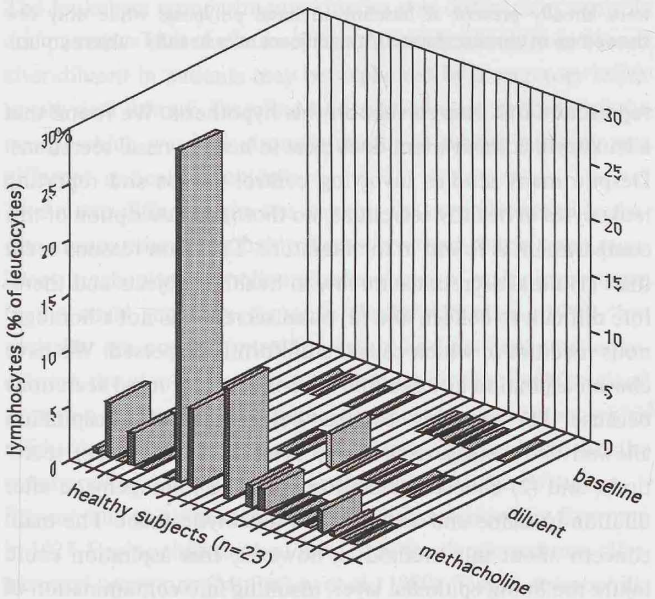

Figure 2. Individual lymphocyte increase in the methacholine-induced compartment of healthy subjects.

phocyte increase was close to significance (Friedman p=0.07; Wilcoxon p=0.04; Figure 2).

Patients

The mean secretion weight at baseline was 70±12 mg. Post-diluent and post-methacholine secretion weights significantly increased to 117±22 mg (p=0.005) and 223±28 mg (p=0.0001), respectively, that is 1.7- and 3.1 times, respectively the baseline weights (Figure 3A). This significant increase of secretion

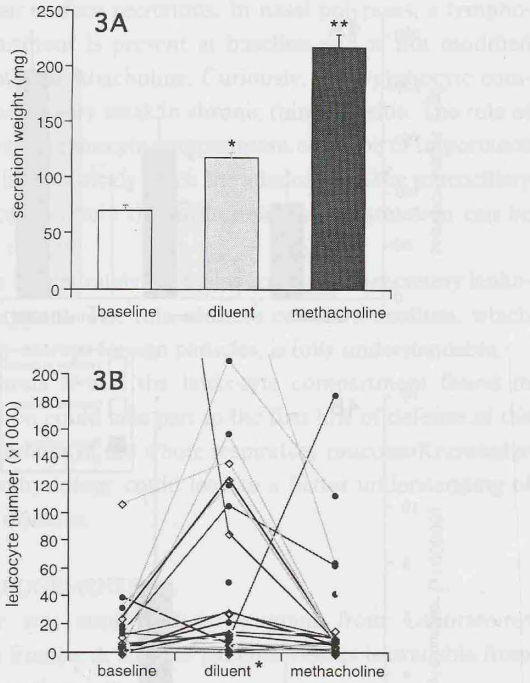

Figure 3. Methacholine significantly increased secretion weights (1A, mean±SEM), but not leukocyte numbers (IB, individual leukocyte numbers) in patients with chronic rhinosinusitis (n=29; *: significant over baseline; ** significant over diluent; diamonds: chronic rhinitis (n=14); filled circles: nasal polyposis [n=15]).

weights was also observed in both the chronic sinusitis and nasal polyposis subgroups ($p < 0.05$).

The mean number of leukocytes counted in baseline secretions was 172,109±95,890. The number of leukocytes significantly increased to 410,503±318,224 after diluent (p=0.02), but decreased after methacholine to 112,774±54,860 (p=0.09; Figure 3B). The same trend was observed in both the chronic sinusitis and nasal polyposis subgroups (Figure 3B). The differential count was considered valid in the same proportion of cases on baseline (20/29), post-diluent (20/29), and post-methacholine samples (24/29). No significant changes of baseline counts were observed, neither after diluent nor methacholine. In chronic sinusitis, baseline counts showed 94.4±2.9% neutrophils, 5.3±3% eosinophils, no basophils, 0.2±0.1% lymphocytes, and 0.1±0.1% monocytes. Baseline counts in nasal polyposis was, however, different with 70.1±10.4% neutrophils, 27.8±10% eosinophils (p=0.03), no basophils, 1.6±0.9% lymphocytes, and 0.5±0.4% monocytes.

Comparison between healthy subjects and patients

Rhinosinusitis and polyposis patients had significantly more secretions (Figure 4A) than healthy subjects at baseline ($p < 0.009$), after diluent ($p < 0.004$), and after methacholine ($p < 0.02$). There was a trend for nasal polyps to have more secretions than chronic rhinosinusitis at baseline (p=0.09) and after diluent (p=0.06), but no difference was observed after methacholine.

Patients had significantly higher number of leukocytes (Figure 4B) than healthy subjects only after diluent (p=0.004). The same trend was observed between nasal polyposis and healthy subjects (p=0.003), and between chronic sinusitis and healthy sub-

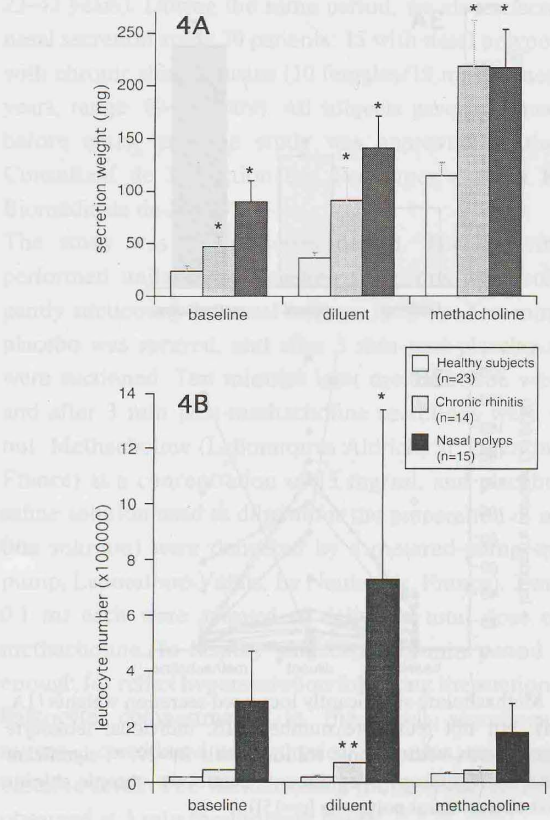

Figure 4. Comparison between healthy subjects and patients. 4A: Both rhinitis and polyposis patients showed significantly higher secretion weights (mean±SEM) than healthy subjects at baseline, after diluent, and after methacholine. 4B: Patients showed significantly higher number of leukocytes (mean±SEM) only after diluent (*: significant over healthy subject group; **: p=0.076 over healthy subject group).

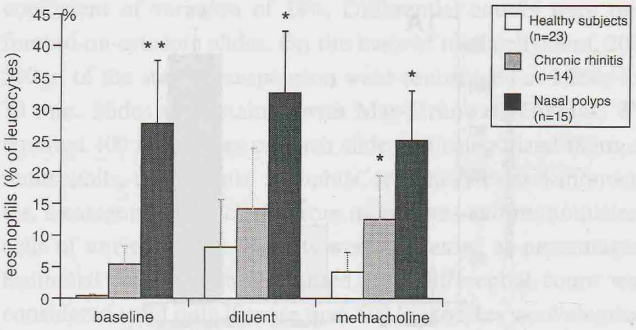

Figure 5. Nasal polyposis secretions showed significantly more eosinophils (mean±SEM) than healthy secretions, without changes after diluent or methacholine over baseline. The same trend was observed in chronic rhinitis (*: significant over healthy subject group; **: significant over healthy and chronic rhinitis group).

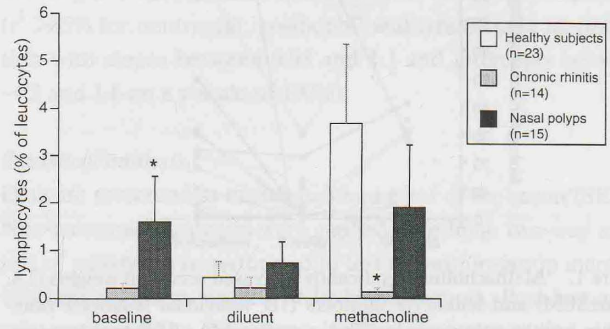

Figure 6. Methacholine-induced leukocyte compartment was only observed in healthy secretions. In contrast, lymphocytes (mean±SEM) were already present at baseline in nasal polyposis while only few showed up in chronic rhinitis (*: significant over healthy subject group).

jects (p=0.07). At baseline, there was a trend for patients to have more leukocytes than controls (p=0.10). After methacholine, these differences disappeared. Eosinophils (Figure 5) were significantly more numerous in nasal polyposis than in control secretion ($p < 0.004$) without changes after diluent or methacholine over baseline. There was a trend ($p < 0.14$) for eosinophils to be more numerous in chronic rhinosinusitis than in healthy subjects, this difference being statistically significant only after methacholine (p=0.003). The same trend ($p < 0.13$) was observed in nasal polyposis over chronic rhinosinusitis, the difference being statistically significant only at baseline (p=0.03). Neither methacholine nor diluent changed the trend observed at baseline.

Lymphocytes (Figure 6) were significantly more numerous in nasal polyposis than in healthy subject secretions at baseline (p=0.02). Methacholine did not modify the lymphocyte compartment in nasal polyposis but lead to a significant increase in healthy subjects. No inter-group differences were noted after diluent. The methacholine-induced lymphocyte compartment was significantly less numerous in chronic sinusitis than in healthy subjects (p=0.02). Methacholine showed no effect on the lymphocyte compartment in chronic sinusitis.

DISCUSSION

Our hypothesis is that the nasal secretion medium contains a leukocyte compartment which plays an active role in the mechanisms of defense of the nose. Results reported in this paper represent a first step to support this hypothesis. We found that a leukocyte compartment does exist in healthy nasal secretions. Despite many studies involving control groups and reporting leukocytes in healthy secretions, no thorough description of this compartment is found in the literature. The main reasons seem that: (1) nasal secretions are few in healthy subjects and therefore difficult to collect, and (2) nasal secretion is not a homogenous medium in which cells are uniformly dispersed. We have chosen aspiration as a method for collection of nasal secretions because: (1) weighing canulas before and after aspiration allowed us to measure precisely even small amount of secretions, and (2) collected secretion could be homogenized after dilution in saline and addition of a mucolytic agent. The main concern about this method is, however, that aspiration could injure the lining epithelial layer, resulting into contamination of specimen by the intraepithelial migrating cells. We have hypothesized that, if a leukocyte compartment is involved in the secretory mechanisms of defense of the nose, gland secretion should participate in its renewal. It has previously been demonstrated that methacholine induces nasal glandular, but not vascular, secretion (Raphael et al., 1988). In our experiment, methacholine has three actions on the leukocyte compartment collected at baseline: (1) it increases the number of leukocytes; (2) it brings out cells of better shape, which are easier to identify under the microscope; and (3) it reveals the presence of a secretory lymphocyte compartment. If leukocytes found in baseline and post-diluent secretions could represent contamina-

tion of specimen by intraepithelial migrating cells, the significant increase of leukocytes in post-methacholine secretions does not support the view of an exclusive contamination origin for leukocytes. Our data argue, therefore, for the existence of a secretory leukocyte compartment, with a subcompartment of surface and an intraglandular subcompartment of reserve. The nasal mucosa usually responds to any aggression by a hypersecretion which can lead to a runny nose. The meaning of this secretory response could be to bring in first-line major actors of immune mechanisms, i.e. leukocytes.

The presence of leukocytes in inflammatory nasal secretions has been known for a long time. We have also found in patients arguments supporting the existence of a glandular compartment of reserve. This compartment seems, however, to be more quickly mobilized in patients as it appears in secretions suctioned after diluent, i.e. after physical stimuli of the mucosa. The following methacholine stimulus, that still increased the amount of secretions, is unable to increase the number of leukocytes, as if the compartment of reserve had been momentarily empty.

The secretion-weight kinetics were identical in controls and in patients. They both increased baseline secretion weight 1.5 times after diluent, and for controls and patients 4.8- and 3 times, respectively, after methacholine. The only difference between the two groups was the significantly higher amount of secretions collected in patients. This difference existed at baseline and remained after diluent and methacholine.

The leukocyte compartments kinetics was different in controls and patients. That the leukocyte number significantly increased after diluent in patients may be explained by a secretory reflex to physical stimuli (baseline and post-diluent probing of the nose), which was not strong enough, or whose pathway was different, in healthy subjects.

These two different kinetics suggest that secretion and leukocyte compartments probably do not respond to the same regulatory mechanisms. Cholinergic signals are of major importance in the nasal secretory response (Jankowski et al., 1993), but probably are not in controlling influx and residence of leukocytes in the glands' lumen. Any stimulation that leads to gland secretion brings out a variable number of leukocytes, which might depend on the number of glands involved and on the number of leukocytes that were staying in the glands' lumen.

Eosinophils in nasal secretion were first described by Eyerman in 1927. Eosinophils can be found in both allergic and non-allergic nasal secretions (Mullarkey et al., 1980). Tissue eosinophilia is a characteristic of nasal polyposis (Jankowski et al., 1989). Our data confirm that eosinophils seem to be more numerous in diseased than in healthy secretions, with very high numbers in nasal polyposis secretion. The functions of this cell are still unclear. Recent knowledge suggests that eosinophils could physiologically be involved in mucosal immunity, but that some of the mechanisms employed by eosinophils in host defense could prove detrimental to the host (Weller, 1991). Little attention has been paid to lymphocytes in nasal secretion, in contrast to intraepithelial (Hellquist et al., 1991; Okuda and Pawankar, 1992) or to tissue lymphocytes (Hameleers et al., 1989). Healthy subjects seem to be able to bring out a lymphocyte compartment in their surface secretions. In nasal polyposis, a lymphocyte compartment is present at baseline and is not modified after diluent or methacholine. Curiously, the lymphocyte compartment seems very weak in chronic rhinosinusitis. The role of this secretory lymphocyte compartment could be of importance as these cells can quickly reach the adenoids via the mucociliary transport route, where the immunological information can be thoroughly treated.

Neutrophils are definitely the major actors of the secretory leukocyte compartment. The role of these cells in a medium, which permanently entraps foreign particles, is fully understandable.

Our hypothesis is that the leukocyte compartment found in nasal secretion could take part to the first line of defense of the nose, and perhaps of the whole respiratory mucosa. Knowledge of its pathophysiology could lead to a better understanding of respiratory diseases.

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REFERENCES

1. Churchil L, Proud D (1990) Response of respiratory epithelial cells to inflammatory stimuli. Am J Rhinology 4: 87–90.
2. Hameleers D, Stoop A, Van der Ven I, Biewenga J, Van der Baan S, Sminia T (1989) Intra-epithelial lymphocytes and non-lymphoid cells in the human nasal mucosa. Int Arch Allergy Appl Immunol 88: 317–322.
3. Hellquist H, Olsen K, Irander K, Karlsson E, Odkvist L (1991) Langerhans cells and subsets of lymphocytes in the nasal mucosa. APMIS 99: 449–454.
4. Jankowski R, Bene MC, Moneret-Vautrin DA, Haas F, Faure G, Simon C, Wayoff M (1989) Immunohistologic characteristics of nasal polyps. A comparison with healthy mucosa and chronic sinusitis. Rhinology Suppl 8: 51–58.
5. Jankowski R, Philip G, Togias A, Naclerio R (1993) Demonstration of bilateral cholinergic secretory response after unilateral nasal cold, dry air challenge. Rhinology 31: 97–100.
6. Kaliner M (1991) Human nasal respiratory secretions and host defense. Am Rev Respir Dis 144: 852–856.
7. Mullarkey M, Hill J, Webb D (1980) Allergic and non- allergic rhinitis: Their characterization with attention to the meaning of nasal eosinophilia. J Allerg Clin Immunol 65: 122–126.
8. Okuda M, Pawankar R (1992) Flow cytometric analysis of intra-epithelial lymphocytes in the human nasal mucosa. Allergy 47: 255–259.
9. Raphael G, Druce H, Baraniuk J, Kaliner M (1988) The pathophysiology of rhinitis. I. Assessment of the sources of protein in methacholine-induced nasal secretions. Am Rev Respir Dis 138: 413–420.
10. Weller P (1991) The immunobiology of eosinophils. N Engl J Med 324: 1110–1118.

Prof. Roger Jankowski
ENT Department
Central Hospital
29 Avenue de Lattre de Tassigny
F-54000 Nancy
France