

Original article

Decreased apoptosis and distinct profile of infiltrating cells in the nasal polyps of patients with aspirin hypersensitivity

Background: Patients with aspirin-hypersensitive rhinosinusitis/asthma suffer from a severe form of hyperplastic rhinosinusitis with recurrent polyposis. We aimed to assess the presence of apoptotic cells in nasal polyps from aspirin-hypersensitive (AH) and aspirin-tolerant (AT) patients with rhinosinusitis as related to the characteristics of local inflammation.

Methods: Nasal polyps obtained from 16 AH patients and 36 AT patients (17 atopic and 19 nonatopic) were stained for eosinophils and metachromatic cells, and in parallel immunocytochemistry was performed to detect CD45RO+, HLA-DR+, CD8+ and CD68+ positive cells. Apoptotic cells were detected by a nick-end labelling technique, TUNEL.

Results: The density of apoptotic cells in AH polyps (5.5 ± 1.5 cells/mm²) was significantly lower as compared to both atopic (18.7 ± 3.8 cells/mm²; $P < 0.02$;) and nonatopic (21.3 ± 5.2 cells/mm²; $P < 0.01$) AT polyps. The number of eosinophils, mast cells, and CD45RO+ cells were significantly increased in AH compared to AT polyps ($P < 0.001$), and the density of HLA-DR+ cells in AH patients was higher than in nonatopic ($P < 0.02$), but not in atopic AT patients. While in AH patients the duration of rhinosinusitis correlated inversely with the number of apoptotic cells ($r = -0.67$; $P < 0.04$), in contrast, in AT atopic patients the duration of rhinosinusitis showed positive correlation with apoptosis ($r = 0.89$; $P < 0.003$).

Conclusions: We conclude, that decreased apoptosis of inflammatory cells in nasal polyps from ASA-hypersensitive patients, reflects a distinct mechanisms of local inflammation and may be related to persistence and severity of the disease in these patients.

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Aspirin hypersensitivity associated with bronchial asthma and nasal polyposis have been widely recognized as a clinical entity, "aspirin triad", with prevalence of between 10 and 20% in the asthmatic population (1-3). Nasal and sinus symptoms are the dominant complaints in the majority patients with aspirin-triad, and there is also evidence that chronic rhinosinusitis aggravates the course of asthma in these patients (4, 5). The incidence of sinusitis by roentgenogram in aspirin-hypersensitive (AH) asthmatics is up to 96.2%, and the frequency of nasal polyps may be as high as 70% (6-9). Rhinosinusitis usually has a protracted course, and the nasal polyp recurrency rate is almost three times higher in AH patients than in aspirin-tolerant (AT) intrinsic asthmatics, and seven times higher than atopic asthmatics (10). Polypoid hypertrophy of the mucosa is not limited to the nasal cavity but usually involves all sinuses. Using CT scans and a semiquantitative scoring scale we have recently demonstrated that the extent of sinus mucosal hypertrophy is significantly higher in AH compared to AT patients (11).

The mechanism underlying the higher severity of rhinosinusitis in AH patients remains unclear, but may be related to basic pathomechanisms of aspirin hypersensitivity. Aspirin hypersensitivity, also involving intolerance of other NSAIDs, is not an IgE-mediated reaction but is related to abnormal metabolism of arachidonic acid (12). Inhibition by aspirin of cyclo-oxygenase, and subsequent release of leukotrienes and other mast cell and eosinophil derived mediators, seems to be crucial for induction of nasal and bronchial symptoms (13-20). Some baseline abnormalities of AA metabolism in nonstimulated nasal polyps from ASA-hypersensitive patients have been also reported, including decrease in PGE₂ production and down-regulation of COX-2 mRNA expression (21-24). Nasal polyp epithelial cells from AH patients may be specifically triggered by aspirin to release 15-HETE which is a potent proinflammatory eicosanoid and may also regulate cell survival (22, 25). However, it is not clear if the pathogenesis of chronic mucosal inflammation involving both the upper and lower airways in ASA-

hypersensitive patients is also related to AA metabolism abnormalities or involves immunological mechanisms (26). Recent studies have shown that not only mast cells and eosinophils, but also T cells are increased in number in the nasal polyps of ASA-hypersensitive patients, and a distinct profile of cytokine expression has been reported (27, 28).

Current understanding of the mechanism of increased accumulation of inflammatory cells involves both enhanced inflammatory cell recruitment (migration) and prolonged survival of cells at the site of inflammation (29). Due to their abundance in nasal polyps, eosinophils seem to be the primary inflammatory cells regulating and perpetuating inflammation (30). Several cytokines (e.g. IL-5, RANTES, GM-CSF, eotaxin), as well as overexpression of VCAM-1 on microvascular endothelium, have been demonstrated in nasal polyp tissue, suggesting some role in the pathogenesis of the eosinophilia (28, 31). Recently delayed programmed cell death (apoptosis) has been implicated in development of nasal polyps, although in these studies the relationship of the apoptosis to patients' characteristics has not been addressed (32-34).

The goal of this study was to elucidate possible involvement of delayed apoptosis in the pathogenesis of nasal polyps, and to assess its relation to cellular infiltration, to markers of inflammation, and to clinical characteristics of aspirin-sensitive and aspirin-tolerant patients. We found that the density of apoptotic cells was significantly decreased in nasal polyps from AH patients as compared to AT patients. Different densities of eosinophils, mast cells, and CD45RO+ cells, as well as a distinct pattern of correlation between clinical features and immunological markers found in AH and AT patients, provide evidence for different mechanisms involved in the persistence of mucosal inflammation.

Material and methods

Study subjects

Nasal polyps were obtained from 16 ASA-hypersensitive (AH) patients (12 atopic and four nonatopic), and 36 ASA-tolerant patients (17 atopic and 19 nonatopic) undergoing elective nasal surgery unrelated to the goals of this study. ASA hypersensitivity was confirmed by oral or inhaled lysine-aspirin challenge, and was excluded in the control group by unequivocal history of good tolerance to aspirin according to methods described previously (15, 16). Atopy was defined by a positive personal history of allergic respiratory symptoms and positive skin prick tests (skin prick test

wheel >3 mm) to the panel of inhalant allergens. Atopic patients with seasonal sensitizations were studied out of the season and patients with perennial allergy during periods of limited exposure to relevant antigens. Nasal or oral steroids and nasal sodium cromoglycate were withdrawn at least four weeks prior to polypectomy. The study was approved by the local ethical committee and informed consent was obtained from all subjects. Patient characteristics are shown in Table 1.

Tissue fixation and staining

Each tissue specimen was cut into pieces about 5 mm thick. One piece was fixed in 10% buffered formalin and another in Carnoy's fluid. After fixation, tissue specimens were embedded in paraffin, cut precisely at 4 µ, and stained by hematoxylin and eosin. Mast cells were detected after staining with toluidine blue, and eosinophils after staining with chromotrope R-2. Anti-CD45RO, anti-HLA DR, anti-CD8, and anti-CD68 antibodies (Dako, Glostrup, Denmark) followed by the PAP technique were used to detect surface cell markers.

Assessment of apoptosis

Apoptotic cell death was detected with a nonradioactive 3'-end DNA-labeling technique using the *In situ* Cell Death Detection Kit, POD (Boehringer Mannheim, Germany). In brief, the paraffin embedded specimens were dewaxed by washing in xylene, rehydrated through a graded series of ethanol and H₂O, and finally rinsed in phosphate buffered saline (PBS). The tissue sections were treated with proteinase K (Boehringer Mannheim, Germany) for 15 min at room temperature and rinsed twice with PBS. Endogenous peroxidase was blocked with 0.3% H₂O₂ in methanol for 30 min at room temperature. Tissue sections were subsequently rinsed with PBS, and then incubated with the labeling-reaction-mixture containing fluorescein-labeled nucleotides and terminal desoxynucleotidyl transferase (TdT) in a humidified chamber for 1 h at 37°C. Slides were rinsed three times with PBS and treated with antifluorescein antibody conjugated with peroxidase for 30 min at 37°C in the humidified chamber. After washing three times with PBS, the diaminobenzidine (DAB Metal Enhanced, Boehringer Mannheim) was used for visualization. Counter staining was performed with hematoxylin. Negative controls were done by omitting TdT. Nuclei exhibiting morphological changes suggestive of apoptosis (condensed nuclei or fragmentation of chromatin into compact masses) were checked for staining, and both the number and the overall morphology of all stained nuclei were taken into consideration.

Morphometry

The thickness of each section was controlled according to the method described by Weibel (35). Histological morphometry was performed by means of an image analysis system consisting of a computer (IBM Pentium 120 MHz) equipped with an optical mouse. A VER 2000 card (frame grabber, true-colour, real-time), produced by ADDA Technologies (USA), and colour TV camera (Panasonic) linked to a microscope (Jenaval Carl Zeiss; Jena). This system was programmed (MULTISCAN produced by CSS-Poland) to calculate the

Table 1. Characteristics of patients with nasal polyps

	ASA-hypersensitive >(n=16)	ASA-tolerant atopic (n=17)	ASA-tolerant nonatopic (n=19)
Mean age in years (range)	54.0 (23-68)	49.0 (19-72)	57.0 (23-76)
Duration of rhinitis in years (range)	15.2 (5-26)	11.4 (3-23)	9.7 (4-24)
Duration of nasal polyposis in years (range)	12.2 (3-25)	10.3 (3-20)	11.36 (3-45)
Number of polypectomies (range)	2.88 (1-9)	1.28 (1-3)	2.9 (1-9)
Duration of ASA-hypersensitivity in years (range)	12.8 (3-27)	-	-

number of objects in a semiautomatic manner. The microscopic images were saved serially in the computer, and then quantitative examinations were carried out in two zones of a tissue. The zone above the basal membrane, and that from the lower border of the basal membrane to 0.18 mm depth, were defined as the epithelial and subepithelial (ES) layers; and the rest of the tissue area as the stromal (STR) layer, as described before (36). For each tissue layer the number of stained cells was determined in a sequence of 7–10 consecutive computer images of high power fields, 0.0049 mm² each. The only adjustments of field were made to avoid large vessels and areas of damaged epithelium. The results were expressed as a mean number of stained cells per mm².

Statistical methods

Differences between groups were tested using analysis of variance (one-way ANOVA) or unpaired Student's *t*-tests preceded by evaluation of normality and Levene's test. The Mann–Whitney *U*-test was used where appropriate. Correlation coefficients were calculated using Spearman's method. Results were considered statistically significant if $P < 0.05$.

Results

Apoptotic cells

Apoptotic cells were detected both within the epithelium and below the basement membrane in the stroma of nasal polyps (Fig. 1A and B). Although we were not able to identify precisely all cells undergoing apoptosis, by morphology most of apoptotic cells appeared to be eosinophils. Density of apoptotic cells was significantly lower in AH patients as compared to both atopic ($P < 0.02$) and nonatopic AT patients ($P < 0.005$) in the superficial epithelial layer of nasal polyps. In the stromal layer the density of apoptotic cells was also lower in AH patients as compared to AT nonatopic polyps ($P < 0.01$) but not to AT atopic polyps ($P > 0.05$) (Fig. 2). Calculation of apoptotic cells as a percentage of eosinophils demonstrated that the mean fraction of apoptotic cells in AT atopic patients did not exceed 2.3%; in AT nonatopic it was close to 2.9%, while in AH polyps it was below 0.37%.

Eosinophils and mast cells

Damaged/detached epithelium or squamous epithelial metaplasia were present in nasal polyp preparations, to a similar extent and frequency in all three groups of patients (Table 2). Visual signs of edema and inflammation in the submucosa were also similar in all groups.

Eosinophils comprised a dominant population of inflammatory cells both in the superficial and deeper layers of nasal polyp tissue in all groups. The density of eosinophils was significantly higher in nasal polyps from AH patients as compared to atopic and nonatopic AT subjects both in superficial and in the deeper layer (Fig. 3A). Atopic and nonatopic polyps did not differ

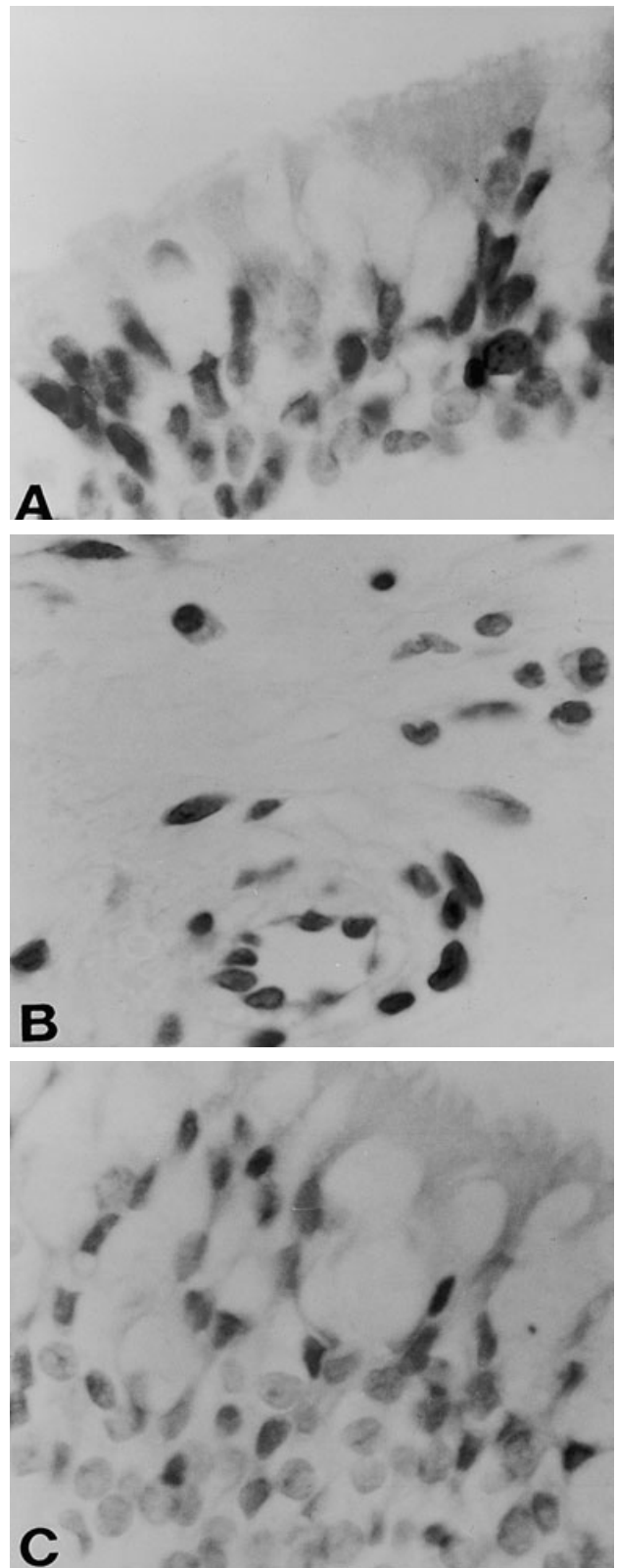


Figure 1. Representative microphotographs of nasal polyp sections. Apoptotic cells labeled by TUNEL method (brown staining) in the epithelial (a) and in the stromal layer (b) of nasal polyp from ASA-tolerant patient. Negative control (c) was performed by omitting TdT. Sections counter-stained with hematoxylin; original magnification $\times 1000$.

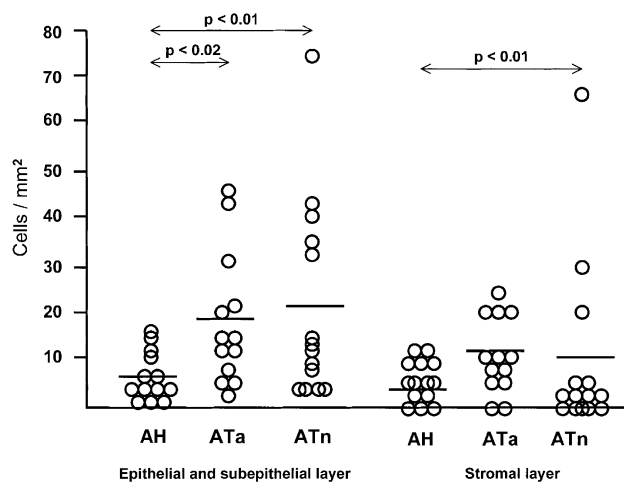


Figure 2. Density of apoptotic cells in nasal polyps from individual 13 ASA-hypersensitive (AH), 13 ASA-tolerant atopic (ATa) and 15 ASA tolerant nonatopic (ATn) patients. Mean values are represented by horizontal bars.

with respect to eosinophil concentration. In all three groups the density of eosinophils was approximately two-fold higher in the superficial as compared to the deeper layer, although only in AH and in AT atopic patients were the differences statistically significant: 1319.2 ± 145.8 vs 779.0 ± 107.3 cells/mm², $P < 0.001$ for AH polyps; 823.4 ± 99.5 vs 463.4 ± 73.5 cell/mm², $P < 0.01$ for atopic AT polyps; and 700.8 ± 153.8 vs 358.7 ± 136.8 cell/mm², NS, for nonatopic AT polyps.

The density of mast cells was significantly higher in AH patients as compared to AT nonatopic patients, but not to atopic patients in both ES and STR layers. In ES layer the density of mast cells was higher in atopic as compared to nonatopic patients (Fig. 3B). In all three groups the density of mast cells was higher in the superficial as compared to the deeper layer, although in only the ASA-hypersensitive and atopic patients were the differences statistically significant: 77.3 ± 8.8 vs

21.8 ± 2.5 cells/mm², $P < 0.001$ for AH patients; 63.1 ± 7.5 vs 15.6 ± 1.8 cell/mm², $P < 0.001$ for atopic patients; and 28.7 ± 8.1 vs 13.0 ± 2.4 cells/mm² = NS for nonatopic patients.

There was a significant correlation between the number of mast cells and eosinophils in the ES layer of nasal polyp tissue in all three groups ($r = 0.66$, $P < 0.01$; $r = 0.83$, $P < 0.001$; $r = 0.83$, $P < 0.001$ for AH, AT atopic, and AT nonatopic, respectively). By contrast mast cells and eosinophils did not show any significant correlation in the STR layer.

Cell surface markers

The density of activated CD45RO+ T cells was significantly higher in AH patients as compared to both atopic and non atopic AT patients (Fig. 3C). The density of CD45RO+ cells was also higher in atopic than in nonatopic AT patients. The density of cells expressing HLA-DR molecule was significantly higher in AH polyps as compared to nonatopic, but not to atopic AT polyps (Table 2). Accordingly, in the AT group the density of HLA-DR+ cells was higher in atopic as compared to nonatopic patients. In the ES layer CD8+ cells were present with similar density in all three groups. In the stromal layer, both AH and AT atopic polyps had significantly more CD8+ cells as compared with nonatopic polyps, although AH and AT atopic polyps demonstrated similar density of CD8+ cells. CD68+ cells, presumably macrophages, were present in all three groups of patients with similar density in both ES layer.

Correlation of apoptotic cells with cell surface markers and other cells

In AH patients a significant inverse correlation was found between the number of apoptotic cells and HLA-DR positive cells in both the superficial epithelial layer

Table 2. Density of cell surface markers positive cells and routine histopathologic findings in nasal polyps from ASA-hypersensitive and ASA-tolerant patients

	AH n = 16	AT atopic n = 17	AT nonatopic n = 19	Statistical significance
Surface marker positive cells (cells/mm²)				
HLA DR + ES	91.4 ± 8.3	89.9 ± 7.3	69.4 ± 5.8	*NS; † < 0.05; ‡ < 0.05
HLA DR + STR	86.3 ± 6.3	88.1 ± 9.8	65.0 ± 5.6	*NS; † < 0.02; ‡ < 0.05
CD8 + ES	72.3 ± 9.3	86.9 ± 15.2	49.0 ± 7.1	*NS; †NS; ‡ < 0.03
CD8 + STR	62.3 ± 6.5	57.8 ± 7.7	31.6 ± 4.6	*NS; † < 0.001; ‡ < 0.01
CD68 + ES	47.3 ± 5.9	56.8 ± 6.6	49.8 ± 6.1	*NS; †NS; ‡NS
CD68 + STR	59.4 ± 6.7	54.7 ± 6.5	50.1 ± 5.0	*NS; †NS; ‡NS
Histopathologic assessment (% of patients with positive findings)				
> 30% damage of the epithelium	31.2	29.4	36.8	*NS; †NS; ‡NS
Epithelial metaplasia	25.0	29.4	21.0	*NS; †NS; ‡NS
Marked edema in the submucosa	56.2	41.2	47.3	*NS; †NS; ‡NS

*ASA hypersensitive vs ASA tolerant atopic patients.

†ASA hypersensitive vs ASA-tolerant nonatopic patients.

‡ASA-tolerant atopic vs ASA-tolerant nonatopic.

NS: not significant; ES: epithelial/subepithelial layer; STR: stromal layer.

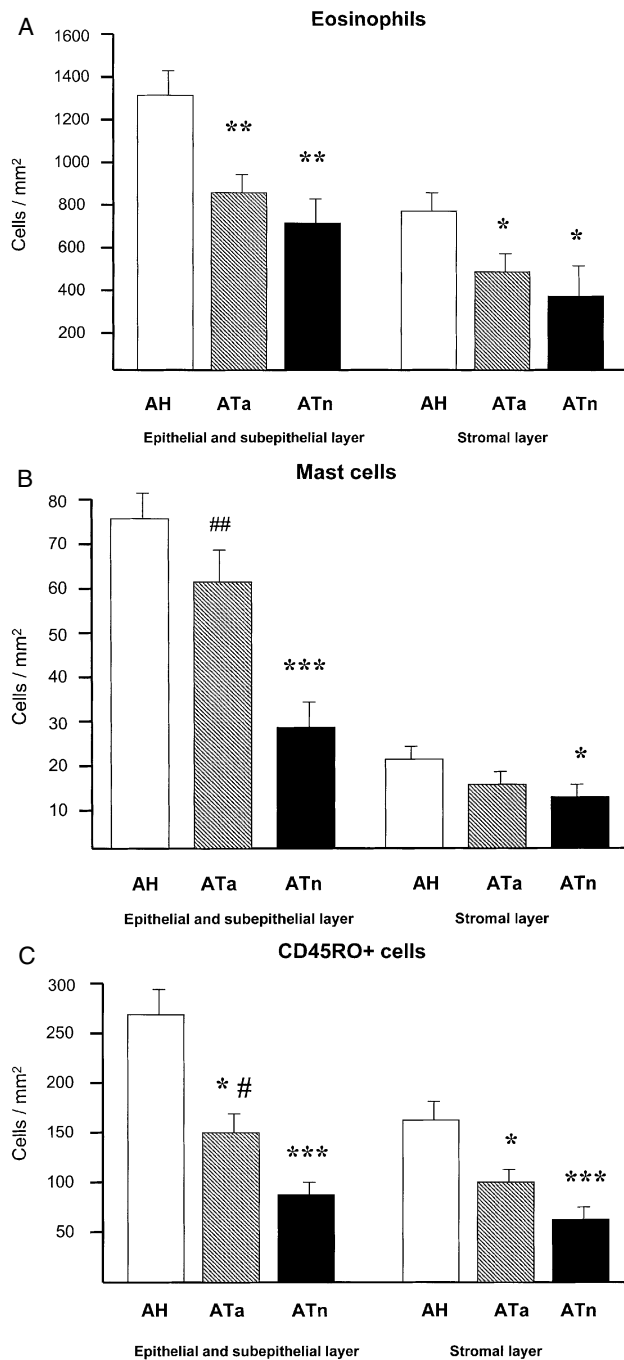


Figure 3. Density of eosinophils (a), mast cells (b), and CD45RO+ cells (c) in nasal polyps from ASA-hypersensitive (AH; open bars), ASA-tolerant atopic (ATa; hatched bars), and ASA-tolerant nonatopic patients (ATn; shaded bars). Mean values + SEM. Asterisks represent statistical difference from ASA-hypersensitive polyps (*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$). #: statistical difference between atopic and nonatopic ASA-tolerant patients (#: $P < 0.05$; ##: $P < 0.01$).

($r = -0.77$; $P < 0.002$) and in the stromal layer ($r = -0.58$; $P < 0.04$) of polyps. The negative correlation between apoptotic cells and CD45RO+ cells in the ES layer was on the edge of significance ($r = -0.55$; $P < 0.051$). There was no significant correlation between

apoptotic cell density and the density of eosinophils, mast cells, CD8+ or CD68+ cells in this group of patients. A distinctive pattern for AH group correlation was observed between CD45RO+ cells and CD68+ cells in both ES and STR layers ($r = 0.8$, $P < 0.001$; $r = 0.6$, $P < 0.01$), and between CD45RO+ and HLA-DR+ cells in the STR layer ($r = 0.6$, $P < 0.01$). CD45RO+ cells in stromal layer correlated also with the number of eosinophils ($r = 0.55$, $P < 0.03$).

In AT atopic patients no significant correlation for apoptotic cells was found. The number of eosinophils correlated with CD45RO+ cells in ES and STR layers: $r = 0.55$; $P < 0.03$; $r = 0.55$, $P < 0.03$.

In AT nonatopic patients there was a significant positive correlation between the number of apoptotic cells and the number of eosinophils in both the superficial epithelial layer ($r = 0.54$; $P < 0.03$) and in the stromal layer ($r = 0.77$; $P < 0.001$) of polyp tissue.

Interestingly the highest and the most consistent correlation in all three groups of polyps (AH, AT atopic and AT nonatopic) was found between CD45RO and CD8+ cells ($r = 0.9$ in ES layer and $r = 0.7$ in STR layer; all $P < 0.001$).

Association of clinical characteristics of rhinosinusitis with parameters of inflammation in nasal polyps

In AH patients the duration of chronic rhinitis symptoms correlated inversely with the number of apoptotic cells ($r = -0.67$; $P < 0.04$). Duration of nasal polyposis and the number of polypectomies correlated negatively with the number of HLA-DR+ ($r = -0.74$; $P < 0.02$ and $r = -0.71$; $P < 0.031$ for duration and number of polypectomies, respectively). In contrast in AT atopic patients the duration of rhinitis symptoms correlated strongly with the number of apoptotic cells ($r = 0.89$; $P < 0.003$). In addition AT atopic patients showed strong positive correlation between the density of apoptotic cells and the duration of nasal polyposis ($r = 0.97$; $P < 0.0001$). In AT nonatopic patients no correlation between clinical features and cellular parameters was observed.

The density of eosinophils or mast cells in nasal polyp tissue showed no correlation with the duration of chronic rhinitis or nasal polyposis nor with the number of polypectomies in any of the three group studied.

Discussion

The mechanism underlying higher severity of rhinosinusitis and high recurrency rate of nasal polyposis in ASA-hypersensitive patients remains unclear. Our study demonstrated for the first time, that the number of apoptotic cells in nasal polyps from aspirin-sensitive patients is significantly lower, than in polyps from AT patients. This observation held true when differences in polyp cellularity between AH and AT polyps were

accounted for, and the percentage of cells undergoing apoptosis in relation to eosinophils was calculated. Thus, not only the number, but actually the proportion of apoptotic cells is decreased in ASA-hypersensitive patients, suggesting that impaired apoptosis may be responsible for increased cellular infiltration in nasal polyps of AH patients.

Tissue eosinophilia is common in most nasal polyp tissue, but prominent eosinophilic infiltration has been considered a distinctive feature of ASA-hypersensitive rhinosinusitis (26). However, in previous studies quantitative differences from ASA-tolerant polyps were not unequivocal, probably due to large variability in the density of eosinophils among different tissue specimens (37-39). We were able to analyze a significant number of polyp tissues, and computerized image analysis technology allowed us to screen a large area of each tissue, thus decreasing variability in cell counting. Aspirin-sensitive patients demonstrated 60–88% higher mean density of eosinophils in the superficial epithelial layer, and 70–120% higher density in the deeper layer of the polyp mucosa as compared to ASA-tolerant patients. Our data are in line with other studies demonstrating that eosinophilic inflammation is a distinctive feature of ASA-hypersensitivity both in upper and the lower airway mucosa (40-42).

It has been suggested that mononuclear cells, in addition to eosinophils, are involved in development and control of inflammation in nasal polyps (31). Although there are studies demonstrating the prominent role of T lymphocytes, and T-cell derived cytokines in the pathogenesis of nasal polyps, few studies addressed the possible differential role of specific immunological mechanisms in nasal polyposis in ASA-hypersensitive patients (27, 43). In addition to assessment of apoptosis, we studied several cellular markers characteristic of activation of immune-competent cells in the polyp tissue. The major difference observed between ASA-hypersensitive and AT nasal polyps was in the number of CD45RO+ cells with AH nasal polyps containing significantly greater number of CD45RO+ cells. CD45RO+ cells are considered to be activated T cells with memory or effector function, and both CD4 and CD8 cells may express CD45RO phenotype (44). Significantly higher densities of CD45RO+ cells in ASA-hypersensitive patients suggests a particular role for this subset of activated T cells in perpetuating the inflammatory response in ASA-hypersensitive patients. Moreover, only in AH group a significant positive correlation was observed between CD45RO+ cells and the number of eosinophils, suggesting possible role of these cells as a source of chemotactic or survival factors for eosinophils. This assumption is further supported by the negative correlation of CD45RO+ cells with the number of apoptotic cells ($r = -0.55$; $P < 0.051$) which was found only in AH polyps.

The contribution of any particular cytokine pattern to

local inflammatory response cannot be directly deduced from our study, since it has been demonstrated, that CD45RO+ T cells may express both Th2 and Th1 cytokine profile (IL-4 and IFN- γ) (45). Our data, indicating a distinct presence of CD45RO+ cells in AH polyps, may be consistent with findings of Hamilos et al. (27) who found a specific profile of cytokine expression in a group of patients with nonallergic (presumably ASA-hypersensitive) polyps, and of Bachert et al. (46) who demonstrated elevated IL-5 protein concentrations in AH-sensitive polyps. The disproportionate increase in IL-5 mRNA as compared to IL-4 mRNA positive cells was also found in nonpolyposus nasal mucosa, suggesting that other than classical “allergic” mechanism may be important in the disease (40).

HLA-DR molecule expression may be determined as another marker of cell activation in the nasal polyp tissue. The number of HLA-DR immunoreactive cells was higher in AH polyps and AT atopic polyps as compared to AT nonatopic polyps suggesting its association with allergic inflammation rather than with aspirin sensitivity clinical phenotype. However, a strong negative correlation for HLA-DR(+) cells with the number of apoptotic cells was observed only in AH polyps suggesting, that HLA-DR expression (or presence of HLA-DR positive cells) is related to an increased survival of inflammatory cells in nasal polyps of AH patients. Another interesting finding was a large number of CD8+ lymphocytes present in nasal polyps from both AH and AT patients, and a high correlation of CD8+ cells number with CD45RO+ immunoreactivity, suggesting that either presumably cytotoxic T cells might express this marker, or these cells may be related to other cells expressing the CD45RO+ marker. CD8+ cells (and specifically CD8+/CD45RO subsets) isolated from peripheral blood have a significant capacity for production of cytokines including IFN- γ , IL-2, GM-CSF and IL-4 but also macrophage inflammatory protein 1a (MIP-1a) and RANTES (44). Bearing in mind the capacity of these cytokines for modulation of eosinophilic inflammation, it may indicate a potential, and not fully appreciated, role for CD8+ cells in the development of inflammation in nasal polyps.

In the present study basic clinical features of chronic rhinosinusitis did not differentiate AH from AT patients, although their correlation with cellular and molecular markers of inflammation were distinctive in both groups. While in AH patients duration of chronic rhinosinusitis correlated negatively with the number of apoptotic cells, in ASA-tolerant atopic patients duration of rhinitis and polyposis showed a positive correlation with apoptosis, and both associations were strongly statistically significant. Similarly, negative correlation was observed exclusively in ASA-hypersensitive patients between duration of nasal polyposis or the number of polypectomies and the number of HLA-DR+ cells.

Since no correlation between clinical characteristics and immunological markers in nasal polyps was noticed in AT patients one may assume, that different pathomechanisms may be responsible for clinical expressions of chronic rhinosinusitis and polyposis in AH and AT patients. Taken together these data indicate that the rate of local apoptosis in nasal polyps may be related not only to distinct immunological features of local inflammation but also to clinical characteristics of patients.

Depressed apoptosis, may result in an increased number of eosinophils in the nasal mucosa and has been implicated in the pathogenesis of nasal polyposis (33, 34). The mechanism responsible for decreased apoptosis and increased eosinophilia observed in AH patients may involve cytokines including IL-5 and GM-CSF which express potent survival activity for eosinophils (27, 45). The distinct profile of cell surface markers in nasal polyps as well as the correlations reported in this study support the assumption that different cytokine profiles could be responsible for decreased rates of apoptosis reported in ASA-hypersensitive polyps. Interestingly, overexpression of IL-5 positive cells in the bronchial mucosa with prominent eosinophilia from ASA-hypersensitive asthmatics was also reported (47). In addition to cytokines, nonpeptide mediators, e.g. 15-HETE or prostaglandin E₂, may be involved in inhibition of cellular apoptosis at the site of inflammation (23, 48). A dominant role of PGE₂ in controlling apoptosis in ASA-hypersensitive patients seems unlikely since PGE₂ release by epithelial cells cultured from nasal polyps is

significantly lower in AH as compared to AT polyps, and ASA-hypersensitive polyps had lower expression of COX-2 responsible for PGE₂ generation (23, 24). However, PGE₂ deficiency observed in nasal polyps from ASA-hypersensitive patients may result in enhanced activation of eosinophils and mast cells, contributing to development of local inflammation.

In summary, we reported the presence of apoptosis in nasal polyp tissue regardless of the presence or absence of atopy or aspirin sensitivity. At the same time a significant difference in the intensity of local apoptosis as well as in cellular and immunologic profiles of inflammation were observed in nasal polyps from aspirin-sensitive patients. Decreased apoptosis, and increased infiltration with eosinophils were associated in these patients with prominent expression of CD45RO+ activated/memory cells, reflecting a distinct pattern of immunologic cell activation, and were also related to clinical features of rhinosinusitis. From the clinical standpoint, these observations provide experimental data to support alternative therapeutic approaches (e.g. aspirin desensitization or antileukotriene agents) for treatment of chronic rhinosinusitis and nasal polyposis in ASA-hypersensitive patients (48, 49).

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