

Intranasal challenge with aspirin induces cell influx and activation of eosinophils and mast cells in nasal secretions of ASA-sensitive patients

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Summary

Background Although the mechanism of aspirin-sensitivity seems to be related to inhibition of cyclo-oxygenase by aspirin (ASA), the chain of biochemical events leading to the ASA-induced adverse reaction is not clear, and the contribution of particular mediators and inflammatory cells has not been elucidated.

Objectives To investigate the involvement of secretory, vascular and cellular mechanisms in the pathophysiology of nasal reactions to aspirin.

Methods Six patients with ASA-sensitive asthma/rhinosinusitis and seven ASA-tolerant patients were challenged intranasally with saline and lysine-acetylsalicylic acid (Lys-ASA) 12 mg, on separate occasions. Nasal lavages were obtained before, and then every 15 min after challenges, and analysed for biochemical and cellular composition.

Results Lys-ASA challenge caused rhinorrhoea, sneezing and nasal congestion with parallel increases in total protein and albumin concentration, albumin % and lysozyme activity in the nasal secretions of ASA-sensitive patients. Concomitant with clinical symptoms, an influx of leucocytes into nasal secretions occurred with significant enrichment in eosinophils (mean prechallenge: $24 \pm 12\%$, post saline $27 \pm 9\%$, post Lys-ASA $51 \pm 10\%$; $P < 0.03$). The influx of eosinophils into nasal secretions was associated with a remarkable increase in Eosinophil Cationic Protein (ECP) immunoreactivity in five of six patients (mean $9.3 \pm 3.8 \mu\text{g/L}$ and $140.9 \pm 45.8 \mu\text{g/mL}$ before and after Lys-ASA, respectively). At the peak of ASA-induced symptoms an increase in the tryptase level was also observed in five of six patients (mean prechallenge: $2 \pm 0.1 \text{ U/L}$; post Lys-ASA $16 \pm 5 \text{ U/L}$; $P < 0.01$) suggesting activation of mucosal mast cells. In ASA-tolerant patients Lys-ASA did not induce significant symptoms or changes in the biochemical and cellular composition of nasal secretions.

Conclusion The results show that the ASA-induced nasal adverse reaction involves changes in vascular permeability and serous cell secretion. Both activated eosinophils and mast cells may contribute to the pathophysiology of the ASA-induced reaction in the nasal mucosa.

Keywords: aspirin-sensitivity, eosinophils, mast cells, tryptase, ECP, nasal washes

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Introduction

Most aspirin (ASA)-sensitive patients present a typical clinical syndrome, i.e. 'Asthma triad' involving aspirin

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sensitivity, bronchial asthma, and chronic rhinosinusitis with nasal polyps [1,2]. These patients suffer from a particularly severe, often steroid-dependent form of bronchial asthma, but also have intractable, rhinosinusitis complicated by recurrent nasal polyps; the management of this syndrome is a difficult clinical challenge [3,4].

The basic mechanism underlying sensitivity to aspirin is not immunological, and seems to involve inhibition of cyclo-oxygenase with subsequent release of leukotrienes and possibly other mediators [2,5]. However, the chain of biochemical events leading to ASA-induced reaction is not clear, and the contribution of particular mediators and inflammatory cells has not been elucidated [6]. Peripheral blood eosinophilia and infiltration of nasal and bronchial mucosa with eosinophils is a prominent feature in ASA-sensitive patients, implicating participation of these cells in the pathophysiology of the disease [7–9]. Several studies suggested also mast cell involvement, although most of these studies were far from conclusive [10,11].

Ortolani *et al.* [12] demonstrated, that intranasal application of Lysine-acetylsalicylic acid (Lys-ASA; a soluble form of aspirin) induced nasal symptoms in ASA-sensitive, but not in ASA-tolerant patients, and this observation has been confirmed by others [13,14]. Since in the majority of asthmatic patients the bronchial reaction to aspirin is accompanied by nasal symptoms, studying nasal secretions induced by intranasal challenges with aspirin may provide insights into the pathomechanism of the mucosal inflammation in ASA-sensitive asthmatics [15–17].

In this study we have documented, that the nasal reaction to aspirin involves mucosal vascular permeability, serous cells secretion and the rapid movement of inflammatory cells, mostly eosinophils, into the nasal secretions. We also investigated the possibility of activation of inflammatory cells by measuring release of Eosinophil Cationic Protein (ECP) and tryptase, specific markers for eosinophil and mast cell activation, respectively.

Materials and methods

Patients

Six ASA-sensitive patients (two male and four female; mean age 37.2 ± 7 years) with chronic rhinosinusitis, nasal polyps and bronchial asthma were studied. Aspirin sensitivity was deduced from the patient's history and confirmed by an oral challenge test with ASA performed at least 3 weeks before the study according to the method previously described [18]. The mean threshold dose of ASA was 97.3 mg (range 40–160).

The control group included seven patients (five male and two female; mean age 32.8 ± 6) with chronic perennial rhinitis (two of them with positive skin-prick tests to house dust mite), but without ASA-sensitivity (negative history and negative challenge with 600 mg of ASA). Three patients had nasal polyps and four suffered from bronchial asthma. None of the patients was on oral or intranasal steroids for at least 4 weeks before the study. Four ASA-sensitive patients, and one ASA-tolerant patient were on chronic inhaled steroids (200–400 μ g budesonide/day), but the medicine was not inhaled on the day of the study. The patients were included in the study after obtaining informed consent, and the study was run according to the protocol approved by the Local Ethical Committee of the Faculty of Medicine.

Challenge procedures and nasal lavages

All patients were challenged intranasally with saline and Lys-ASA solution on separate days.

The protocol of the study was designed to study unilateral secretory responses, but both sides were challenged in order to improve subjective scoring of symptoms. Thus, diluent or Lysine-Aspirin (Aspegic, Lek Ljubljana, kindly provided by Mrs Lucija Burger) were delivered via a nasal spray pump (120 μ L per actuation) to both nostrils. Preliminary experiments with 2 mg, 6 mg and 12 mg of Lys-ASA demonstrated, that only the latter dose gave reproducible responses in ASA-sensitive patients (data not shown), thus 12 mg of Lys-ASA was used throughout the study. Nasal responses were evaluated based on clinical symptoms recorded with a symptom score technique grading the responses as none = 0, mild = 1, moderate = 2 or severe = 3. A composite nasal score was calculated as the sum of individual scores for blockage and sneezing.

Nasal lavages were performed according to the method previously described [19,20]. Patients were seated in an upright position and a soft rubber catheter connected to a suction device was atraumatically inserted along the floor of the nasal cavity and secured to the cheek allowing for continuous collection of secretions. The catheter was inserted only once, about 10 min before the start of the procedure, thus allowing the mucosa time for adaptation. No increases in basal secretion were noticed during basal washes, and following application of control solution. A hand-held nebulizer was used to deliver nasal lavages with normal saline (4 mL each). Before the challenge three prewashes were obtained and discarded and the fourth one was retained and served as a baseline. After Lys-ASA instillation the lavages were performed every 15 min until the first symptoms of a reaction occurred. Then at least three nasal lavages were

performed during the next 30 min, before the reaction disappeared or was stopped with an appropriate treatment. In ASA-tolerant patients, the nasal lavages were performed every 15 min for 120 min after saline or Lys-ASA challenge. All washes were performed on one side of the nose only. The lavages were centrifuged for 10 min, 400 *g* at 4°C; the cell pellet was processed on the same day and supernatants frozen at -20°C, and subsequently assayed for proteins and inflammatory mediators.

Cell counts in nasal lavage fluids

After centrifugation, cells in the pellet were resuspended in 1 mL of RPMI 1640 and the total cell number was counted with a Neubauer haemocytometer; the cell number was expressed per mm³ of lavage fluid. The number of eosinophils in the nasal washes was counted after an aliquot of cell suspension was stained with Dungen's stain. Metachromatic cells (basophils and mast cells) were counted after staining another aliquot with Toluidine blue in the chamber. Differential cell counts were performed on slides after staining one specimen from each sample with May-Grünwald/Giemsa stain. Cells were classified according to their morphology as epithelial, neutrophils, eosinophils and mononuclear cells. The epithelial cells were disregarded.

Protein and mediator assays

The total protein concentration of the nasal lavage fluids was determined by the Lowry method [21], albumin concentration by rocket immunoelectrophoresis, and lysozyme activity by the lysoplate assay method [22]. ECP and tryptase were measured by a radioimmunoassay using ECP and Tryptase RIA kits (Pharmacia, Sweden). The range of standard curve was 2–200 µg/L and 2–50 U/L for ECP and for tryptase, respectively. All assays were run in duplicate.

Calculations and statistical analysis

Results are presented as mean ± SEM. In order to assess Lys-ASA induced changes of biochemical and cellular parameters in ASA-sensitive patients, mean values from two to three consecutive washes performed at the peak of nasal symptoms were compared with mean values in basal washes, or with mean values in washes obtained after saline challenge at 2–3 time points corresponding to the reaction after Lys-ASA. Since, in ASA-tolerant patients no symptoms occurred, their basal washes were compared with mean values from washes obtained at 60 and 75 min after challenge with saline or Lys-ASA, i.e. at the time when a typical reaction in ASA-sensitive

patients was observed. Mean values in washes obtained after Lys-ASA were also compared with corresponding washes after saline challenges. A one-tailed Wilcoxon Matched Pairs Signed Rank test was used to analyse data within groups.

Results

Symptoms

Intranasal challenge with Lys-ASA, but not with diluent, induced in all ASA-sensitive patients clinical symptoms of nasal congestion, sneezing and rhinorrhoea. Symptoms typically appeared 30–60 min after challenge, reached maximum at 45–90 min, and lasted up to 2 h. Only two ASA-tolerant patients demonstrated transient sneezing 5–15 min after Lys-ASA instillation. In ASA-tolerant patients the mean composite symptom score was 0.3 ± 0.2 and 0.9 ± 0.5 postsaline and postLys-ASA challenge, respectively ($P > 0.05$). In ASA-sensitive patients the mean composite symptom score was 0.5 ± 0.2 after saline and 5.2 ± 1.7 after Lys-ASA ($P < 0.01$).

Proteins in nasal lavages

The volume of secretions recovered in the lavage fluid increased significantly in ASA-sensitive patients in parallel with nasal symptoms (mean 4.3 ± 0.5 mL and 7.5 ± 0.7 mL postsaline, and post Lys-ASA, respectively; $P < 0.01$) and was not changed in ASA-tolerant patients (mean 4.1 ± 0.4 mL and 4.7 ± 0.6 postsaline and postLys-ASA, respectively). Aspirin increased the mean concentration of total protein, albumin and the relative concentration of albumin to total protein (Alb%) only in ASA-sensitive patients at the peak of the clinical symptom score ($P < 0.01$) (Fig. 1 a,b,c). There was also a significant increase in the lysozyme activity at the peak of the clinical symptom score ($P < 0.01$) (Fig. 1d). The biochemical changes usually started 30–45 min after Lys-ASA and reached maximum 45–75 min after the challenge.

Cellular findings

The total number of cells recovered in nasal washes increased significantly in ASA-sensitive patients at the peak of clinical symptoms after Lys-ASA challenge ($P < 0.01$) (Fig. 2a). Aspirin induced a significant increase in the number of eosinophils recovered in nasal secretions, in four of six ASA-sensitive patients, but smaller increases were also seen in two ASA-tolerant patients (Fig. 2b). Increased nasal eosinophilia was noticed as early as 30 min (usually 60–90 min) after

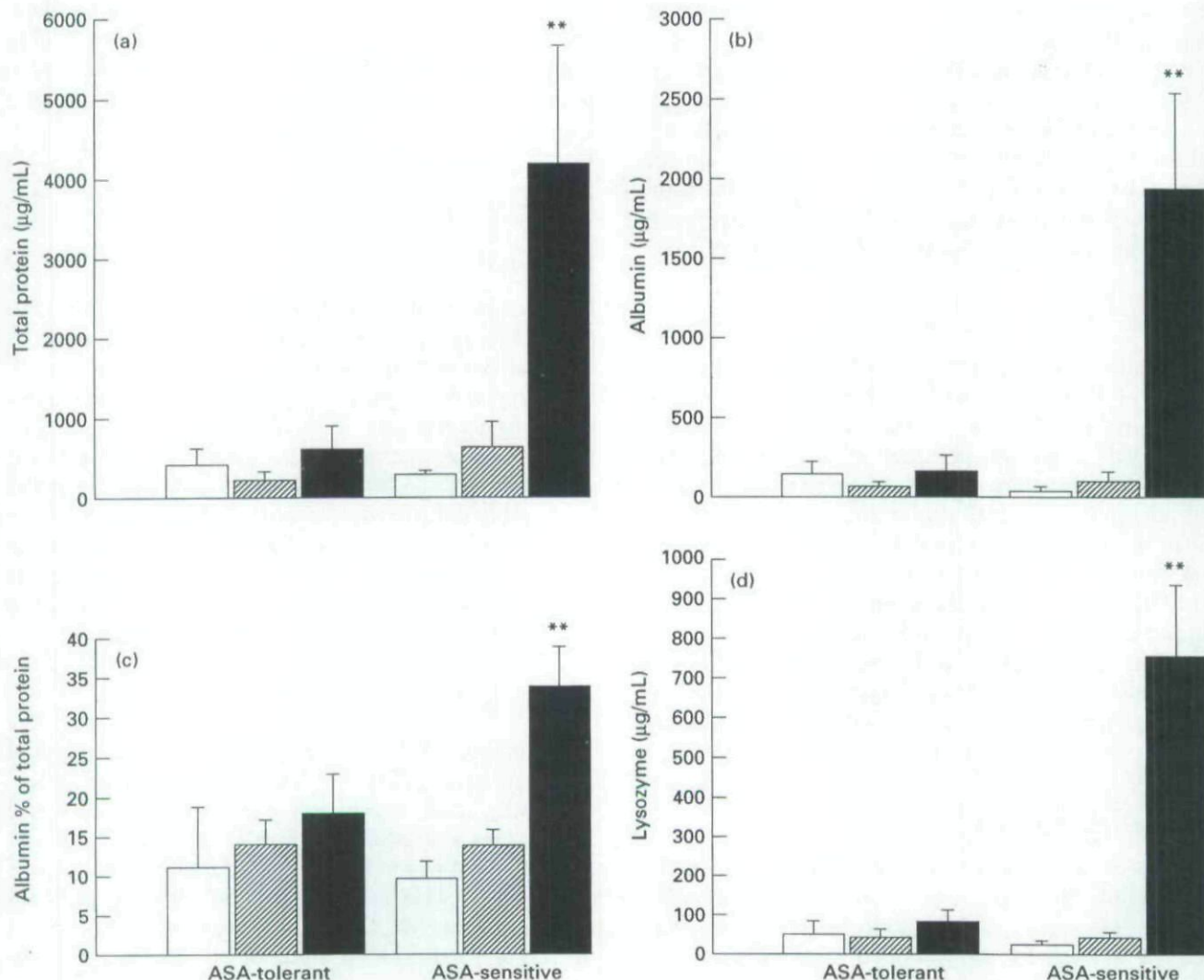


Fig. 1. Protein concentration in basal nasal lavages (□), after control (▨) and after Lys-ASA challenge (■) in ASA-tolerant and ASA-sensitive patients. ** $P < 0.01$ compared with post-saline values (Wilcoxon Matched Pairs Signed Rank test). (a) total protein; (b) albumin; (c) albumin to total protein ratio; (d) lysozyme.

ASA challenge, and at 120 min the mean number of eosinophils returned to basal values. On differential count a significant enrichment in eosinophils (expressed as percentage of total number of leucocytes) was seen (Fig. 2c).

Metachromatic cells in nasal washes of ASA-sensitive patients showed a tendency to increase from baseline counts of 24 ± 9 cell/mm³ to 42 ± 21 cell/mm³ after saline, and 61 ± 18 cell/mm³ after Lys-ASA challenge, but the changes were not statistically significant ($P > 0.05$). In ASA-tolerant patients the number of metachromatic cells in basal washes was 21 ± 8 cell/mm³, after saline 31 ± 22 cell/mm³ and after Lys-ASA 64 ± 48 cell/mm³; the differences were not statistically significant ($P > 0.05$).

Inflammatory mediators in nasal lavages

Since, in ASA-tolerant patients, ASA-challenges did not induce symptoms or changes in protein and cellular composition of nasal washes, inflammatory mediators were determined only in ASA-sensitive patients before and 30, 60, and 120 min following saline and Lys-ASA challenge. Lys-ASA challenges produced an increase in ECP concentration in nasal lavage fluids at the peak of nasal symptoms in all but one patient (Fig. 3). The mean concentration of ECP was 3.5 ± 2.3 μg/mL before, and 140.9 ± 45 μg/mL after Lys-ASA ($P < 0.01$). A significant increase in ECP concentration was observed as early as 30 min after the challenge (mean level

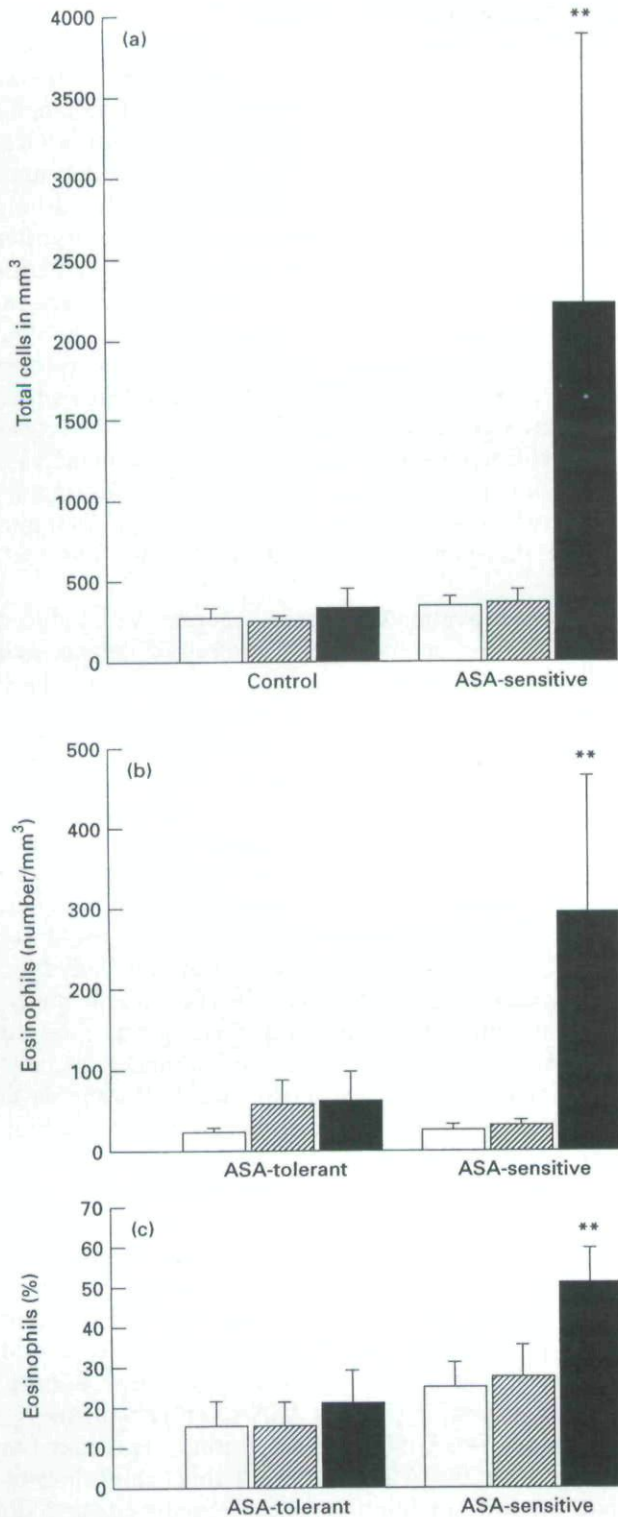


Fig. 2. Cellular composition of basal nasal lavages ($\square$), after control (hatched) and after Lys-ASA challenge ($\blacksquare$) in ASA-tolerant and ASA-sensitive patients. ** $P < 0.01$ compared with post-saline values (Wilcoxon Matched Pairs Signed Rank test). (a) total cell number; (b) eosinophil number; (c) eosinophil percentage.

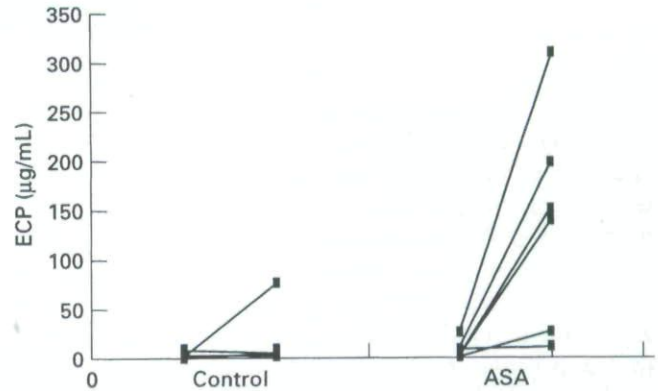


Fig. 3. Changes in ECP immunoreactivity concentration in nasal lavages after control and Lys-ASA challenges in ASA-sensitive patients.

$96.1 \pm 51.9 \mu\text{g}/\text{mL}$; $P < 0.01$), the maximal increase being seen at 60 min (mean $140.9 \pm 45.8 \mu\text{g}/\text{mL}$; $P < 0.01$). At 120 min ECP was still significantly elevated (mean $41.2 \pm 11.5 \mu\text{g}/\text{mL}$; $P < 0.03$). Challenge with saline increased ECP concentrations in one patient only, without changing significantly the mean ECP concentration (mean basal concentration $3.4 \pm 1.0 \mu\text{g}/\text{mL}$, mean post-saline $18.9 \pm 14.8 \mu\text{g}/\text{mL}$; $P > 0.05$).

Tryptase levels in nasal fluids increased in all but one patient 30–60 min after Lys-ASA challenge. At 120 min they returned close to baseline, and only in one patient remained elevated. By contrast no significant changes in tryptase levels were observed in any patient after challenge with the diluent (Fig. 4).

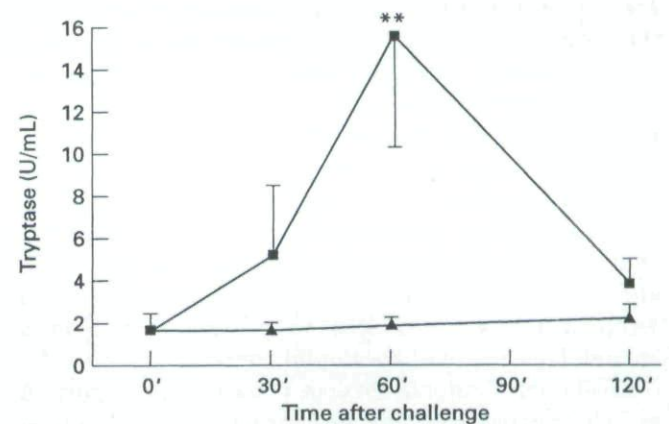


Fig. 4. Mean values of tryptase immunoreactivity in nasal secretions after control ($\blacktriangle$) and Lys-ASA challenge ($\blacksquare$) in ASA-sensitive patients ($n = 6$). ** $P < 0.01$ compared with prechallenge values and 60 min post-saline values (Wilcoxon Matched Pairs Signed Rank test).

Discussion

Nasal provocation with Lys-ASA solution induced nasal symptoms almost exclusively in ASA-sensitive patients indicating the specificity of intranasal challenge with ASA in the dose employed. The fact that the reaction was accompanied not only by a rise in albumin concentration, but also by a significant enrichment of total lavage proteins with albumin suggests that mucosal vascular permeability and plasma protein exudation contributed to this reaction [23]. This was in contrast to our previous study in which oral provocation with ASA increased only albumin concentration without affecting the albumin percentage [17]. Similarly to oral aspirin challenge, intranasal aspirin also induced lysozyme release into the nasal secretions suggesting activation of serous secretory cells. The pattern of vascular and secretory glandular proteins in nasal secretions after Lys-ASA was similar to that observed after antigen challenge [20], but quite different from that after intranasal metacholine [19], capsaicin [24] or metabisulfite [25], where only secretory responses were noticed.

Although nasal and blood eosinophilia are prominent features in patients with ASA-sensitive rhinosinusitis/asthma, quantitative differences from allergic rhinitis/asthma are not unequivocal [7–9]. The eosinophil, a potent proinflammatory cell, has already been implicated in the pathogenesis of ASA-induced reactions as a possible source of mediators, although no convincing evidence for eosinophil activation during ASA-induced reactions has been presented [2,26]. Recently, it has been shown that aspirin-induced bronchospasm was accompanied by a fall in blood eosinophilia, probably reflecting eosinophil recruitment into the respiratory tract [27]. In the present study we observed an early influx of eosinophils into nasal secretions concomitant with development of nasal symptoms after Lys-ASA, and probably reflecting influx of eosinophils into the nasal mucosa. Similarly, eosinophils infiltrate the nasal mucosa early after specific allergen challenge, although they appear again in the lavage fluids during the late reaction [28,29]. Our study was terminated 120 min after the challenge, and at this time point the number of eosinophils had returned to baseline, so it is not possible to rule out a second, later phase of eosinophil recruitment.

Eosinophil Cationic Protein (ECP) is an important protein released from activated eosinophils, and has been suggested as a major factor in allergic disease [30,31]. Our study demonstrated the rise in ECP associated with eosinophil influx into nasal lavage fluid, indicating that ASA-induced hypersensitivity reactions involve activation of eosinophils (in the light of absence of clinical,

biochemical and cellular responses to Lys-ASA in control patients we omitted measurement of ECP and tryptase in these patients due to high costs of the radioimmunoassay [RIA]). These data provide direct evidence of eosinophil participation in the pathophysiology of aspirin intolerance. Sladek and Szczeklik [27] demonstrated that oral, but not inhalation, challenge with aspirin induced only negligible increases in ECP in plasma several hours after reaction. It is possible, that an early activation of eosinophils may occur only locally, at the site of the reaction, and is not detectable in the peripheral blood. The time course of eosinophil activation by Lys-ASA seems to be similar to that observed after allergen challenge where ECP was released 30–60 min after antigen [29]. Activation of eosinophils in the nasal mucosa, and an increase in ECP in nasal lavage fluid are also observed during the late-phase allergic reaction, so it would be interesting to look at this parameter several hours after Lys-ASA [32,33].

Possible activation of mast cells during ASA-induced asthmatic reaction has been a matter of debate, and studies both favouring or denying its involvement have been published [10,11,34]. Previous studies employing measurement of histamine in nasal secretions yielded inconsistent results demonstrating small increases in histamine in three of four patients [15] or similar increases in some ASA-sensitive patients and in control groups [17]. Furthermore, there is the possibility of non-mast cell sources of histamine in nasal secretions. Thus, studies employing measurement of histamine could not answer the question of possible mast cell activation during ASA-induced reactions. In the present study, mast cell activation in the nasal mucosa was assessed by measurement of tryptase, a specific marker of mast cell degranulation [35]. Tryptase concentrations in nasal washes increased from values below the level of detection 2 U/L to 8–30 U/L at the peak of symptoms, clearly indicating mast cell activation in ASA-induced nasal reactions. Only in one patient, despite a clinical reaction to Lys-ASA, was the tryptase level in nasal lavage fluid not increased. Interestingly, this patient did not show any increase in ECP level after Lys-ASA. In a recent study Fischer *et al.* [36] also demonstrated, that oral aspirin challenge induces significant, but less prominent, increases in nasal tryptase in ASA-sensitive patients.

Although ASA-induced respiratory reactions in patients with the ASA-sensitive asthma/rhinosinusitis syndrome are not immunologically mediated, and do not involve IgE-mediated mechanisms, they do seem to involve an early activation of the same effector cells, i.e. mast cells and eosinophils. Both mast cells and eosinophils, in addition to releasing the cell-specific proteins tryptase and ECP, may be a source of potent

lipid mediators — peptidoleukotrienes — which may be responsible for symptoms after ASA provocation. Peptidoleukotrienes have been shown to be released into the nasal lavage fluids following both intranasal [16] and oral provocation with aspirin [17,36] in sensitive patients. Similarly, an increased urinary excretion of leukotrienes was well documented after oral ASA provocation, and both oral and inhaled provocations were effectively inhibited by specific leukotriene antagonists [37–39]. It is conceivable that mast cells and eosinophils, by releasing leukotrienes, may contribute to ASA-induced respiratory reactions in sensitive patients.

The early activation of mast cells and eosinophils during IgE-mediated, antigen-induced early reactions in the nasal mucosa, is the initiating event in the pathophysiology of the allergic late-phase reaction. It seems possible, that ASA-induced mast cell activation reaction may also be followed by a late inflammatory phase. This suggestion is compatible with the observation that the ASA-induced asthmatic reaction, similarly to the antigen-induced reaction, is followed by an increase in the airway responsiveness to methacholine [27]. Further studies are required to determine whether or not local challenge with aspirin involves protracted inflammatory responses in the airway mucosa.

In summary, we have demonstrated that nasal reactions to Lys-ASA in ASA-sensitive patients are accompanied by an increased vascular permeability and secretory cell activation. Concomitantly, an influx of cells into nasal secretions occurs with significant enrichment in the percentage of eosinophils. The influx of eosinophils into nasal secretions is associated with an increase in the concentration of Eosinophil Cationic Protein (ECP) indicating activation of eosinophils, and parallel increase in the tryptase level suggests activation of mucosal mast cells. Our study indicates that both mast cells and eosinophils may be important sources of mediators contributing to the pathophysiology of the airway response to aspirin.

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