

ORIGINAL ARTICLE

Chemokine CC-ligand 5 production and eosinophil activation into the upper airways of aspirin-sensitive patients

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Summary

Background Airway eosinophilia is a hallmark of aspirin-sensitive asthma/rhinitis.**Objective** We have investigated chemokine CC-ligand 5 (CCL5) production and its association with eosinophil activation in the upper airways of aspirin-sensitive patients both *in vivo* and *in vitro*.**Methods** Twenty aspirin-sensitive asthma/rhinosinusitis patients, 18 atopic-tolerant asthma/rhinosinusitis patients and 15 healthy control subjects took part in the study. All subjects were challenged with saline and lysine-acetylsalicylic acid (L-ASA) on separate occasions. Nasal lavages were obtained at baseline and 120 min after challenge and analysed for mediators' release.**Results** When compared with control subjects, the baseline levels of CCL5 were significantly increased in both sensitive and tolerant patients (there was no significant difference in CCL5 concentrations between these two groups, $P > 0.05$). However, L-ASA nasal challenge induced significantly increased levels of CCL5 in the sensitive patients but not in the tolerant subjects (median: 380 vs. 140 pg/mL, $P < 0.0001$). Similarly, the concentrations of both eosinophil cationic protein (ECP) and cysteinil leukotriene (cys-LTs) were increased significantly in the aspirin-sensitive but not in the tolerant patients. There was a trend towards a significant correlation between CCL5 and ECP concentrations in the sensitive patients following L-ASA challenge. On incubation with aspirin, nasal tissue derived from aspirin-sensitive but not that derived from tolerant subjects released increased CCL5 levels in culture. As determined by immunohistochemistry, CCL5 was predominantly localized to the nasal airway epithelium.**Conclusion** Altogether, these findings suggest that CCL5 is released in aspirin-sensitive asthma/rhinosinusitis.**Keywords** aspirin sensitivity, CCL5/RANTES, ECP, eosinophils, leukotrienes

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Introduction

Aspirin-exacerbated respiratory disease (AERD) is characterized by the presence of chronic rhinosinusitis, nasal polyposis and asthma, which is precipitated in response to aspirin [1]. The aetiology of aspirin acetylsalicylic acid (ASA) sensitivity is complex and not fully understood. Most studies conclude that aspirin causes inhibition of cyclooxygenase-1 (COX-1), shunting arachidonic acid (AA) metabolism towards an overproduction of cysteinil leukotrienes (cys-LTs), while there is a rapid decrease in the synthesis of COX-1 products including prostaglandin E2 (PGE2), which is known to have bronchodilator properties [1–4]. These changes of the AA metabolism in the airways of AERD patients result in bronchoconstriction

and increased mucus production. Increased cys-LTs levels have been found in the airways of ASA-sensitive patients [5, 6]. Moreover, leukotriene receptor antagonists improve lower airway parameters in these patients [7, 8]. Similarly, patients exposed to an oral aspirin challenge do not experience a respiratory reaction when they are pre-treated with inhaled PGE2 [9, 10]. To date, however, the sequence of biochemical events regulating the ASA-induced reaction is not fully understood.

Eosinophil recruitment is considered to play a key role in the inflammatory response that characterizes AERD. Indeed, eosinophil numbers in bronchial biopsies and nasal polyps derived from ASA-sensitive patients are fourfold greater than in airway biopsies from ASA-tolerant asthmatics and 15-fold more numerous than in

Table 1. Clinical characteristics of the subjects

	ASA-sensitive (n = 20)	ASA-tolerant (n = 18)	HCS (n = 15)
Age (years)	44.4±14.3	29.8±9	35.25±11.3
Methacholine PC ₂₀ (mg/mL)	2.5±1	3.0±2	> 32
Mean basal FEV _{1s} (% predicted)	97±12	103±22	107±6
Mean basal nasal flow (mL/s)	446±24	431±27	742±86
Nasal polyps	11	1	0
Serum IgE (UI/L)	156±49	561±17*	90±37
Atopics	4	7	0

* $P < 0.05$.

HCS, healthy control subjects; ASA, acetylsalicylic acid; FEV_{1s}, forced expiratory volume in 1 s.

healthy subjects [6]. On activation, these cells release a vast array of mediators including leukotrienes, basic proteins (major basic protein and eosinophil cationic protein), cytokines and oxygen free radicals that cause local tissue damage [11, 12]. Moreover, eosinophils have been identified as a major cell source of LTC₄ synthase, which regulates leukotriene production [6, 13]. Thus, understanding the mechanisms by which eosinophils are recruited into the airways may shed new light on the pathogenesis of this disease.

The chemokine CC-ligand 5 (CCL5) [previously named: RANTES (regulated on activation, normal T-cell expressed and secreted)] is a potent eosinophil attractant. The *in vitro* effects of CCL5 on eosinophils include chemotaxis, transendothelial migration, induction of Ca²⁺ mobilization and actin polymerization, degranulation and stimulation of the respiratory burst [14]. In addition, CCL5 activates monocytes and lymphocytes [15]. This chemokine has been associated with eosinophil recruitment into the airways of atopic asthmatics [14, 16]. In 2001, Pods et al. [17] reported increased CCL5 expression in nasal polyps but did not detect any difference in the expression of this chemokine between ASA-sensitive and ASA-tolerant patients. In contrast, they showed increased expression of both CCL11/eotaxin and CCL24/eotaxin-2 in nasal polyps derived from ASA-sensitive when compared with those from ASA-tolerant patients. In the present study, we performed a lysine-ASA (L-ASA) nasal challenge and studied CCL5 release in ASA-sensitive patients.

Methods

Subjects

Fifty-three subjects participated in this study (Table 1). Their atopic status was investigated by skin-prick testing with different allergens. Neither oral corticosteroids nor antileukotrienes were given to patients 4 weeks before the study and β_2 -agonists were withdrawn 48 h before the

study. ASA sensitivity was established by clinical history and a nasal L-ASA challenge. Before the challenge, all patients were subjected to a rhinological examination with anterior rhinoscopy to evaluate the presence of nasal polyps. Eleven out of the 20 ASA-sensitive patients had small nasal polyps (Lund-Mackay score = 1). Patients with nasal polyps of grades 2 and 3 did not participate in the study as they could not undergo reliable rhinometry measurements. The subjects' characteristics are shown in Table 1. The hospital's ethic committee approved the study.

Study design

Subjects were divided into three groups [ASA-sensitive ($n = 20$), ASA-tolerant ($n = 18$) and healthy control subjects (HCS) ($n = 15$)]. The ASA-sensitive group had been diagnosed previously by both clinical history and a positive response to a nasal L-ASA (total = 25 mg) challenge. A baseline nasal lavage was carried out in all subjects during the screening visit. Subsequently, they were challenged with saline and L-ASA, and a nasal lavage was performed 120 min later. In order to study the kinetics of release of CCL5, eight of the 20 ASA-sensitive patients were rechallenged 6 weeks after the initial study. There was a week's washout period between each study visit ($n = 3$).

Nasal lysine-acetylsalicylic acid challenge

A nasal L-ASA challenge was performed using a modified protocol of that described by Casadevall et al. [18]. The nasal challenge was performed with 25 mg of L-ASA (12.5 mg L-ASA was given in a small filter strip and placed on each inferior turbinate). The nasal response was evaluated by rhinometry and clinical response by scoring the subjective nasal symptoms using a visual analogue scale ranging from 0 (no symptoms) to 30 (severe symptoms) as described previously [19]. The clinical symptoms score included rhinorrhoea, nasal blockage, itching of the nose, palate and/or throat and sneezing. The positive nasal reaction was defined as the appearance of nasal symptoms such as rhinorrhoea, nasal congestion, sneezing and a 25% decrease of the total nasal flow value, as compared with the baseline measurement, or a 40% bilateral decline of inspiratory nasal flow, as compared with the baseline value assessed by rhinomanometry. Subjects with baseline nasal inspiratory flow < 250 mL/s did not participate in the study. The bronchial response was also evaluated by performing spirometry.

Quantitation of eosinophil cationic protein and cysteinyl leukotrienes in nasal lavage fluid

Detection of eosinophil cationic protein (ECP) and cys-LTs in nasal lavage fluid (NALF) was performed by

commercial enzyme immunoassays (MBL, Nagoya, Japan and R&D Systems, Minneapolis, MN, USA, respectively) [20, 6]. The total concentration of cys-LTs was determined after purification on a silica column (AccuBond II, Agilent Technologies, Sta Clara, CA, USA) as described previously [6]. The sensitivities for ECP and cys-LTs were 0.12 and 26 pg/mL, respectively. The concentrations of all mediators were calculated from the standard curve generated from the standards.

Quantitation of chemokine CC-ligand5 in nasal lavage fluid

Measurement of CCL5 in NALF was performed using a two-antibody sandwich ELISA (capture antibody: MAB 678, detection antibody: BAF 278, R&D Systems) as described previously [16]. The concentration of CCL5 was calculated from the standard curve using a CCL5 recombinant protein (278-RN, R&D Systems). The lower limit of detection was 15 pg/mL.

Quantitation of chemokine CC-ligand5 from nasal tissue in vitro and immunohistochemistry

To investigate whether CCL5 is released *in vitro*, nasal tissues (NTs) were obtained from six ASA-sensitive patients and six ASA-tolerant subjects undergoing nasal surgery and processed as described previously [21]. After washing with culture medium [minimum essential medium (MEM), NTs were then placed on nitrocellulose paper to absorb excess fluid and weighed. After cutting NTs into 1–2 mm large specimens, they were placed in a six-well culture plate, 1 mL of culture medium [MEM with 5% bovine fetal serum (BFS), 1 mmol/L glutamine, 2 mmol/L pyruvate, 100 U/L streptomycin, 100 U/L penicillin] was added and then stimulated with 10 µL of L-ASA 10× (900 mg of L-ASA in 1000 mL of PBS) to obtain a final concentration of 500 µM and cultured at 37 °C in a 0.5% CO₂ atmosphere. After incubation for 24, 48 and 96 h, the supernatants were collected and kept at –70 °C for CCL5 measurements. CCL5 expression was investigated in NTs by immunohistochemistry as described above. Immunohistochemistry for CCL5 was performed by indirect immunoperoxidase staining as described previously [22]. The sections were counterstained using Meyer's haematoxylin.

Statistical Analysis

The concentrations of CCL5, ECP and cys-LTs in nasal lavages and tissue culture were tested by the Kruskal–Wallis test. Correlations between chemokine levels and ECP were evaluated using the Pearson correlation coefficient test. A *P*-value < 0.05 was considered statistically significant. A post-hoc analysis of the sample size using G power (version 3.0.10) showed that 18 ASA-sensitive and

20 ASA-tolerant subjects would have sufficient power (80%) to test our hypothesis.

Results

Steady-state aspirin-exacerbated respiratory disease

There was no significant difference in the volume of baseline lavage fluid recovered from any of the three groups of patients. When compared with HCS, the baseline concentrations of ECP (median: 0.12 ng/mL) were significantly increased in ASA-sensitive (median: 28 ng/mL) and ASA-tolerant patients (median: 20 ng/mL), *P* < 0.001 (Fig. 1a). Similarly, the baseline levels of cys-LT were significantly increased in these two groups of subjects (ASA-sensitive: median, 130 pg/mL; ASA-tolerant: 100 pg/mL) as compared with HCS (median: 45 pg/mL), *P* < 0.001 (Fig. 1b). There was no significant difference in ECP concentrations and cys-LT levels between ASA-sensitive and ASA-tolerant patients (Fig. 1a and b).

Chemokine CC-Ligand5 concentrations in nasal lavage fluid

Measurements of CCL5 showed that when compared with HCS, the baseline concentrations of this chemokine were almost threefold greater in the ASA-sensitive and ASA-tolerant patients (Fig. 1c) (*P* < 0.05). There was no significant difference in the levels of CCL5 between these two groups of patients (median: 76 vs. 66 pg/mL, *P* > 0.05), and there was no significant correlation between CCL5 levels and ECP concentrations in any of the three groups of subjects (data not shown).

Lysine-acetylsalicylic acid induced rhinosinusitis

Intranasal challenge with L-ASA, but not with saline solution, induced clinical symptoms, including rhinorrhoea, nasal congestion and sneezing, 30–60 min after challenge (symptoms lasted up to 2 h) in all ASA-sensitive patients. The mean composite score in ASA-sensitive patients was 8.2 ± 0.5 and 16.8 ± 1.5 after saline and L-ASA challenge, respectively (*P* < 0.05). No difference in the clinical response to L-ASA was found in both ASA-tolerant patients and HCS. The mean composite score in ASA-tolerant patients was 2.3 ± 0.3 and 3.2 ± 0.8 after saline and L-ASA challenge, respectively. The corresponding values for HCS were 0.9 ± 0.2 and 1.2 ± 0.3 (*P* > 0.05). Following L-ASA challenge, there was a significant decline in the mean nasal inspiratory flow in ASA-sensitive patients (68 ± 10%). In contrast, neither ASA-tolerant nor HCS showed a significant decrease of the nasal inspiratory flow (7% and 5%, respectively). There was a significant correlation between the decrease in the mean nasal inspiratory flow and the mean composite score in the

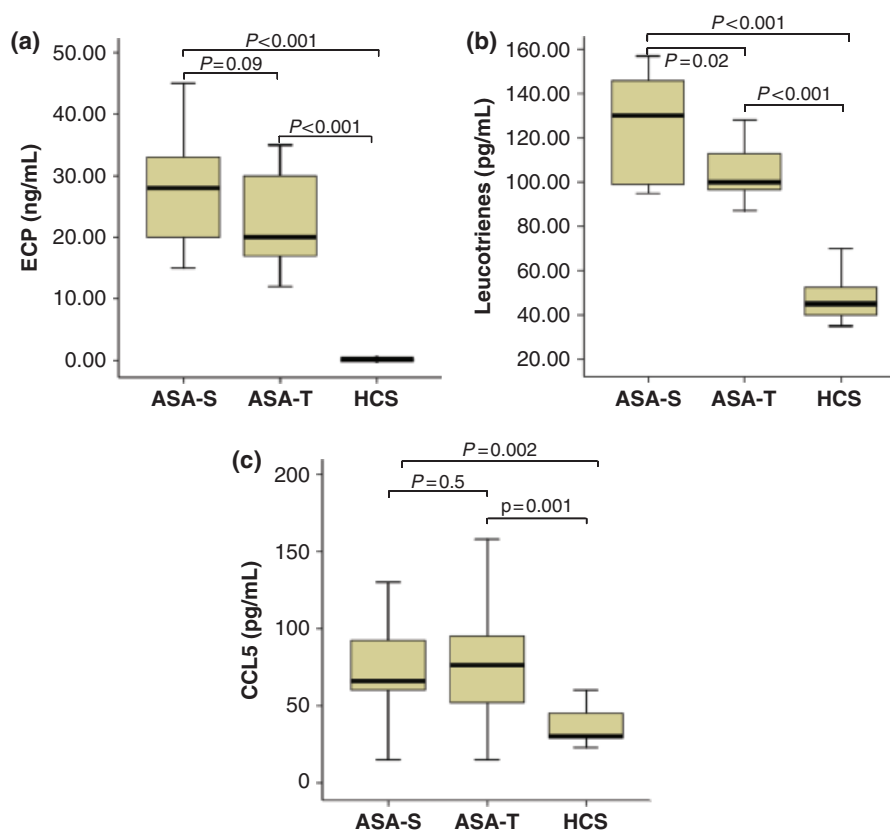


Fig. 1. Baseline concentrations of the chemokine CC-ligand (CCL5), eosinophil cationic protein (ECP) and leukotrienes in nasal lavages derived from acetylsalicylic acid-sensitive patients (ASA-S), ASA-tolerant patients (ASA-T) and healthy control subjects (HCS). Horizontal lines represent median values.

Table 2. Correlation between clinical findings and CCL5 and ECP release

	Pearson's correlation	Significance (<i>P</i>)
NIF vs. NCS	0.60	0.01
CCL5 vs. NIF	0.39	0.02
CCL5 vs. NCS	0.44	0.01
ECP vs. NIF	0.59	0.001
ECP vs. NCS	0.45	0.01

CCL5, chemokine CC-ligand 5; ECP, eosinophil cationic protein; NCS, mean nasal composite score; NIF, mean nasal inspiratory flow.

ASA-sensitive patients ($r=0.6$, $P<0.01$) (Table 2). The volume of secretions recovered in the lavage fluid increased significantly in the ASA-sensitive patients following L-ASA challenge (Table 3).

Eosinophil cationic protein and cysteinil leukotrienes

The concentrations of ECP were significantly increased following the L-ASA challenge when compared with the saline challenge in the ASA-sensitive group (median: 72 vs. 28 ng/mL, $P<0.0001$). In contrast, there was no statistical difference between the saline- and the L-ASA-

Table 3. Lavage fluid recovered

Nasal lavage (mL)	ASA-sensitive	ASA-tolerant	HCS
Basal	10.1±0.5	10.2±0.8	9.8±1.2
Saline challenge	10.4±0.4	9.9±0.9	10.0±0.3
Allergen challenge	13.8±1.5*	10.7±0.8	10.1±0.7

* $P<0.05$.

HCS, healthy control subjects; ASA, acetylsalicylic acid.

challenged lavages in both ASA-tolerant patients (median: 33 vs. 20 ng/mL, $P>0.05$) and in HCS (median: 0.17 vs. 0.12 ng/mL, $P>0.05$) (Fig. 2a). Likewise, the levels of cys-LTs were found to be increased in nasal lavage from the L-ASA-challenged as compared with the saline-challenged patients (median: 260 vs. 130 pg/mL) in the ASA-sensitive group ($P<0.001$) (Fig. 2b). No differences were found between the L-ASA- and saline-challenged lavages in both the ASA-tolerant group (median: 147 vs. 100 pg/mL, $P>0.05$) and the HCS (median: 67 vs. 45 pg/mL, $P>0.05$). Both ECP and cys-LTs levels correlated with either the mean composite score ($r=0.45$, $P=0.01$; $r=0.675$, $P<0.01$, respectively) or the mean

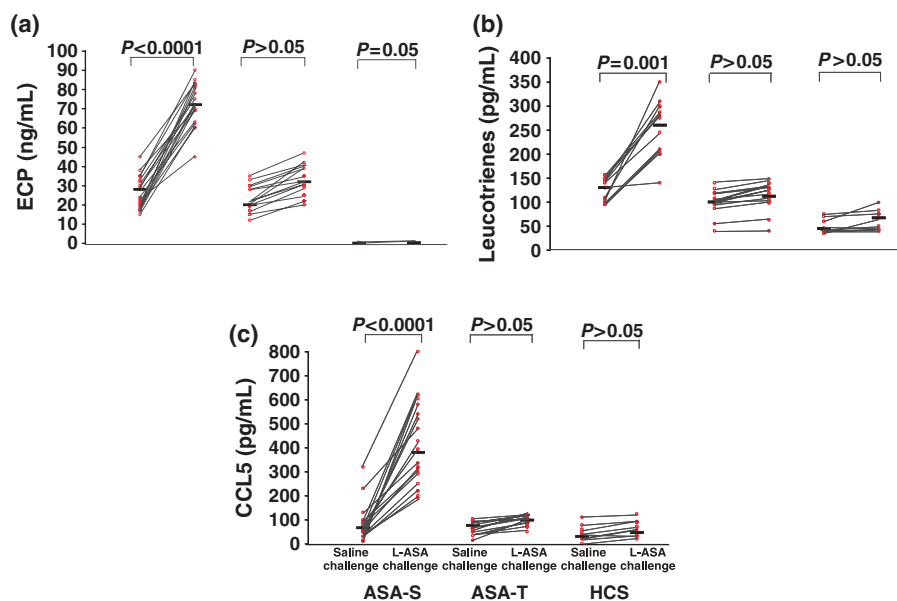


Fig. 2. Effect of L-ASA and saline challenges on the release of chemokine CC-ligand (CCL5), eosinophil cationic protein (ECP) and leukotrienes in nasal lavages derived from acetylsalicylic acid-sensitive patients (ASA-S), ASA-tolerant patients (ASA-T) and healthy control subjects (HCS). Horizontal lines represent median values. L-ASA, lysine-acetylsalicylic acid.

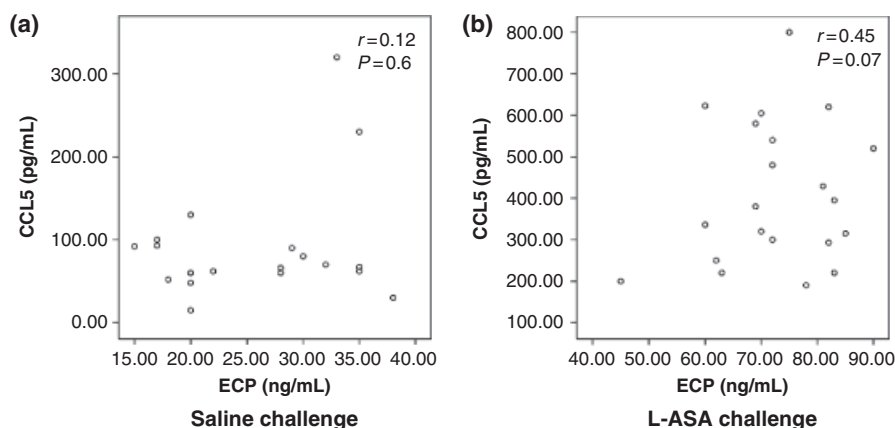


Fig. 3. Correlation between chemokine CC-ligand (CCL5) levels and eosinophil cationic protein (ECP) concentrations following L-ASA and saline challenge in ASA-sensitive patients. L-ASA, lysine-acetylsalicylic acid.

nasal inspiratory flow ($r = 0.59$, $P < 0.001$; $r = 0.632$, $P = 0.001$, respectively) in the ASA-sensitive patients.

Lysine-acetylsalicylic acid induces chemokine CC-ligand5 production in vivo

CCL5 levels increased significantly following L-ASA challenge when compared with the saline challenge in the ASA-sensitive patients (median: 380 vs. 66 pg/mL, $P < 0.0001$), but neither in ASA-tolerant patients (median: 140 vs. 76 pg/mL, $P > 0.05$) nor in healthy control subjects (median: 47 vs. 30 pg/mL, $P > 0.05$), Fig. 2c. There was a trend towards a significant correlation between CCL5

levels and ECP concentrations after L-ASA- ($r = 0.45$, $P = 0.07$), but not after saline challenge ($r = 0.12$, $P = 0.6$), in the ASA-sensitive patients (Fig. 3). Interestingly, CCL5 levels correlated with the mean nasal composite score ($r = 0.44$, $P = 0.01$) and the mean nasal inspiratory flow ($r = 0.39$, $P = 0.02$) in the L-ASA-sensitive group (Table 2).

Kinetics of chemokine CC-ligand5 release

The nasal time-response to lysine-ASA was studied in order to investigate the kinetics of release of CCL5. Measurements of this chemokine showed increased levels

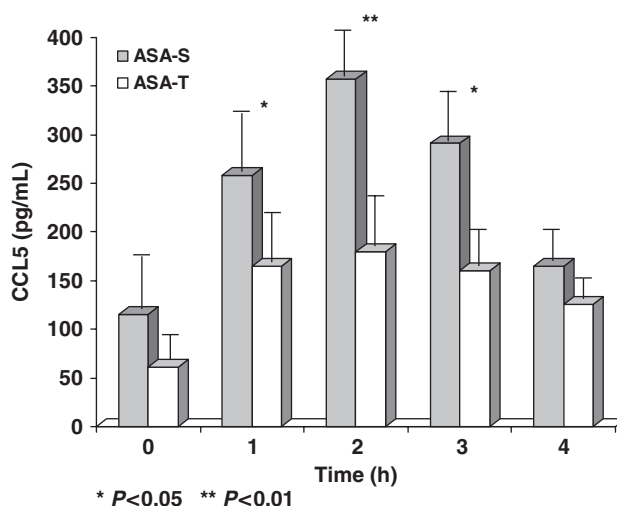


Fig. 4. Kinetics of chemokine CC-ligand (CCL5) release in nasal lavage from ASA-sensitive (ASA-S) and ASA-tolerant patients (ASA-T).

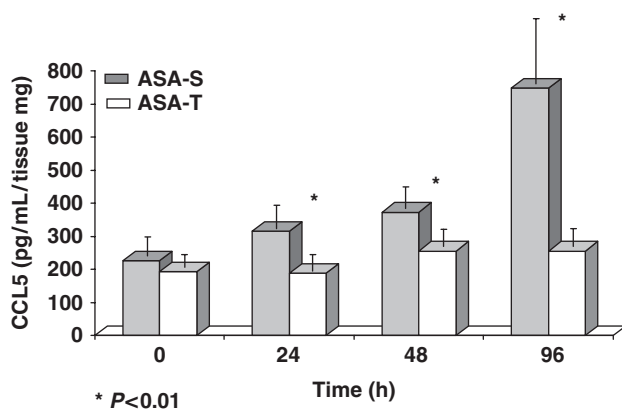


Fig. 5. Effect of L-ASA on the *in vitro* release of chemokine CC-ligand (CCL5) from cultured nasal tissue explants derived from ASA-sensitive (ASA-S) and ASA-tolerant patients (ASA-T). L-ASA, lysine-acetylsalicylic acid.

1 h after challenge, which peaked at 2 h ($P < 0.05$) (Fig. 4). By 4 h CCL5 returned towards baseline values.

Lysine-acetylsalicylic acid induces chemokine CC-ligand5 production *in vitro*

Measurements of CCL5 released by nasal tissue explants into the culture medium demonstrated that although this cytokine was released and synthesized constitutively at a very low concentration, incubation of nasal tissue with L-ASA induced a significant release of CCL5 from nasal tissue derived from ASA-sensitive patients but not from that derived from ASA-tolerant subjects (Fig. 5). Indeed, time-response studies of nasal tissue explants demonstrated that levels of CCL5 were increased significantly by

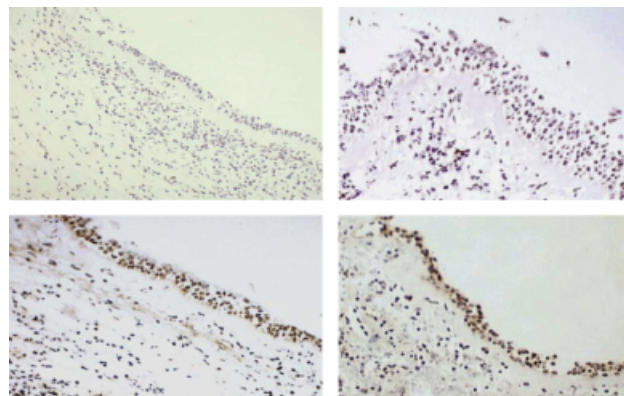


Fig. 6. Immunohistologic staining of chemokine CC-ligand (CCL5) in nasal tissue at baseline (top right panel) and 24 h after lysine-acetylsalicylic acid (L-ASA) challenge *in vitro* (bottom panel shows nasal tissue from two different patients). Immunohistological staining of negative control with an anti-IgG, mAb (top left panel). Nasal tissue specimens were obtained from acetylsalicylic acid-sensitive patients (ASA) who underwent surgery for therapeutic reasons.

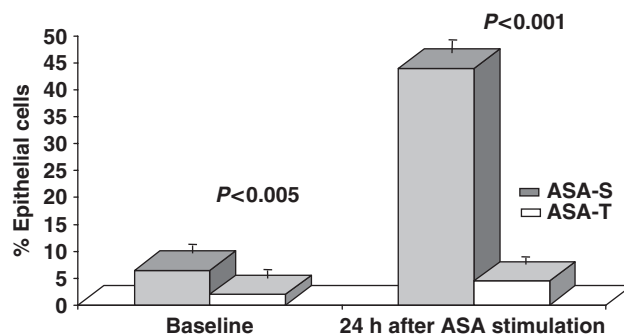


Fig. 7. Effect of lysine-acetylsalicylic acid (L-ASA) on the epithelial expression of chemokine CC-ligand (CCL5) in the nasal tissue explants derived from acetylsalicylic acid-sensitive patients (ASA-S) both at baseline and 24 h after L-ASA stimulation.

24 h of incubation and peaked by 96 h, suggesting a long-lasting production of this cytokine (Fig. 5).

Detection of chemokine CC-ligand5 in nasal tissue by immunohistochemistry

Figure 6 shows nasal tissue sections stained with goat anti-CCL5 antibody (top right panel) and an irrelevant antibody from the same species (top left panel), and demonstrates that CCL5 was mainly localized to the airway epithelium although there was sparse immunoreactivity localized to mononuclear cells in the subepithelial tissue at baseline. Moreover, L-ASA stimulation *in vitro* induced further CCL5 expression (bottom left and bottom right panel). Figure 7 shows quantitation, by colour image analysis, of the percentage of epithelium staining both at baseline and 24 h after *in vitro* stimulation with L-ASA.

Discussion

Although it is well known that cys-LTs play a prominent role, the cascade of biochemical events that take place during the ASA-induced reaction is not fully understood. This is the first study to demonstrate that CCL5 is involved in the pathogenesis of ASA-sensitive patients. Levels of this cytokine were found to be increased in the steady state. In ASA-sensitive patients, L-ASA challenge induced further CCL5 production. Interestingly, nasal tissue derived from ASA-sensitive patients also released CCL5 upon L-ASA stimulation *in vitro*. By immunohistochemistry, CCL5 was localized predominantly to the airway epithelium. These findings altogether suggest that this cytokine may contribute to the inflammatory response that takes place in aspirin-sensitive asthma.

Aspirin-exacerbated respiratory disease (AERD) is characterized by overproduction of cys-LTs and tissue eosinophil infiltration into the airways. Cys-LTs are potent bronchoconstrictor mediators while the eosinophils are considered to cause tissue damage leading to airway inflammation and bronchial hyperactivity. Several eosinophil activators have been associated with ASA sensitivity including IL-5, CCL7/MCP-3, CCL11/eotaxin and CCL24/eotaxin-2 [17, 23, 24]. However, the role of CCL5 in this disease has not been established. In the present study, we have used the nasal provocation with aspirin that has been used with increasing frequency to study inflammatory changes induced by the local application of L-ASA aspirin in the nasal airways. This method has a number of advantages over both the whole-lung inhalation challenge and the oral challenge: it is safe as it induces predominantly upper respiratory symptoms, it allows both direct deposition and sampling and it is not invasive. Indeed, in the present study, L-ASA challenge induced symptoms only in the upper airways, including rhinorrhoea, sneezing and nasal congestion, with a parallel release of cys-LTs in nasal secretions, indicating the specificity of intranasal challenge in ASA-sensitive patients.

Having characterized the response to aspirin, we next investigated CCL5 release and demonstrated increased levels of this cytokine in nasal lavages obtained at baseline, suggesting that it is involved in steady-state AERD: levels of CCL5 in ASA-sensitive patients were comparable with those observed in ASA tolerant (allergic rhinitis patients) and threefold greater concentrations than in control subjects. Of particular interest was the finding that L-ASA challenge increased further CCL5 release in ASA-sensitive patients. Indeed, concentrations of this chemokine increased almost sixfold following L-ASA challenge, which further supports the hypothesis that this cytokine is involved in the ASA-induced reaction. We have previously purified natural CCL5 from the bronchoalveolar (BAL) fluid derived from asthmatic subjects

exposed to segmental allergen challenge and demonstrated that BAL CCL5 is biologically active on eosinophils [25], which suggests that nasal CCL5 released into the upper airways of ASA-sensitive patients may also activate these cells.

To investigate eosinophil activation and recruitment, we measured ECP and showed that L-ASA challenge induces ECP release. This finding is consistent with the report of Kowalski et al. [20], who reported ECP following L-ASA challenge. Interestingly, there was a trend towards a significant correlation between ECP and CCL5 concentration following L-ASA challenge, which is consistent with the view that the release of this chemokine is associated with eosinophil recruitment in this situation. Alternatively, other eosinophil attractants may also recruit and activate these cells. In previous studies, we and other groups have identified the eosinophil-activating chemokines CCL7, CCL11 and CCL24, IL-5 and LTD4 associated with aspirin sensitivity [17, 23, 24, 26]. The finding that CCL5 is released into the airways of ASA-sensitive patients suggests that this chemokine, in concert with other eosinophil-activating chemokines, may recruit eosinophils into the airways of ASA-sensitive patients. Interestingly, the chemokine receptor CCR3 serves as a receptor for CCL5, CCL7, CCL11 and CCL24. Thus, it is tempting to hypothesize that neutralizing the effects of CCR3 may have therapeutic implications in patients having aspirin sensitivity.

Once the involvement of CCL5 in ASA-sensitive patients was clearly established, we stimulated NT with L-ASA and demonstrated that this chemokine is also released *in vitro*. Indeed, NT derived from ASA-sensitive, but not that derived from ASA-tolerant patients, released CCL5 in a time-dependent manner upon L-ASA stimulation. At 24 h, CCL5 concentrations increased and peaked at 96 h, which suggests a long-lasting production of this chemokine.

In order to investigate the cellular source of CCL5, we have performed immunohistochemical staining of nasal tissue obtained from patients undergoing nasal surgery. CCL5 can be produced by several cells implicated in the airway inflammatory response in AERD including eosinophils [27], T-lymphocytes [15], monocytes [28] fibroblasts [29], vascular endothelial cells [30, 31] and airway epithelial cells [32, 33]. However, we have demonstrated that immunoreactivity was predominantly localized to the nasal epithelium, which was found to be increased in ASA-sensitive as compared with ASA-tolerant patients. This finding is consistent with previous observations that CCL5 immunoreactivity is localized to the epithelium in nasal polyp biopsies and in nasal biopsies from rhinitis and non-rhinitis patients and in bronchial biopsies from non-asthmatic and unchallenged asthmatic subjects [33, 34]. Moreover, the finding that L-ASA stimulated the expression of CCL5 in the nasal epithelium *in vitro* further supports the hypothesis that CCL5 may derive from the

airway epithelium. Platelets are another important potential source of CCL5 [35]. Thus, platelet-derived CCL5 may also be released into tissue as a result of the vascular leakage that takes place during the local inflammatory reaction in aspirin-sensitive asthma [20].

In summary, we have shown that patients having aspirin sensitivity release high concentrations of the chemokine CCL5 under basal conditions, which is further enhanced by nasal L-ASA challenge. The parallel increase in ECP and CCL5 concentrations suggests that CCL5 may recruit and activate eosinophils in ASA-sensitive patients. Interestingly, these clinical findings were further supported by *in vitro* experiments showing that L-ASA-stimulated NT, derived from ASA-sensitive patients, release CCL5 in culture. By immunohistochemistry, CCL5 was localized predominantly to nasal epithelial cells. Taken together, these findings suggest that CCL5 play an important role in the recruitment and activation of eosinophil in aspirin sensitivity.

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