

# Systemic responses after bronchial aspirin challenge in sensitive patients with asthma

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**Background:** In aspirin-sensitive patients with asthma, bronchial obstruction induced by oral aspirin may be associated with extrabronchial symptoms, suggesting the systemic character of the response.

**Objective:** Go assess potential systemic effects of local aspirin challenge, hemopoietic progenitors were measured in the peripheral blood of challenged patients.

**Methods:** In 19 patients with a history of aspirin-induced asthma, placebo-controlled bronchial challenges with lysine-aspirin were performed. Peripheral blood was collected before and then 1 hour and 20 hours after challenge (placebo or aspirin). Using the flow-cytometric method, the numbers of leukocyte (CD34<sup>+</sup> cells) and eosinophil (CD34<sup>+</sup>CD125<sup>+</sup> cells) progenitors were determined.

**Results:** The challenge was positive in 13 patients; 6 patients had isolated local bronchial reaction, and 7 patients developed systemic symptoms (bronchial and extrabronchial). In patients with positive challenge ( $n = 13$ ), leukocyte progenitors increased significantly at 1 hour and 20 hours after challenge (mean, 0.04% at baseline, 0.066% at 1 hour after challenge, and 0.073% at 20 hours;  $P < .05$ ). Eosinophil progenitors raised significantly from mean 0.017% before challenge to 0.04% ( $P < .05$ ) at 20 hours after the challenge. At 20 hours after the challenge, the increase in leukocyte and eosinophil progenitors was observed only in patients with systemic reactions. Positive aspirin challenge was associated with a significant increase in eotaxin 2 serum concentration.

**Conclusion:** This study demonstrated that bronchial challenge with aspirin may involve systemic reactions and is associated with mobilization of leukocyte and eosinophil progenitor cells from the bone marrow. (*J Allergy Clin Immunol* 2008;121:348-54.)

**Key words:** Aspirin hypersensitivity, bronchial asthma, AERD, progenitor cells, CD34, aspirin bronchial challenge

Hypersensitivity to nonsteroidal anti-inflammatory drugs (NSAIDs) manifesting with bronchial obstruction can affect 10% to 20% of adult patients with asthma.<sup>1,2</sup> Aspirin-induced obstruction in most patients with asthma is associated with upper

## Abbreviations used

EP: Eosinophil progenitor

LP: Lymphocyte progenitor

NSAID: Nonsteroidal anti-inflammatory drug

airway symptoms (nasal obstruction and rhinorrhea), and in some patients, other extrabronchial symptoms may occur (urticaria/angioedema, dermal flush, conjunctival irritation, or nausea), indicating a systemic character of the reaction.<sup>3,4</sup> The presence of hypersensitivity to aspirin is a hallmark of more severe asthma often complicated with intractable chronic rhinosinusitis and nasal polyposis. These patients have been referred to as having "aspirin triad."<sup>5,6</sup> Because chronic respiratory symptoms are present even in the absence of exposure to NSAIDs, more recently the term *aspirin-exacerbated respiratory disease* (AERD) has been proposed to define this group of patients.<sup>7,8</sup>

The mechanisms of hypersensitivity to aspirin and other NSAIDs is related to pharmacologic properties of these drugs, namely inhibition of cyclooxygenase enzyme,<sup>9</sup> and associated with subsequent activation of inflammatory cells (mast cells and eosinophils)<sup>10,11</sup> and release of mediators including cysteinyl leukotrienes.<sup>12-14</sup>

Allergen-induced IgE-mediated asthmatic responses are usually followed by late-phase reactions related to recruitment of inflammatory cells into the target organ.<sup>15</sup> Bronchial challenge with allergen in sensitive patients with asthma is associated with an increase in the number of eosinophil/basophils progenitors in bone marrow,<sup>16,17</sup> although the changes in eosinophils progenitors in peripheral blood have been less consistent.<sup>18,19</sup>

In this study we aimed to assess, using the flow-cytometric method, possible recruitment of bone marrow-derived leukocyte progenitors into peripheral circulation after diagnostic bronchial challenge with a soluble form of aspirin (lysine-aspirin) in patients with asthma with a history of bronchial reaction to oral NSAIDs. In addition, to get some insight into the potential mechanism of cell recruitment, hemopoietic cytokines were measured in the serum in parallel with challenge with lysine-aspirin.

## METHODS

### Patients

Nineteen patients with asthma (6 men and 13 women; age range, 19-57 years) with a history of bronchial hypersensitivity reactions to oral aspirin or other NSAIDs were studied. To confirm/exclude aspirin hypersensitivity, bronchial challenges with lysine-aspirin were performed. Table 1 summarizes clinical characteristics of recruited patients.

### Challenge procedure

A single-blind bronchial challenge with lysine-aspirin (Aspisol, Bayer, Germany) was performed according to the protocol described by

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**TABLE I.** Clinical characteristics of patients with asthma recruited to the study and distinguished on the basis of the results of bronchial aspirin challenge

	All	Results of bronchial challenge		
		Bronchial reaction	Systemic reaction	No reaction
No. of patients	19	6	7	6
Sex (female/male)	13/6	2/4	6/1	5/1
Mean age (y)	37.5	44.33	31.81	37.5
Age range (y)	19-57	19-57	21-48	24-53
Patients with nasal polyps	12	5	3	3
History of chronic rhinitis	19	6	7	6
History of recurrent urticaria	7	1	5	1
Patients with positive SPT	12	3	5	4
Patients on inhaled glucocorticosteroids:	15	5	5	5
mean daily dose ( $\mu$ g budesonide)	566.67	733.33	457.15	433.33
Patients taking antileukotrienes	5	2	2	1
Eosinophilia (cells/mm <sup>3</sup> ), mean	296.32	224.83	459.57	177.33

Nizankowska et al.<sup>13</sup> The study protocol was approved by local ethics committee. The patients were required to have stable bronchial asthma and FEV<sub>1</sub> higher than 70% of predicted value. Inhaled glucocorticosteroids were withheld 12 hours, long-acting  $\beta_2$ -agonists and theophylline 24 hours, and antileukotrienes 3 days before challenge. Short-acting  $\beta_2$ -agonists were discontinued 8 hours before challenge.

On the first day, only isotonic sodium chloride (diluent-placebo) was administered by inhalation from dosimeter-controlled jet nebulizer (SpiraE-lectro 2; Respiratory Care Center, Hameenlinna, Finland). On the second day, challenge started with inhalation of sodium chloride solution, and if there was no drop of FEV<sub>1</sub> higher than 15%, 30 minutes later increasing doses of lysine-aspirin (1, 2, 5, 13, 40, 90, 220, and 640  $\mu$ mol) were inhaled every 30 minutes until the reaction was considered positive or a cumulative dose of 181.98 mg was reached. Spirometry was performed 10, 20, and 30 minutes after administration of each dose, and extrabronchial symptoms (rhinorrhea, eye itching, nasal congestion, or dermal flush) were recorded and scored on a 5-grade scale). The challenge was considered positive and stopped if a patient developed bronchial symptoms with a fall of FEV<sub>1</sub> >20% or >400 mL and/or if significant extrabronchial symptoms occurred (cumulative score  $\geq$  12). The challenge was also considered positive if an isolated objective cutaneous reaction appeared (rush, local angioedema, and/or urticaria).

Bronchial reactions were treated with nebulization of 2.5 mg albuterol, repeated if necessary. Before being discharged, all patients with a positive reaction received oral prednisone (mean dose, 35 mg), and those with cutaneous symptoms in addition received clemastine intravenously.

### Peripheral blood collection

Blood was collected by venipuncture at 5 time points: before placebo challenge (P0), 1 hour (P1) and 20 hours (P2) after placebo challenge was completed (this time was also the baseline – A0 for aspirin challenge), and then at 1 hour (A1) and at 20 hours (A2) after positive reaction, or in patients with negative response, 1 hour and 20 hours after the last dose of aspirin was inhaled (Fig 1).

### Assessment of circulating leukocyte progenitors and cytokines

Hemopoietic cell progenitors in the whole blood were assessed with a flow cytometer (DAKO Galaxy; DAKO, Basel, Switzerland) according to CD34<sup>+</sup> cell enumeration protocol ISCHAGE.<sup>10</sup> In the consecutive samples, tricolor staining (mAbs from Becton-Dickinson Co, New York, NY) was used to assess subsets of leukocyte progenitors (CD34<sup>+</sup>CD45<sup>+</sup>), eosinophil progenitors (CD34<sup>+</sup>CD45<sup>+</sup>CD125<sup>+</sup>), lymphocyte T progenitors (CD34<sup>+</sup>CD45<sup>+</sup>CD7<sup>+</sup>), and lymphocyte B progenitors (CD34<sup>+</sup>CD45<sup>+</sup>CD19<sup>+</sup>). Isotypic control was performed for every sample. The results for progenitor subsets were expressed as the percentage of CD45<sup>+</sup> cells.

The absolute number of eosinophils in the whole blood was counted. The concentrations of cytokines and chemokines in serum were measured by the ELISA method (R&D Systems, Minneapolis, Minn). The immunoassays had the following sensitivity as reported by the manufacturer: IL-5 (3 pg/mL), GM-CSF (0.26 pg/mL), stem cell factor (9 pg/mL), eotaxin 1 (5 pg/mL), eotaxin 2 (1.83 pg/mL), and eotaxin 3 (2.33 pg/mL).

### Statistical analysis

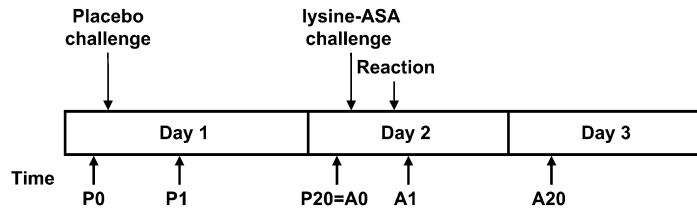
Data are presented as median, 25% and 75%, and minimum and maximum. Statistical analysis to compare 3 groups of patients (negative challenge, patients with systemic and bronchial response) was carried out by 2-way Friedman ANOVA followed by least significant difference (LSD) *post hoc* test and Wilcoxon signed-rank test.  $P < .05$  was considered statistically significant.

## RESULTS

### Clinical response to inhalation of lysine-aspirin

The challenge was assessed as positive in 13 and as negative in 6 patients. There was no significant difference in mean age, FEV<sub>1</sub> % predicted value, daily dose of chronic inhaled glucocorticosteroids, or blood eosinophilia between patients with negative and positive challenge (Table II summarizes clinical response to aspirin challenge).

Of 13 patients with positive response, 6 had isolated local bronchial reaction with significant fall of FEV<sub>1</sub> (median fall, 24% or 790 mL; range, 460-830 mL;  $P < .05$ ), and 7 patients developed extrabronchial symptoms and were considered to have a systemic reaction. Two patients with a systemic reaction presented only extrabronchial symptoms: 1 developed angioedema of the lips and rhinorrhea, and the second patient had rhinorrhea, lacrimation, and dermal flush within 15 minutes after the inhalation of lysine-aspirin. All 5 remaining patients with systemic reactions had nasal symptoms, and 4 in addition had ocular symptoms. In this group, 5 patients experienced lower respiratory symptoms (dyspnea and/or coughing), but only 3 of them had a significant fall of FEV<sub>1</sub>. Median drop of FEV<sub>1</sub> in the group of patients with systemic reaction was 10% (median, 300 mL; range, 210-550 mL) and was significantly smaller than in the group of patients with an isolated bronchial reaction ( $P < .05$ ). Patients with extrabronchial symptoms (ie, systemic reaction) inhaled a significantly higher mean cumulative dose of lysine-aspirin compared with those with an isolated bronchial reaction (median, 181.98 mg and 6.8 mg, respectively;  $P < .05$ ).



**FIG 1.** Blood for flow cytometry and cytokine analysis was collected at the following time points: P0, before placebo inhalation; P1, 1 hour after completion of placebo administration; P20 = A0, 20 hours after placebo administration (the baseline for aspirin challenge); A1, 1 hour after aspirin-induced reaction (in patients with positive reaction) or 1 hour after completion of aspirin administration (in patients with negative challenge); A20, 20 hours after reaction caused by aspirin (in patients with positive reaction) or 1 hour after completion of aspirin administration (in patients with negative challenge).

**TABLE II.** Pattern of clinical reactions in patients subjected to inhaled aspirin challenge

No.	Early reaction after lysine-ASA	Late reaction after lysine-ASA	Clinical reaction to oral NSAID by history*	Cumulative dose of lysine-ASA (mg)	Mean drop of FEV <sub>1</sub> % (mL)	Dose of prednisone (mg) received after reaction
Patients with isolated bronchial reaction						
1	BR	No reaction	BR/ Cut	10.98	20% (830)	30
2	BR	No reaction	BR	2.78	26% (750)	30
3	BR	No reaction	BR	27.18	40% (990)	40
4	BR	No reaction	BR	0.54	26% (460)	100
5	BR	BR	BR	2.78	20% (460)	30
6	BR	No reaction	BR	27.18	22% (830)	30
Patients with systemic reaction						
1	BR, N	U	BR/Cut	181.98	18% (800)	20
2	BR, N, C	U	BR	181.98	9% (300)	30
3	BR, N, C, DF, P	N, BR, C	BR	1.44	20% (420)	20
4	BR, N	BR, U	BR/Cut	181.98	8% (280)	30
5	N, DF, C	No reaction	BR	181.98	6% (140)	30
6	N, AO, C	No reaction	BR/Cut	181.98	10% (210)	30
7	BR, N	No reaction	BR/Cut	66.78	13% (550)	30
Patients with negative provocation						
1	No reaction	No reaction	BR	181.98		
2	No reaction	No reaction	BR	181.98		
3	No reaction	No reaction	BR	181.98		
4	No reaction	No reaction	BR	181.98		
5	No reaction	No reaction	BR	181.98		
6	No reaction	No reaction	BR	181.98		

AO, Angioedema; ASA, aspirin; BR, bronchial; BR/Cut, both bronchial and cutaneous symptoms; C, conjunctivitis; DF, dermal flush; N, nasal; P, puritus; U, urticaria.

\*Pattern of past reaction to oral ingestion of NSAIDS as reported by patient.

The subgroups with isolated bronchial reactions or systemic reactions did not differ with respect to maintenance dose of inhaled steroids, mean FEV<sub>1</sub> % of predicted, or blood eosinophilia.

In 8 of 13 patients with a positive reaction, symptoms started within 30 minutes after aspirin inhalation (early reaction). Five patients developed symptoms later than 3 hours after the challenge (late reaction): 2 patients had urticaria, 2 dyspnea with a fall in peak expiratory flow, and 1 patient developed urticaria and dyspnea with a fall in peak expiratory flow (more than 30% decrease from baseline).

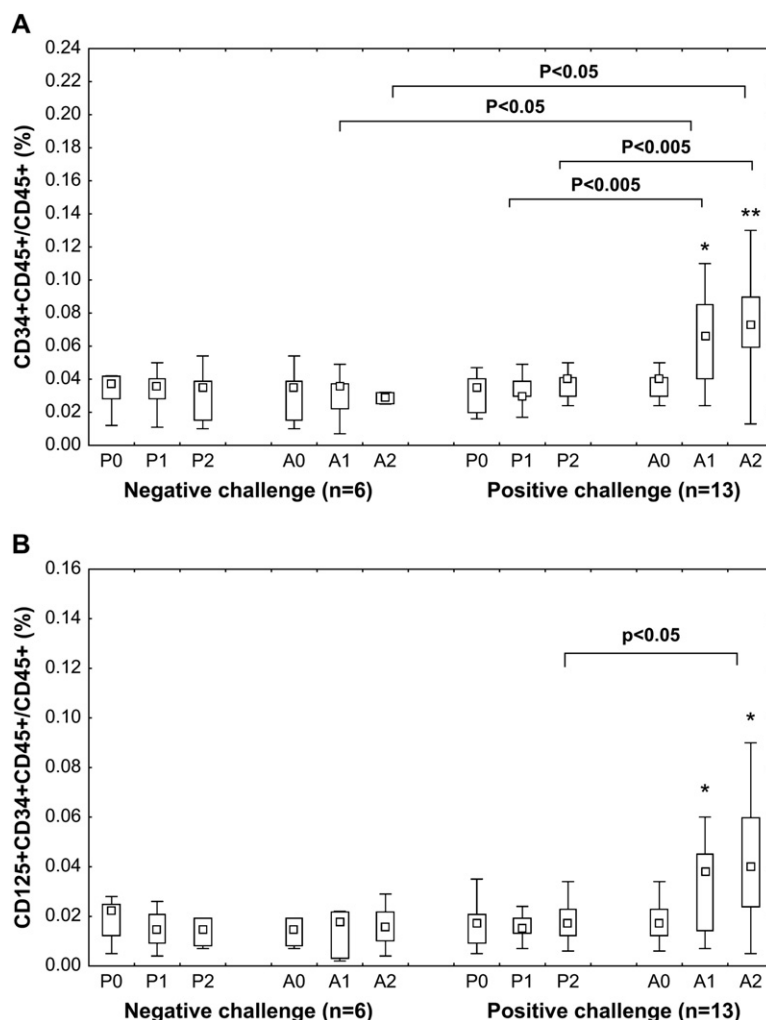
### Circulating progenitor cells after aspirin challenge

In patients with a positive reaction, there was a significant increase in the number of circulating CD34<sup>+</sup>CD45<sup>+</sup> cells (leukocyte progenitors; LPs) and CD125<sup>+</sup>CD34<sup>+</sup>CD45<sup>+</sup> cells (eosinophil progenitors; EPs) in the peripheral blood at 1 hour and 20 hours after challenge (Fig 2, A and B). The percentage of LP cells

increased from a median of 0.04% (0.3% to 0.41%) at baseline to 0.066% (0.04% to 0.085%) at 1 hour ( $P < .05$ ) and to 0.073% (0.059% to 0.09%) at 20 hours ( $P < .05$ ). The percentage of EP cells increased from a median of 0.017% (0.012% to 0.023%) at baseline to 0.038% (0.014% to 0.045%) at 1 hour ( $P < .05$ ) and to 0.04% (0.024% to 0.06%) at 20 hours ( $P < .05$ ).

After negative challenge, no changes in LP cells or EP cells were observed; median values were 0.035% (0.019% to 0.039%) at baseline, 0.036% (0.019% to 0.039%) at 1 hour, and 0.029% (0.026% to 0.036%) and 0.015% (0.009% to 0.018%) at baseline, 0.018% (0.006% to 0.022%) at 1 hour, and 0.016% (0.011% to 0.021%) for LP and EP cells, respectively. No significant changes in LP or EP cells were noticed after placebo administration.

A different pattern of changes in the percentage of circulating progenitors was observed in patients with isolated bronchial reactions ( $n = 6$ ) and in patients with systemic responses ( $n = 7$ ; Fig 3, A and B). Although in both subgroups a significant increase in LP cells was observed at 1 hour after



**FIG 2.** The percentage of leukocyte progenitor cells (**A**) and eosinophil progenitor cells (**B**) after bronchial challenge with lysine-aspirin in patients with negative and positive reaction (\* $P < .05$ , \*\* $P < .005$  compared with A0).

the challenge, only in patients with a systemic reaction the number of LP cells increased significantly at 20 hours. In patients with isolated bronchial response, there was a nonsignificant increase in EP cells at 1 hour after the challenge, but at 24 hours, the number of EP cells returned to baseline (median, 0.017% at baseline, 0.035% at 1 hour, and 0.033% at 20 hours;  $P > .05$ ). In contrast, in patients with a systemic response, a small increase in EP at 1 hour (from 0.017% to 0.027%) was followed by a significant rise in EP cells at 22 to 24 hours (to 0.047%;  $P < .05$ ).

Among all circulating CD34<sup>+</sup>CD45<sup>+</sup> leukocytes, the eosinophil progenitors (CD125<sup>+</sup> cells) were the main population of progenitor cells in patients with either negative or positive aspirin challenge, both before and after the challenge (approximately 50% of LP cells). B-cell progenitors (CD19<sup>+</sup>CD34<sup>+</sup>CD45<sup>+</sup>) were detected in 32 of 95 samples and T-cell progenitors (CD7<sup>+</sup>CD34<sup>+</sup>CD45<sup>+</sup>) were detected in 55 of 95 samples examined before and after placebo or aspirin challenges.

The percentages of circulating B-cell and T-cell progenitors were very low and varied between 0% and 0.015% of CD45<sup>+</sup> cells. No significant differences in the number of circulating

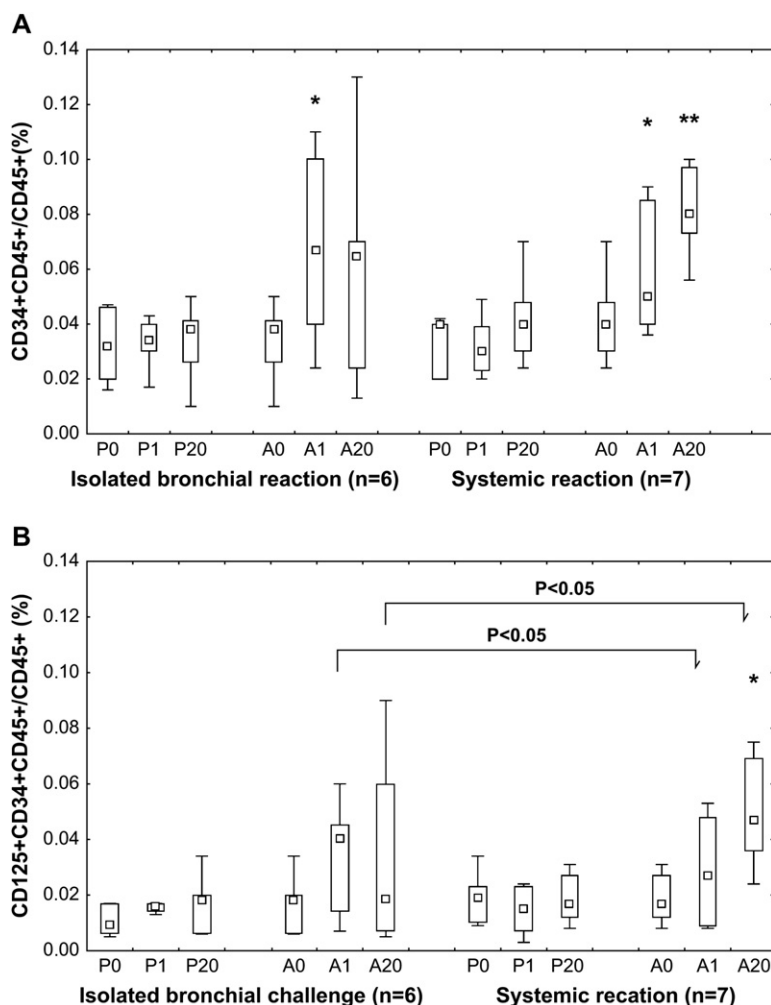
B-cell or T-cell progenitors were observed after placebo or either positive or negative aspirin challenge.

No differences in baseline eosinophil count (eosin staining) were observed between patients with positive or negative challenge outcomes (data not shown). After placebo challenge, the mean eosinophil count, from 319/mm<sup>3</sup> (range, 177-350) before challenge to 232/mm<sup>3</sup> (range, 199-248;  $P = .06$ ), was not changed; however, a nonsignificant fall of eosinophil count was observed 20 hours after aspirin challenge in the group of patients with systemic reactions.

### Circulating chemokine and cytokine levels after aspirin challenge

Serum concentrations of eotaxin 1, 2, and 3, IL-5, GM-CSF, and SCF were measured in 10 patients with positive (6 with isolated bronchial and 4 with systemic reactions) and in 4 with negative aspirin challenges.

Median eotaxin 2 concentration before aspirin administration was significantly higher in patients with positive aspirin challenge (117 pg/mL; range, 88-145 pg/mL) compared with patients with



**FIG 3.** The percentage of leukocyte progenitor cells (**A**) and eosinophil progenitor cells (**B**) after bronchial challenge with lysine-aspirin in patients with isolated bronchial reactions, systemic reactions, and negative response (\* $P < .05$ ; \*\* $P < .005$  compared with A0).

negative challenge (median, 65 pg/mL; range, 44-92 pg/mL;  $P < .05$ ). In patients with positive aspirin challenge, there was a significant increase in median serum eotaxin 2 concentration at 20 hours (to 191 pg/mL; range, 83-347 pg/mL) compared with baseline ( $P < .05$ ; Fig 4). There were no significant differences between baseline level of eotaxin 1 in patients with positive (96 pg/mL; range, 90-127 pg/mL) or negative (137 pg/mL; range, 80-169 pg/mL) challenges. In patients with a positive challenge, the level of eotaxin 1 decreased significantly at 1 hour to 77 pg/mL (range, 66-88 pg/mL), but at 20 hours after the challenge, no significant change in eotaxin 1 serum concentration was observed.

Although SCF was detectable in all samples tested, there were no changes in SCF concentration after aspirin challenge. IL-5 was detectable only in 3 patients with a positive challenge, and no significant change after aspirin challenge occurred. GM-CSF and eotaxin 3 serum concentrations were less than the sensitivity threshold of the methods.

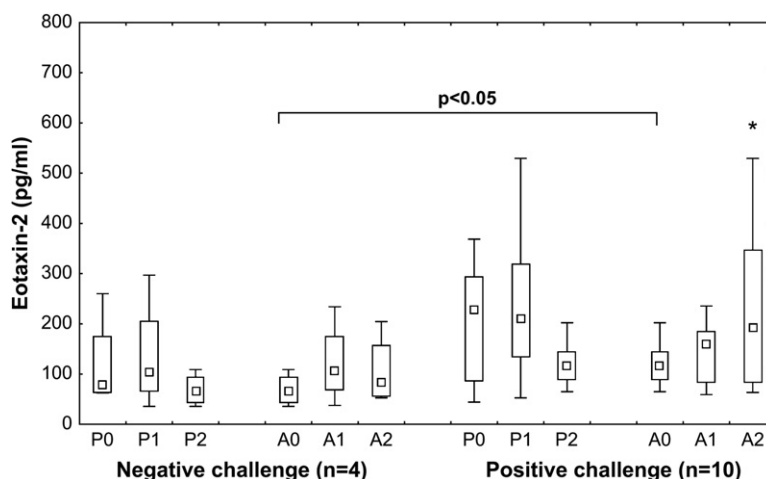
## DISCUSSION

In this study, we report that a bronchial reaction induced by inhalation of lysine-aspirin may be accompanied by extrabronchial (nasal and/or cutaneous) symptoms in half of aspirin-

hypersensitive patients, and in some patients, inhalation of lysine-aspirin may result in development of isolated extrabronchial symptoms. The mechanism leading to a systemic response after the local challenge with lysine-aspirin is not clear. Nasal reactions observed in 7 patients could be explained by aspiration of lysine-aspirin into the nasal cavity during bronchial challenge, and local oral reactions observed in 1 patient could result from a direct effect of aspirin deposition on the oral mucosa. However, in 6 of 7 patients with defined systemic reactions, various skin symptoms (urticaria, skin rash, or angioedema) were observed as well. The role of locally (ie, in the bronchi) released mediators in development of symptoms in distal organs seems to be unlikely because isolated extrabronchial responses were also observed. Because the dose of inhaled aspirin received by patients with systemic reactions was 10 times higher than in patients with isolated bronchial reactions, it is possible that lysine-aspirin was absorbed in the lungs and reached the skin and other organs via the circulation. In fact, extrabronchial symptoms often coincide with bronchoconstriction after oral aspirin challenge.<sup>4,20,21</sup>

The second important observation is the development of delayed symptoms in 5 (38%) patients with a positive reaction





**FIG 4.** Serum concentrations of eotaxin 2 after bronchial challenge with lysine-aspirin in patients with positive and negative response (\* $P < .05$  compared with A0).

to aspirin, a prevalence similar to 30% of late reactions reported previously.<sup>22</sup> Late reactions, which appeared several hours after lysine aspirin challenge in all 5 subjects, were preceded by early reactions, suggesting late responses may be secondary to earlier events. Late-phase response to allergen is a common feature after bronchial or nasal allergen challenge, and its mechanism seems to be related to the recruitment of inflammatory cells from the circulation into the target organ as a result of early mast cell degranulation and release of chemoattractant mediators and chemokines.<sup>22</sup> Although the pathomechanism of lysine-aspirin induced late reaction has not been studied, several data suggest that it may be also secondary to early mast cell activation. Mast cell activation was observed after both bronchial<sup>23,24</sup> and nasal<sup>11,25</sup> challenges with aspirin. This is the first report describing an increase in circulating leukocyte and eosinophil progenitor cells in peripheral blood after positive bronchial aspirin challenge. A significant increase in the median percentage of leukocyte progenitors and eosinophil progenitor cells was observed at 1 hour after the reaction and was maintained as long as 20 hours after the challenge. However, further analysis revealed that the increase in eosinophil progenitor cells (CD125<sup>+</sup>CD34<sup>+</sup>CD45<sup>+</sup> cells) was significant at 20 hours after challenge only in patients with systemic but not with isolated reactions. Thus, in patients with isolated local reactions, only a mobilization of less differentiated leukocyte progenitors within the first hour after aspirin administration is observed, whereas in patients with systemic reactions, it is followed by recruitment from the bone marrow of more specialized cells bearing IL-5 receptor (CD125) on their surface, suggesting that eosinophil progenitor recruitment may contribute to the pathomechanism of late systemic response to aspirin. The major limitation of our study is that subjects with immediate positive reactions received systemic glucocorticosteroids within 2 to 3 hours of challenge to decrease the risk of late asthma exacerbations. This therapy likely affected the 20-hour assessment of leukocyte progenitors. Thus, we cannot exclude the possibility that the rate of late clinical reactions was in fact underestimated, that the late mobilization of cell progenitors could be suppressed to some extent by systemic glucocorticosteroids, and that without this pretreatment the cellular changes may have been greater than observed.<sup>18</sup>

To explain the mechanism of allergen-induced inflammatory cell recruitment, it has been suggested that cytokines (eg, IL-5 or eotaxins) released in the target organ were sending signals to the bone marrow, resulting in upregulation of specific receptors on progenitor cells and leading to migration of cells into the circulation.<sup>23,27-29</sup> Alternatively, it has been proposed that allergen specific T cells might traffic from the airways to the bone marrow to enhance hematopoiesis.<sup>24,25</sup> Because aspirin-induced reaction is not immunologic but results from cyclooxygenase inhibition in the setting of inherited arachidonic acid abnormalities, any involvement of specific T cells seems to be unlikely. On the other hand, early activation of mast cells and eosinophils observed after bronchial or nasal aspirin reaction suggests that chemoattractant cytokines released by these cells in the airways could be responsible for leukocyte progenitor cell recruitment. To test this hypothesis, plasma levels of several molecules chemotactic for eosinophils (IL-5, eotaxins, SCF, and GM-CSF) were assessed in parallel with measurement of progenitor cells. Only eotaxin 2 concentration was increased significantly 20 hours after positive aspirin challenge, implicating the role of this chemokine in the late recruitment of eosinophil progenitors into the peripheral circulation. In earlier studies, eotaxin 2 has been shown to be involved in eosinophil progenitor mobilization in a mouse allergy model based on inhibition of the allergen induced increase in progenitor cells in the airway by mAbs against eotaxin 2.<sup>26</sup> Furthermore, in our study, patients with positive aspirin challenge (ie, with confirmed hypersensitivity to aspirin) demonstrated a higher baseline level of eotaxin 2 compared with patients with negative challenge. These observations are consistent with a previous study reporting the expression of eotaxin 2 and eotaxin 1 mRNA was significantly greater in the airways of patients with aspirin triad.<sup>30</sup>

In addition to chemoattractant cytokines, lipid mediators, such as cysteinyl leukotrienes, are released by mast cells and eosinophils into the systemic circulation after a positive aspirin challenge.<sup>31,32</sup> A role for cysteinyl leukotrienes in aspirin-induced bone marrow response is supported by the expression of cysteinyl leukotriene receptor type 1 on CD34<sup>+</sup> progenitor cells and the inhibition of eosinophilopoiesis by pranlukast, a cysteinyl leukotriene receptor type 1 inhibitor.<sup>33</sup> Thus, a variety of cytokines and chemoattractants may participate in the systemic response to aspirin.

In conclusion, this study demonstrates an increase in peripheral blood eosinophil progenitors after a positive lysine-aspirin bronchial challenge. We speculate that this change in progenitor cells is a result of multiple mechanisms, including a systemic increase in eotaxin 2. Further study is needed to determine the relative importance of each mechanism resulting in a bone marrow response after a positive bronchial aspirin challenge.

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**Clinical implications: Patients challenged with inhaled aspirin should be monitored for extrabronchial symptoms as well.**

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