

Original article

The course of allergen-induced leukocyte infiltration in human and experimental asthma

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Background: Although the timing of allergen-induced bronchoconstriction is well defined, there is little information about the kinetics of allergen-induced leukocyte infiltration in asthma and its comparability between human and animal models of asthma.

Objective: To investigate systematically allergen-induced leukocyte infiltration into the airway lumen in human and experimental asthma by using bronchoalveolar lavage.

Methods: Patients with allergic asthma were lavaged at different time points as long as 1 week after segmental allergen challenge. Allergen-sensitized mice were lavaged as long as 3 weeks after allergen challenge. Differential cell counts, lymphocyte subsets, and cytokines were assessed in bronchoalveolar lavage fluid.

Results: In both models, neutrophil infiltration was a relatively early event (maximum: 18 hours after challenge). In contrast, eosinophil infiltration peaked 42 hours (human model) to 4 days (mouse model) after allergen challenge, paralleled by an IL-5 peak in this period. There were elevated macrophage counts over a period of several days after allergen challenge in both models. Lymphocytes (predominantly CD4⁺ T cells) peaked 18 hours after challenge in the human model, but not until 2 weeks after challenge in the murine model.

Conclusion: Early neutrophil accumulation (within hours after challenge) and delayed eosinophil accumulation (within days after challenge) in the airway lumen are common features of allergen-induced airway inflammation, whereas lymphocyte kinetics are dependent on the asthma model.

Clinical implications: Similarities in the infiltration kinetics of granulocytes after allergen challenge suggest a common role for these cells in asthma, whereas the presumed orchestration of allergic inflammation by lymphocytes appears to differ between the models. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Asthma, allergen challenge, allergic airway inflammation, kinetics, leukocytes, eosinophils, bronchoalveolar lavage

Allergic asthma is associated with a characteristic airway inflammation, airway hyperresponsiveness, and a variable degree of airway obstruction.¹ Both in human and experimental asthma, allergen challenge results in a characteristic biphasic pattern of bronchoconstriction: the early asthmatic response (within minutes after challenge) and the late asthmatic response (4-12 hours after challenge).^{2,3} There is convincing evidence that early phase bronchoconstriction is attributable to IgE-mediated mast cell degranulation.⁴ In contrast, the underlying mechanisms of the late asthmatic response are still in dispute. Eosinophils, the most characteristic leukocyte subpopulation within allergen-challenged airways,⁵ are one example for this debate.⁶ Animal studies suggested a role for eosinophils in the development of late phase bronchoconstriction.³ However, a specific reduction of endobronchial and peripheral eosinophils did not affect the development of a late asthmatic response in human asthma.⁷

One of the major obstacles in this ongoing debate on the relationship between leukocyte infiltration and airway obstruction⁸⁻¹⁰ is a lack of information concerning the kinetics of leukocyte infiltration in asthma. Although there is a plethora of publications that examine cellular subsets in allergic airway inflammation, leukocyte kinetics have not been explored systematically. In human asthma, models of allergen challenge with subsequent fiberoptic bronchoalveolar lavage (BAL) and biopsy have been developed to study allergen-induced leukocyte infiltration.^{11,12} Segmental allergen challenge has been widely used because of its safety and the possibility to compare BAL fluid and biopsy specimens from challenged and unchallenged segments intraindividually.¹³ Most protocols include 2 bronchoscopies. The first bronchoscopy is performed during the early asthmatic response (5-10 minutes after challenge). Because of concerns regarding safety, the second bronchoscopy has generally not been performed during the expected bronchoconstriction of the late phase asthmatic response (4-12 hours after challenge), but after its resolution.⁵ The time points chosen for the second bronchoscopy vary between 18 and 48 hours after challenge¹⁴⁻¹⁷; however, a clear rationale for choosing one of these time points has not been documented. The mouse

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Abbreviations used

BAL: Bronchoalveolar lavage
FACS: Fluorescence-activated cell sorting

model of allergen-induced airway inflammation is currently the most popular animal model of asthma.^{3,18-20} Animals are sensitized to an allergen by intraperitoneal injections and subsequently challenged with the allergen via the airways. In nearly all protocols, animals are analyzed within 24 hours after challenge. Again, a clear rationale for choosing this time frame is not documented in the literature.

It was the aim of this study, therefore, systematically to investigate and compare the kinetics of allergen-induced leukocyte infiltration in 2 established models of human and experimental asthma, and to provide a scientific basis for future research on leukocyte physiology in asthma and its relationship to functional changes within allergen-challenged airways.

METHODS

Human model of allergen-induced airway inflammation

Human model. Twenty-five nonsmoking patients with mild allergic asthma (mean age, 25.9 ± 5.4 years; duration of asthma, 12.5 ± 5.6 years; FEV₁, 94.1 ± 12.6 % predicted) were included in the study (Table I) using previously described²¹ criteria: (1) airway hyperresponsiveness, (2) positive allergen skin prick tests, (3) elevated total or specific IgE concentrations, and (4) a dual reaction after allergen inhalation (FEV₁ fall of >20% of baseline after 5-10 minutes and >15% after 4-6 hours). Inhaled allergen provocation and the calculation of the individual provocation dose were performed as described.¹⁴ Inhaled and segmental allergen challenge were separated by at least 4 weeks. Cromoglycates or corticosteroids were withdrawn at least 7 days before challenge. Patients gave their written informed consent. The study was approved by the local ethics committee. Segmental allergen challenge was performed as described.²¹ Briefly, 2.5 mL saline was instilled into the left S8 and S5 segment, and the left S8 was then lavaged by using 100 mL prewarmed saline. Subsequently, allergen (diluted in 2.5 mL saline) was instilled into the right S8 and S5 segment, and the right S8 was lavaged by using 100 mL prewarmed saline after 10 minutes. The second BAL was performed in the left and right S5 segment, in protocol A, 18 hours (n = 16), B, 42 hours (n = 16), C, 3 days (n = 6), and D, 7 days (n = 6) after challenge. Some patients participated in several protocols, with at least 6 months between challenges (Table I). Before each bronchoscopy, venous blood samples were obtained (for differential blood cell counts).

Analysis of cell subsets and cytokines in the BAL fluid. Bronchoalveolar lavage fluid samples were filtered through a 2-layer sterile gauze into sterile plastic vials, centrifuged at 4°C and 500g for 10 minutes. Supernatants were removed and stored at -80°C until measured. Cells were resuspended in PBS. A fraction of the suspension was used for cell counts (using a Neubauer chamber) and for cytopspins. Cytopspins were stained with May/Grünwald/Giemsa-solution, and differential cell counts were determined by using standard morphologic criteria. Results were expressed as total number of cells per milliliter of recovered fluid. Flow-cytometric analysis of

lymphocyte markers was performed as described.⁵ Lymphocyte subsets were expressed as a percentage of total cell counts in the lymphocyte gate. Cytokines in BAL fluid supernatants were measured by using ELISA as described.²¹

Mouse model of allergen-induced airway inflammation

Animal model. Female BALB/c mice 6 to 8 weeks old (obtained from Harlan-Winkelmann, Borcheln, Germany) were sensitized to ovalbumin (10 µg/injection) adsorbed to 1.5 mg Al(OH)₃ by intraperitoneal injections on days 1, 14, and 21 as described.¹⁹ Aerosol challenges were performed in a dedicated chamber with 1% ovalbumin (wt/vol) diluted in PBS (allergen-challenged cohort) or with PBS alone (sham-challenged cohort) on days 26 and 27 as described.¹⁹ Both cohorts were then divided into 6 subgroups and analyzed 18 hours (n = 18, control: n = 18), 42 hours (n = 10, control: n = 8), 4 days (n = 14, control: n = 6), 7, 14, or 21 days (n = 10, control: n = 6, in each group) after the last challenge. For analysis of lymphocyte subsets, a separate experiment was performed in which ovalbumin-sensitized and ovalbumin-challenged BALB/c mice were analyzed 18 hours (n = 4) or 7 days (n = 5) after the last challenge. On the day of analysis, all animals were killed by cervical dislocation, and their tracheae were cannulated. Afterwards, lungs were lavaged twice with 0.8 mL ice-cold PBS (recovery $1.4 \text{ mL} \pm 0.2 \text{ mL}$ in all groups), and the obtained BAL fluid was placed on ice. Animal experiments were approved by the local animal care committee.

Analysis of cell subsets and cytokines in the BAL fluid. Bronchoalveolar lavage fluid samples were processed as described in the human model. Cytopspins were stained with hematoxylin/eosin solution, and differential cell counts were determined by using standard morphologic criteria. Results were expressed as total number of cells per milliliter of recovered fluid. For flow cytometry, BAL fluid was centrifuged at 4°C and 350g for 10 minutes. After erythrocyte lysis, the solution was washed twice in fluorescence-activated cell sorting (FACS) buffer (PBS with 2% FCS and 0.01% NaN₃), and the cells resuspended in 9 µL FACS buffer. Afterward, 1 µL normal mouse serum (Dianova, Hamburg, Germany) and 1 µL fluorochrome-conjugated antibody solution (Becton Dickinson [BD], San Jose, Calif) were added. After incubation (30 minutes at room temperature), cells were washed in FACS buffer and resuspended in 300 µL Cell Fix solution (BD). At least 10,000 cells were analyzed by using a FACScan Flow Cytometer (BD). Lymphocyte subsets were expressed as a percentage of total cell counts in the lymphocyte gate. Cytokines in BAL fluid supernatants were measured by using ELISA as described.¹⁹

Statistical analysis

Data were analyzed by using SPSS (SPSS Inc, Chicago, Ill). Most parameters were not normally distributed. Therefore, parameters are expressed as median values (minimum – maximum). Groups were compared by using the Mann-Whitney *U* test. *P* values < .05 were regarded as significant. Boxplot graphs display the median (line within the box), interquartile range (edges of the box), and the range of all values less distant than 1.5 interquartile ranges from the upper or lower quartile (vertical lines).

RESULTS

Total cell counts in human and murine BAL fluid

In the human model, there was no difference in total cell counts between allergen-challenged and sham-challenged

TABLE I. Patient characteristics

#	Sex	Allergen	Dose (AU)	Total IgE (kU)	Specific IgE (kU)	Medication	Protocol
1	F	Birch	160	72	6	BA	A(2),B(2)
2	F	Birch	2.5	199	8	IC, BA	A
3	M	DP	5.5	214	25	IC, BA, CR	A
4	M	Birch	10	229	6	IC, BA	A(2),B(2),C,D
5	M	Birch	526	314	5	BA	A
6	M	Rye	100	986	100	BA	A,B
7	M	DP	4700	234	26	BA, CR	A
8	F	DP	1	483	100	IC, BA	A(2),B
9	M	Birch	2220	204	21	CR	A,B
10	F	DP	650	247	>100	IC, BA	A,B(2),D
11	F	DP	2650	551	715	IC, BA	A,B(2)
12	M	Rye	1.8	282	171	CR, BA	A
13	M	Birch	775	65	64	BA	A,B
14	M	Birch	6950	314	ND	BA	B
15	F	Birch	12.9	221	18	None	B
16	F	DP	190	264	>100	None	B,D
17	M	DP	1000	65	20	None	B
18	F	Rye	127	512	>100	BA	D
19	F	Rye	140	224	13	IC, BA	D
20	M	DP	1000	64	10	BA	D
21	F	DP	9.8	226	57	BA	C
22	F	Rye	76	545	13	BA, OC	C
23	F	DP	1.8	64	14	CR, BA	C
24	F	Birch	28	121	35	BA	C
25	F	Birch	14	20	5	BA	C

Sex of the patients (male/female), the allergen (DP, *Dermatophagoides pteronyssinus*) and the dose (in arbitrary units, AU) used for segmental allergen challenge, serum levels of total and allergen-specific IgE in kU, the medication before the study (BA, inhaled β_2 -agonist; IC, inhaled corticosteroid; OC, oral corticosteroid; CR, cromoglycates), and the protocol (ND, not done). Some patients were included in different protocols or twice (2) in the same protocol.

TABLE II. Total cell counts in BAL fluid after allergen challenge

	Human model		Mouse model	
	n	Median (range) (10^6 cells/mL BAL fluid)	n	Median (range) (10^6 cells/mL BAL fluid)
Control	44	0.11 (0.03-1.25)	18	0.05 (0.02-0.10)
10 min	44	0.08 (0.03-1.14)	—	—
18 h	16	0.34 (0.10-1.23)*	18	0.15 (0.07-0.36)*
42 h	16	0.95 (0.10-2.88)*	14	0.21 (0.14-0.46)*
3 d	6	0.33 (0.13-0.69)*	—	—
4 d	—	—	10	0.38 (0.21-0.68)*
7 d	6	0.21 (0.15-0.30)*	10	0.29 (0.18-0.93)*
14 d	—	—	10	0.18 (0.10-0.33)*
21 d	—	—	10	0.12 (0.10-0.18)*

Number of subjects (n) and the median values (minimum-maximum) in all groups. Control: 10 minutes after saline challenge (protocols A-D) in the human model, and 18 hours after saline challenge in the mouse model.

*Significant differences ($P < .05$) in cell counts between allergen-challenged segments or animals and the corresponding controls.

segments after 10 minutes ($n = 44$; $P > .05$). At all other time points, cell counts were elevated after allergen challenge. Cell counts peaked after 42 hours, although this was not significantly different compared with 18 hours after challenge ($P = .08$; Table II, human model). In the mouse model, total cell counts were elevated at all time points after allergen challenge (compared with sham-challenged mice). Cell counts peaked 4 days after challenge ($P < .05$ compared with the time points 42 hours and 7 days; Table II, mouse model).

Differential cell counts in human BAL fluid

There was no difference in the differential cell counts between allergen-challenged and sham-challenged segments after 10 minutes ($n = 44$; protocols A-D, Fig 1). Macrophage counts were elevated after 42 hours and 3 days compared with corresponding control segments and with those lavaged after 10 minutes. Macrophages returned to control levels after 7 days (Fig 1, Mac). At all time points (except 10 minutes after challenge), eosinophil counts were elevated compared with the control segments.

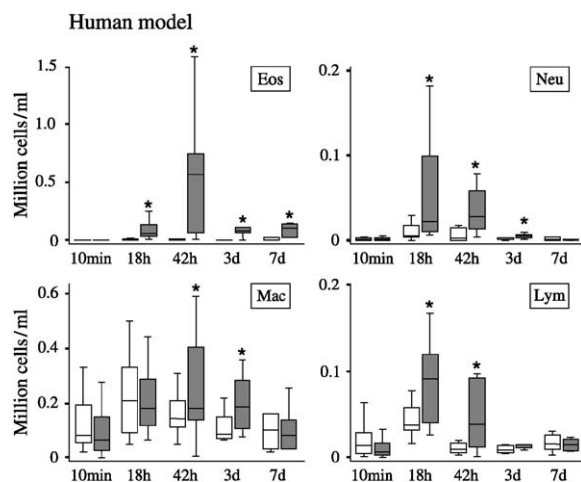


FIG 1. Leukocyte kinetics in human BAL fluid after segmental allergen challenge. Eosinophils (*Eos*), neutrophils (*Neu*), macrophages (*Mac*), and lymphocytes (*Lym*) in human BAL fluid at different time points after allergen challenge. Boxplots show sham-challenged (white boxes) and allergen-challenged (grey boxes) segments at each time point. *Significant difference ($P < .05$) compared with the corresponding control.

Eosinophils peaked 42 hours after challenge (Fig 1, *Eos*). Although highest neutrophil counts were found 18 hours after challenge, there was no significant difference between the time points 18 and 42 hours ($P = .86$). Neutrophil counts returned to control levels after 7 days (Fig 1, *Neu*). Lymphocyte counts peaked 18 hours after challenge ($P < .05$ compared with 10 minutes and 42 hours) and returned to control levels after 3 days (Fig 1, *Lym*). T cells were the predominant lymphocyte subpopulation. The high percentage of $CD4^+$ cells among T cells was increasing, whereas the low percentage of $CD8^+$ cells among T cells was decreasing with time. In contrast, the percentage of $CD4^+/CD25^+$ among T cells did not change significantly (Table III).

Differential cell counts in murine BAL fluid

Compared with corresponding controls, macrophage counts were increased 4 days ($P < .05$), but not at other time points after allergen challenge ($P > .05$ for all groups; Fig 2, *Mac*). There was a constant increase in eosinophil counts, reaching a maximum 4 days after allergen challenge ($P < .01$ for each step). Eosinophil counts began to decline after 7 days ($P < .01$ compared with the numbers measured after 4 days) and returned to control levels 21 days after challenge (Fig 2, *Eos*). There was no difference between elevated neutrophil counts 18 and 42 hours after challenge ($P = .25$), although the highest numbers of neutrophils were observed after 18 hours. Neutrophil counts 4 and 7 days after challenge were lower than 18 hours after challenge ($P < .05$ for both groups) and returned to control levels after 14 days (Fig 2, *Neu*). There was a constant increase of lymphocyte counts, reaching a maximum 14 days after allergen challenge ($P < .01$ for each step). Three weeks after challenge, lymphocyte

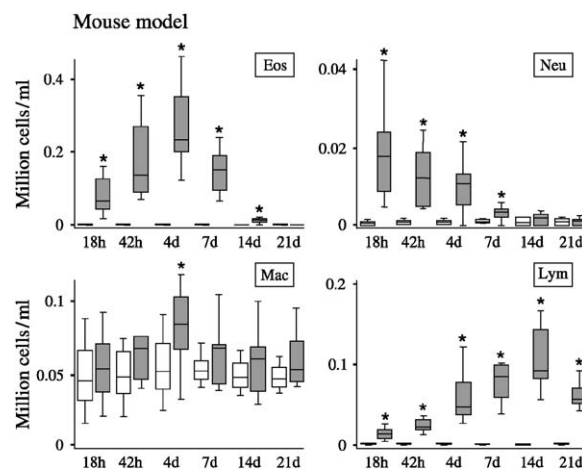


FIG 2. Leukocyte kinetics in murine BAL fluid after ovalbumin challenge. Eosinophils (*Eos*), neutrophils (*Neu*), macrophages (*Mac*), and lymphocytes (*Lym*) in murine BAL fluid at different time points after allergen challenge. Boxplots show sham-challenged (white boxes) and allergen-challenged (grey boxes) mice at each time point. *Significant difference ($P < .05$) compared with the corresponding control.

counts were still higher than 18 or 42 hours after challenge ($P < .05$; Fig 2, *Lym*). The percentages of T cells among lymphocytes were nearly identical to those in the human model. Similarities were also observed in the percentages of $CD4^+$ cells and $CD4^+/CD25^+$ cells among T cells. The percentage of $CD8^+$ cells among T cells was lower in the murine than in the human model, as reflected by a higher $CD4/CD8$ ratio in the murine model (Table III).

Percentages of leukocyte subpopulations in human and murine BAL fluid

In both models, there were no significant differences in the relative percentages of BAL fluid leukocytes in control segments/animals at all time points after sham challenge (Fig 3, lower panel). Both models were characterized by a peak in the percentage of neutrophils 18 hours after allergen challenge, which was constantly declining at the following time points. In both models, eosinophils represented more than 40% of the leukocytes in BAL fluid gathered between 2 and 7 days after challenge. Eosinophil percentages peaked 42 hours (human model) and 4 days (mouse model) after challenge (Fig 3, upper panel). The highest percentage of lymphocytes in BAL fluid was found 18 hours after challenge in the human model (20%), but not until 14 days after challenge in the mouse model (60 %; Fig 3, upper panel).

Cytokines in human and murine BAL fluid

Elevated IL-4 and IL-5 levels in human BAL fluid peaked 42 hours after challenge ($P < .05$ compared with 18 hours after challenge) and returned to control levels after 3 days (Fig 4, human model). In mice, IL-5 levels peaked 42 hours after challenge, whereas IL-4 levels peaked already 18 hours after challenge. Both cytokines

TABLE III. Lymphocyte subsets in BAL fluid after allergen challenge

	Human model		Mouse model	
	18 h	7 d	18 h	14 d
B cells (% of gated cells)	ND	3.3 ± 2.5	12.0 ± 2.3	3.8 ± 3.0*
Natural killer cells (% of gated cells)	7.8 ± 6.1	5.7 ± 5.2	ND	ND
T cells (% of gated cells)	44.0 ± 20.5	75.5 ± 8.8*	46.8 ± 3.3	77.0 ± 9.7*
CD4 ⁺ cells (% of T cells)	73.8 ± 11.2	83.7 ± 3.7	75.3 ± 2.4	88.2 ± 5.1*
CD8 ⁺ cells (% of T cells)	28.9 ± 11.2	16.0 ± 2.2*	14.7 ± 1.2	4.7 ± 1.2*
CD4/CD8 ratio	3.5 ± 2.4	5.8 ± 1.2*	5.2 ± 0.6	19.7 ± 5.0*
CD4 ⁺ /CD25 ⁺ cells (% of T cells)	28.3 ± 13.8	28.9 ± 9.0	34.2 ± 0.7	37.4 ± 8.5

N = 16 (18 h) and n = 6 patients (7 d), and n = 4 (18 h) and n = 5 animals (14 d) were analyzed. B cells (CD19⁺ in the human model; CD45R⁺ in the mouse model), natural killer cells (CD3⁺/CD16⁺/CD56⁺) and T cells (CD3⁺) are expressed as percentages of total cell counts in the lymphocyte gate. T-cell subsets are expressed as percentages of all T cells. Parameters are displayed as means ± SDs (ND, not done).

*Significant differences between the 2 time points in 1 model ($P < .05$).

returned to control levels after 7 days (Fig 4, mouse model). There were no differences in IFN- γ levels between allergen-challenged and sham-challenged mice at any time point (data not shown). IFN- γ levels in human BAL fluid were detectable in only a small portion of the samples (unrelated to a specific time point). Therefore, a complete analysis of IFN- γ kinetics was not possible in the human model.

Differential cell counts in human peripheral blood

Monocyte and neutrophil counts increased significantly 18 hours after challenge and returned to control levels 42 hours after challenge. Neutrophils (but not monocytes) were significantly decreased 3 days after challenge, before returning to control levels after 7 days. Lymphocyte counts did not change significantly at all time points. In contrast, eosinophils were significantly elevated at all time points, with a maximum 42 hours after challenge (Fig 5).

DISCUSSION

Despite a large body of literature on the mechanisms of allergic airway inflammation in asthma,¹ there is little information about the kinetics of the leukocyte influx into the airways after allergen challenge and its comparability between human and animal models of asthma. The current study therefore attempts systematically to characterize the course of allergen-induced leukocyte infiltration in human and experimental asthma.

In both human and experimental asthma, eosinophils were among the last leukocyte subpopulations to infiltrate allergen-challenged airways. Previous human studies have shown that eosinophils and IL-5 levels in BAL fluid increase between 4 and 24 hours after allergen challenge.²² In our human model, we found a maximum of eosinophil counts (both in BAL fluid and in the blood) and IL-5 levels 42 hours after challenge. This peak coincides with the reported maximum of eosinophilopoiesis in human bone marrow after allergen challenge.²³ In the mouse model, the maximum of eosinophilic infiltration occurred even

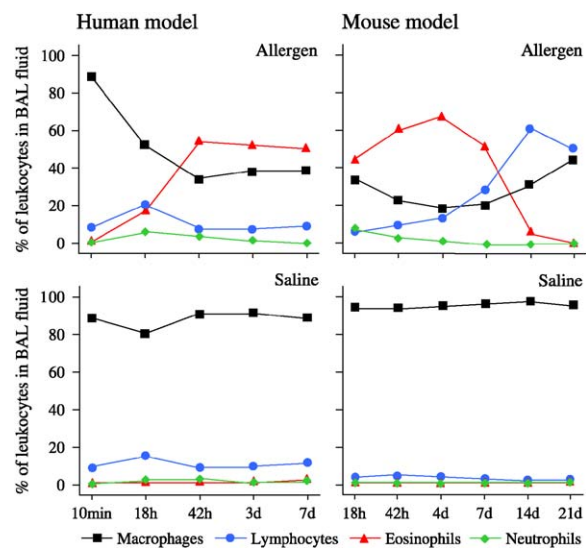


FIG 3. Percentages of leukocyte subpopulations in BAL fluid. The percentages of macrophages (black squares), lymphocytes (blue circles), eosinophils (red triangles), and neutrophils (green diamonds) in BAL fluid are displayed as percentages (median values) of the total number of leukocytes. The interquartile ranges were omitted in the graph for clarity. *Upper panel*, Kinetics in allergen-challenged segments or mice. *Lower panel*, Kinetics in corresponding sham-challenged segments or mice.

later, according to data from other animal models.^{20,24,25} However, the peak of IL-5 in BAL fluid in the murine model was identical to the human model (42 hours after challenge). These data suggest that, in both human and experimental asthma, the recruitment of eosinophils peaks approximately 2 days after allergen exposure.

This time point of eosinophil recruitment is in considerable contrast with the reported time frame of allergen-induced bronchoconstriction in asthma.¹ Studies using the same models as in the current study reported a maximum of late-phase bronchoconstriction approximately 6 hours after allergen exposure.^{3,13} This discrepancy does not support the concept that bronchoconstriction and eosinophil recruitment are directly related.^{3,26} Indeed, there is evidence to question this concept.^{2,6,9,27} Treatment

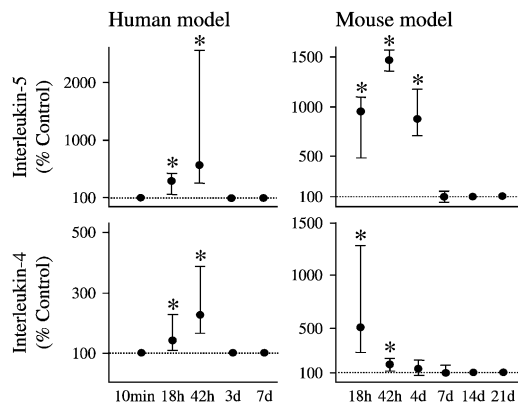


FIG 4. Cytokine kinetics in BAL fluid. Kinetics of IL-5 (upper panel) and IL-4 (lower panel) in BAL fluid after allergen challenge. Concentrations are displayed in percentages of the mean concentration measured in the corresponding controls. Graphs display the median (dot) and interquartile range (vertical line). *Significant differences ($P < .05$) compared with controls.

with anti-IL-5 antibodies was virtually ineffective against late-phase bronchoconstriction, although it was highly effective in reducing endobronchial and peripheral blood eosinophilia in patients with asthma.⁷ Treatment with anti-IgE antibodies had a similar outcome: large effects on airway eosinophilia were not accompanied by beneficial effects on airway dysfunction.^{10,28} In mice, eosinophil counts do not predict the severity of airway dysfunction,¹⁸ and a total ablation of the eosinophil lineage fails to reduce bronchoconstriction.²⁹ Notably, hypereosinophilia can even prevent airway dysfunction, possibly because of the release of anti-inflammatory mediators by eosinophils.³⁰ Therefore, it can be speculated that the delayed infiltration of eosinophils might be related to the resolution rather than the initiation of allergen-induced bronchoconstriction in asthma.

The recruitment and infiltration of neutrophils peaked already 18 hours after allergen challenge in both models. The maximum of neutrophil infiltration may occur even earlier.^{31,32} An early and transient infiltration of neutrophils into the airways, which precedes the infiltration of eosinophils, has also been described in other models of asthma.^{25,33} These data suggest that neutrophil recruitment may be an early event after allergen challenge. There is evidence suggesting that neutrophils are more closely related to airway obstruction and the severity of asthma than eosinophils,^{34,35} and that neutrophils are the dominant leukocyte subpopulation within airways during status asthmaticus.³⁶ Thus, given the kinetics observed in our study, the temporal relationship of neutrophils to late-phase bronchoconstriction might indeed be closer than that of eosinophils.

The relative percentages of infiltrating lymphocyte subpopulations did not differ between human and experimental asthma. CD4⁺ T cells represented the majority among infiltrating lymphocytes, whereas the percentages of CD8⁺ T cells were relatively low. The previously reported percentage of B cells (12%) among lymphocytes

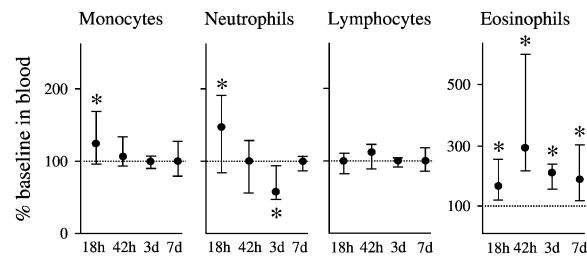


FIG 5. Leukocyte kinetics in human peripheral blood after segmental allergen challenge. Shown are the cell counts of leukocyte subpopulations in human peripheral blood at different time points after allergen challenge, in percentages of the individual baseline directly before allergen challenge. Graphs display the median (dot) and interquartile range (vertical line) at each time point. *Significant differences ($P < .05$) compared with baseline.

in human BAL fluid collected 18 hours after segmental allergen challenge⁵ corresponds well with the data from our murine model. There were also striking similarities regarding B-cell percentages at the late time points. These data suggest that a high percentage of CD4⁺ T cells and a low percentage of B cells and CD8⁺ T cells among infiltrating lymphocytes represent a common feature in allergic asthma.

In contrast, we found a marked difference in lymphocyte kinetics between human and experimental asthma. In the human model, lymphocytes peaked 18 hours after allergen challenge. This is in line with a previous human study showing that T-lymphocyte counts in BAL fluid are lower 4 days compared with 48 hours after allergen challenge.¹² Current hypotheses hold that lymphocytes are recruited into the lung after allergen challenge to orchestrate the activity and differentiation of various effector cells.³⁷ This would be compatible with the finding that lymphocytes infiltrate the airways relatively early after allergen challenge. However, these kinetics were not observed in the murine model: lymphocytes peaked 14 days after allergen exposure, at a time point where other cell populations had already returned to baseline.

One explanation for this discrepancy might be species-specific differences. However, the discrepancy might also be a result of the different modes of allergic sensitization. In human asthma, the airways are most likely the primary site of both allergic sensitization and allergen challenge, whereas different sites of allergic sensitization (peritoneal cavity) and allergen challenge (airways) are used in the mouse model. In a rat model, in which animals are sensitized to allergen by subcutaneous injections and challenged via the airways, there was a peak of lymphocyte infiltration into the airways 6 days after challenge.²⁴ Therefore, the delayed infiltration of lymphocytes in animal models (compared with the human model) may be a result of the different sites of allergen sensitization. There may be an additional influence of the frequency or dose of allergic sensitization. In a similar mouse model of ovalbumin-induced airway inflammation with only 2 separate intraperitoneal ovalbumin injections (compared with 3 separate injections in our model), there was a peak of lymphocyte accumulation in BAL fluid

approximately 1 week after challenge (compared with 2 weeks after challenge in our model).²⁵ Thus, lymphocyte kinetics might be determined by both the sites and the pattern of allergic sensitization before allergen challenge. Irrespective of the reason for the difference in lymphocyte kinetics between human and experimental asthma, our data suggest that lymphocyte kinetics represent a model-dependent feature in allergic airways inflammation. This has to be considered when comparing lymphocyte pathophysiology between different models of asthma.

In conclusion, this is the first study to describe the differential kinetics of allergen-induced leukocyte infiltration into the airway lumen in men and mice. Although there are marked similarities regarding the kinetics of granulocyte and macrophage infiltration into the airways, there is a clear difference in lymphocyte kinetics between human and experimental asthma.

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