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 2006;118:91-7.)

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 obstruction[8-10] 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 challenge[14-17]; however, a clear rationale for choosing one of these time points has not been documented. The mouse model of allergen-induced airway inflammation is

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Abbreviations used
BAL: Bronchoalveolar lavage
FACS: Fluorescence-activated cell sorting

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; FEV_1, 94.1 ± 12.6 % predicted) were included in the study (Table I) using previously described[21] 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 (FEV_1 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 500*g* 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 cytospins. Cytospins 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, Borchen, 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 mL ± 0.2 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. Cytospins 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 350*g* 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% NaN_3), 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