Gradual increase in priming of human eosinophils during extravasation from peripheral blood to the airways in response to allergen challenge

Bart Luijk, MD,[a] Caroline A. Lindemans, MD,[a] Deon Kanters, BSc,[a] Roos van der Heijde, MD, PhD,[a] Paul Bertics, PhD,[b] Jan-Willem J. Lammers, MD, PhD,[a] Mary-Ellen Bates, PhD,[b] and Leo Koenderman, PhD[a] *Utrecht, The Netherlands, and Madison, Wis*

Background: Eosinophils isolated from the blood of patients with allergic asthma exhibit enhanced responsiveness to multiple stimuli compared with cells from normal controls, a phenomenon generally referred to as *priming*. This priming response is essential for optimal activation with augmented responses including chemotaxis, cytotoxicity, respiratory burst, and the release of proinflammatory lipid mediators.
Objective: To monitor the kinetics of priming of eosinophils in the peripheral blood and in the bronchoalveolar lavage fluid of patients with allergic asthma before and after allergen challenge.
Methods: Priming of blood eosinophils obtained from patients with allergy and donors without allergy was measured by labeling with monoclonal phage antibodies A17 and A27 recognizing priming-associated epitopes on phagocytes. In addition, blood and bronchoalveolar lavage fluid eosinophils from subjects with allergy after segmental and whole lung allergen challenge were similarly analyzed.
Results: A dose-dependent cytokine-induced upregulation of priming-associated epitopes on blood eosinophils was found. Patients with allergic asthma exhibited an *in vivo* partially primed eosinophil phenotype, which is further primed *in vitro* after cytokine or chemokine incubation. Priming was increased in peripheral blood 6 hours after whole lung challenge as well as after segmental allergen challenge. Interestingly, eosinophils obtained from the bronchoalveolar lavage fluid 48 hours after segmental allergen challenge exhibited a higher primed phenotype.
Conclusion: These data are consistent with a model in which local allergic inflammatory reactions induce partial systemic eosinophil priming in the peripheral blood. Eosinophils found in the airway are highly primed, consistent with the markedly upregulated inflammatory capacity observed in these cells. (J Allergy Clin Immunol 2005;115:997-1003.)

Key words: *Priming, allergen challenge, eosinophils, allergic asthma, peripheral blood*

Allergic asthma is accompanied by a chronic inflammatory reaction in the airways.[1] This inflammation is characterized by the presence of eosinophils and $CD4^+$ T cells in the airway, and several lines of evidence indicate that this inflammatory reaction is an important determinant in allergic asthma.[2] These data were obtained by analyzing biopsies taken from the airways of patients with allergic asthma at different stages of this disease.[3] Indeed, many studies using anti-inflammatory treatment have shown that the inflammatory reaction is an integral part of the pathogenesis of the disease.[4,5] Treatment with inhaled glucocorticosteroids is the therapy of choice to inhibit inflammatory processes seen in the airways of patients with moderate to severe asthma.[6]

Evaluation of the extent of inflammation in the bronchial tissue is hampered by the required bronchoscopies that are tedious and expensive and impose a substantial discomfort on the patients. This has provoked many investigators to develop disease markers that can monitor this inflammatory reaction through less invasive methods. These methods range from the analysis of induced sputum to exhaled gases like nitric oxide and H_2O_2.[7,8] Several approaches have also been developed to measure markers in peripheral blood such as serum eosinophilic cationic protein and soluble adhesion molecules.[9,10] Several of these markers have proven to be applicable in population-based studies but appeared to have their limitations to monitor the disease using repeated measures in individual patients.

Several studies have shown that eosinophils from subjects with allergic asthma exhibit a primed phenotype likely as a consequence of their interaction with cytokines in peripheral blood, which facilitates extravasation to the airways with an increased chemotaxis and endothelial adhesion response.[11-14]

Despite the recognition of the importance of eosinophil priming in the pathogenesis of allergic diseases, no antibodies have yet been described that can identify these primed eosinophils with a sufficient dynamic range to

From [a]the Department of Pulmonary Diseases, University Medical Center, Utrecht; and [b]the Department of Biomolecular Chemistry, University of Wisconsin.

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Reprint requests: Leo Koenderman, PhD, Department of Pulmonary Diseases, University Medical Center, Heidelberglaan 100, NL 3584 CX Utrecht, The Netherlands. E-mail: L.Koenderman@hli.azu.nl.

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Abbreviations used
AU: Arbitrary units
BAL: Bronchoalveolar lavage
FITC: Fluorescein isothiocyanate
FMLP: N-formyl-methionyl-leucyl-phenylalanine
MCF: Median channel fluorescence
MoPhab: Monoclonal phage antibody
SBP: Segmental bronchial provocation

allow detailed priming studies *in vivo* and *in vitro*. We recently developed 2 mAbs from a semisynthetic phage antibody library.[15] These antibodies identified neutrophils and monocytes in blood primed *in vitro* with low doses of cytokines (picomolar range) and *in vivo* in patients with unstable chronic obstructive pulmonary disease.[15] Here we studied the priming of eosinophils in allergic asthma both in tissue and in peripheral blood.

METHODS

Patients

Thirty-eight patients who had mild asthma according to the definition of the Global Initiative for Asthma guidelines were selected from patients attending the outpatient clinic of the University Medical Center, Utrecht, The Netherlands, or via advertisements.[6] All subjects had a history of episodic wheezing and periods of impaired lung function (Table I). Twenty-two of these patients with asthma underwent an inhaled allergen challenge according to a standardized protocol as described previously (for patient details, see the Journal's Online Repository at www.mosby.com/jaci).[16] Healthy controls were selected from the laboratory and clinical staff without a history of asthma or presence of atopy (exclusion of atopics was performed by skin prick testing for common allergens[17]). The study was approved by the hospital ethical committee of the University Medical Center, and all patients and healthy controls gave their written informed consent.

In addition, 7 individuals were included in the bronchoscopic study who were heterogeneous with regard to diagnosis (5 patients with asthma and 2 with allergic rhinitis) and underwent bronchoalveolar lavage (BAL) 48 hours after intrasegmental bronchial challenge with allergen (segmental bronchial provocation [SBP]) as previously described.[18] Airway eosinophils were obtained from BAL fluid 48 hours after segmental bronchoprovocation of atopic human subjects with relevant antigen. Antigen dose for segmental bronchoprovocation was defined as previously described (for details, see the Online Repository at www.mosby.com/jaci).[19,20] Bronchoscopic procedures were approved by the University of Wisconsin-Madison Health Sciences Human Subjects Committee, and informed consent was obtained from all subjects before participation.

Blood sampling

Blood was obtained from healthy donors without allergy (from the laboratory staff) and patients with allergic asthma. Furthermore, blood samples were obtained from atopic subjects who underwent a segmental allergen challenge, and in a subgroup of patients with asthma, blood samples were obtained before and at 3 time points after an inhaled allergen challenge or sham challenge (using inhaled saline as control, at 3, 6, and 24 hours after the challenge, respectively).

TABLE I. Characteristics of patients with asthma (n = 38) from whom whole blood was collected (A) and from the subpopulation of this group (n = 22) who underwent an inhaled allergen challenge with a late asthmatic response (B)

	Controls Mean (SD)	A Mean (SD)	B Mean (SD)
Age, y	31 (5.6)	22 (5.6)	22 (5.5)
Gender (M/F)	22/16	26/12	16/6
Atopy	−	+	+
Baseline FEV_1 (% predicted)	101 (2.5)	94 (10)	91 (10)
Methacholine PC_{20}*	—	—	0.52 (0.07-4.43)
Late asthmatic response (% fall in FEV_1)	—	—	30(10)
Allergen†	—	—	17 House dust mite 2 Cat 3 Grass

*Geometric mean (range).
†Inhaled allergen during challenge.

Procedure for staining eosinophils with monoclonal phage antibodies A17 and A27

Priming in vitro. Blood was collected in tubes containing sodium heparin as anticoagulant and incubated at 37°C immediately after venapuncture. Blood was treated with buffer (control) or with different amounts of cytokines (GM-CSF and IL-5 incubated for 30 minutes at 37°C) and for different periods (concentration 0.1 nmol/L for 30 minutes) as indicated in the different figures. Hereafter, the blood was chilled to 4°C, erythrocytes were lysed, and the leukocytes were stained with antibodies.

Priming in vivo. Unstimulated and N-formyl-methionyl-leucyl-phenylalanine (FMLP)–stimulated (1 μmol/L, 10 minutes at 37°C) blood samples from normal donors and patients with asthma were immediately put on ice until further processing.

The used reagents are described in the Online Repository (www.mosby.com/jaci).

Flow-cytometric evaluation of eosinophil labeling by monoclonal phage antibodies A17 and A27. Blood samples were stained with fluorescein isothiocyanate (FITC) directly labeled phage antibodies A17 and A27.[15] In short, monoclonal phage antibodies (MoPhabs) A17 and A27 were diluted 1:10 with PBS:4% milk powder. This mix at 100 μL was added to whole blood samples of 50 μL each and incubated for 60 minutes on ice. Hereafter, the red cells were lysed in ice-cold isotonic NH_4Cl and centrifuged at 1500 rpm for 7 minutes. Pelleted cells were washed and resuspended in ice-cold PBS/1% human serum albumin for analysis.[15] Cells were analyzed in a FACS vantage flow cytometer (Becton & Dickinson, Mountain View, Calif). Eosinophils were identified according their specific side scatter and forward scatter signals.[21] Data from individual experiments are reported as fluorescence intensity in arbitrary units (AU) or summarized as the median channel fluorescence (MCF) of at least 5000 events.

Analysis of airway eosinophils. Data on airway eosinophils were acquired by using the flow-cytometric analysis of eosinophils from BAL fluid obtained 48 hours after SBP.[17] The unprocessed BAL fluid containing resident airway cells was incubated with FITC-labeled A17 or A27 phage antibody, and the eosinophils were identified on the basis of scatter characteristics.

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