Expression of activated FcγRII discriminates between multiple granulocyte-priming phenotypes in peripheral blood of allergic asthmatic subjects

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Background: Allergic asthma is associated with chronic airway and systemic immune responses. Systemic responses include priming of peripheral blood eosinophils, which is enhanced after allergen challenge. In a subpopulation of asthmatic subjects, neutrophils are associated with bronchial inflammation.
Objective: We sought to monitor systemic granulocyte priming in allergic asthmatic subjects as a consequence of chronic and acute inflammatory signals initiated by allergen challenge.
Methods: Blood was taken at baseline and 6 to 24 hours after allergen challenge in asthmatic subjects with and without late asthmatic responses. Systemic granulocyte priming was studied by using expression of cellular markers, such as α-chain of Mac-1 (αm)/CD11b, L-selectin/CD62L, and an activation epitope present on FcγRII/CD32 recognized by monoclonal phage antibody A17.
Results: Eosinophils of asthmatic subjects have a primed phenotype identified by cell-surface markers. Neutrophils of these patients were subtly primed, which was only identified after activation with N-formyl-methionyl-leucyl-phenylalanine. After allergen challenge, an acute increase in eosinophil priming characterized by enhanced expression of activated FcγRII was found in patients experiencing a late asthmatic response and not in patients with a single early asthmatic response. In contrast, expression of αm/CD11b and L-selectin on granulocytes was not different between control and asthmatic subjects and was not affected by allergen challenge. Interestingly, expression of both adhesion molecules was positively correlated, and αm expression on eosinophils and neutrophils correlated positively with bronchial hyperresponsiveness.
Conclusion: Different phases, phenotypes, or both of allergic asthma are associated with distinct priming profiles of inflammatory cells in peripheral blood.
Clinical implications: Insight in differences of systemic innate responses will lead to better definition of asthma subtypes and to better designs of new therapeutic options. (J Allergy Clin Immunol 2007;120:1073-81.)

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

Allergic asthma is accompanied by a chronic inflammation in the airways.[1] This inflammation is characterized by the presence of T_H2-type cytokines, including IL-3, IL-4, IL-5, IL-13, and GM-CSF. These cytokines stimulate growth, differentiation, and functionality of inflammatory cells that have been implicated in asthma, such as B/T cells, mast cells, basophils, and eosinophils.[2,3] In addition to these typical T_H2 cytokines, increasing evidence indicates the involvement of other cytokines, such as TNF-α, in the chronic inflammation of asthma.[3,4]

Next to eosinophils, neutrophils are also implicated in the pathogenesis of asthma.[5-10] The presence of neutrophils is associated with an increase in the concentration of the proteolytic enzyme matrix metalloproteinase 9 in bronchoalveolar lavage fluid, tissue, and sputum,[5,11-13] suggesting a role of neutrophils in the remodeling process in asthma.

In several studies eosinophils of asthmatic patients have been reported to exhibit a preactivated or primed phenotype in peripheral blood, which can be demonstrated by an enhanced expression of priming-associated epitopes.[14,15] Priming of these inflammatory cells is particularly associated with an enhanced functionality of adhesion-associated responses that facilitates the recruitment of eosinophils from the blood to the airways. This was shown by increased chemotactic responses and transendothelial movement of these cells, which were in part mediated by upregulation of adhesion molecules.[16-20]

In contrast, priming of neutrophils in peripheral blood of allergic asthmatic subjects has not been described as clearly as for eosinophils. In comparison with healthy donors,

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Supported by a research grant of GlaxoSmithKline and the Netherlands Asthma Foundation (NAF) project numbers 3.2.99.16, 3.2.98.40, and 3.2.01.49.
Disclosure of potential conflict of interest: L. Koenderman and J.-W. J. Lammers have received grant support from GlaxoSmithKline. J. A. M. Raaijmakers is employed by GlaxoSmithKline. The rest of the authors have declared that they have no conflict of interest.
Received for publication June 22, 2006; revised June 1, 2007; accepted for publication June 7, 2007.
Available online August 15, 2007.
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0091-6749/$32.00

doi:10.1016/j.jaci.2007.06.021

Abbreviations used
αm: α-Chain of Mac-1
AHR: Airway hyperresponsiveness
EAR: Early asthmatic response
FITC: Fluorescein isothiocyanate
fMLF: N-formyl-methionyl-leucyl-phenylalanine
LAR: Late asthmatic response
MoPhab: Monoclonal phage antibody

neutrophils of asthmatic subjects are not characterized by differences in expression of (pre-)activation markers, such as the α-chain of Mac-1 (αm)/CD11b and L-selectin/CD62L.[15,21] However, we have previously shown that preactivation of peripheral blood neutrophils can be measured by means of gene expression analysis in asthma[22] and chronic obstructive pulmonary disease.[23] The determination of priming of neutrophils probably needs these very sensitive measures because (primed) neutrophils are thought to quickly leave the peripheral blood in response to inflammation induced after, for example, exposure to allergens.[24-26]

Little is known regarding priming phenotypes of inflammatory cells in the context of different phases, phenotypes, or both of allergic asthma. Our first study on the kinetics of priming of inflammatory cells showed that allergen challenge lead to a short burst of priming of eosinophils, but not of neutrophils, in the peripheral blood after 6 hours.[15] The data corroborated other data showing differential activation of inflammatory cells in response to allergen challenge in allergic asthmatic subjects.[27,28] Allergen exposure is characterized by 2 phenomena in the airways of allergic asthmatic patients, the early asthmatic response (EAR) and the late asthmatic response (LAR), which are associated with airflow limitation and an increase of airway hyperresponsiveness (AHR).[28] The LAR, which is maximal after 6 to 8 hours,[29] exhibits characteristics that resemble the chronic inflammatory phase of allergic asthma in the bronchial tissue, such as infiltration of inflammatory cells starting as early as 3 hours after allergen challenge.[27,30] Systemic inflammatory responses during the LAR have been described in several studies and were associated with an increase of blood eosinophil numbers that likely had a primed phenotype.[30-32]

In this study the mechanisms involved in the systemic innate immune responses of allergic asthmatic subjects were investigated before and after allergen challenge. The allergen-induced priming of peripheral blood eosinophils and neutrophils was measured in atopic subjects with mild asthma with and without allergen-induced LAR.

METHODS

Cloning of CD32 into pMT2SM_VSV

CD32 (FcγRIIA) was cloned into a pMT2 vector containing a vesicular stomatitis virus (VSV)-epitope tag. U937 cDNA was used as a template, and a PCR reaction was performed with the following primers: CD32_Fw, TCCCCCGGGATGGCTATGGAGACCCAA; CD32_Rev, TAAAGCGGCCGAGTTATTACTGTTGACATG. The PCR product was digested with *Sma*I and *Not*I restriction enzymes for cloning into the pMT2SM_VSV vector. Therefore the existing pMT2_FcαRI_VSV[33] was used to replace the FcαRI/CD89 insert by the FcγRIIA/CD32 PCR product. This new construct was verified by means of sequencing.

Generation of stable transfectants

A Ba/F3 cell line expressing FcγRIIA/CD32 was generated similarly as described for Ba/F3_FcαRI/CD89 cell lines, as described by Bracke et al.[33] In short, Ba/F3 cells were cultured at a cell density of 10^5 to 10^6 cells/mL in RPMI 1640 supplemented with 8% Hyclone serum (Gibco, Carlsbad, Calif) and recombinant mouse IL-3. For the generation of polyclonal transfectants, pMT2_VSV containing FcγRIIA was electroporated into Ba/F3 cells (0.28 V; capacitance, 960 μ-Farad [μFD]) together with pSG5-CMV-Hygro containing the hygromycin resistance gene. Cells were cultured in the presence of IL-3 and selected in 500 μg/mL hygromycin (Boehringer Mannheim, Mannheim, Germany). After 2 weeks of selection, cells were tested for CD32 expression, and positive cells were sorted with a FACSvantage flow cytometer (Becton-Dickinson Immunocytometry Systems, Mountain View, Calif). Briefly, CD32-transfected Ba/F3 cells were incubated with the CD32 mAb IV.3 for 30 minutes at 4°C and subsequently with a fluorescein isothiocyanate (FITC)–conjugated secondary antibody. Fluorescence of the cells was quantified with the flow cytometer, and IV.3-positive cells were sorted and cultured. Polyclonal cell lines were generated expressing CD32. Stable cell lines were grown continually on murine IL3 (mIL3) and hygromycin. Expression of FcγRII/CD32 was checked regularly with the flow cytometer.

Patients and healthy control subjects

We included 45 nonsmoking, steroid-naive patients with a diagnosis of asthma according to the Global Initiative for Asthma guidelines (Table I).[34] All patients had stable asthma without a respiratory tract infection in the last 4 weeks before entering the study. They had positive allergen skin prick test responses,[35] with at least 1 positive reaction to common inhaled allergens, and they all had documented AHR (PC_{20} methacholine, <8 mg/mL). When patients used low doses of inhaled or nasal corticosteroids, this medication was stopped at least 4 weeks before study entry. No other antiasthma drugs were allowed during the study, except short-acting β_2-agonists. Patients receiving an inhalation challenge had a baseline FEV_1 of greater than 70% of the predicted value after withholding β_2-agonists for 8 hours at both the control visit and on the day of the allergen challenge. Healthy subjects without asthma symptoms or presence of atopy (Table I) were selected from the laboratory and clinical staff. The medical ethics committee of the University Medical Center Utrecht (Utrecht, The Netherlands) approved the study, and all patients provided written informed consent.

Inhalation challenges

Methacholine challenge. AHR was measured by using an inhalation provocation test with methacholine. Methacholine was inhaled in doubling concentrations at 5-minute intervals in a range starting from 0.038 mg/mL to a maximum of 8 mg/mL methacholine, according to a standardized challenge protocol, to determine PC_{20} methacholine.[36] FEV_1 was measured at 30 and 90 seconds after 2-minute tidal breathing through a calibrated nebulizer (model 646; Devilbiss, Inc, Somerset, Pa; 0.13 mL/min) while the nose was clipped. The challenge with inhaled methacholine was performed until the FEV_1 decreased by at least 20% from baseline FEV_1 to determine PC_{20}. Methacholine (Sigma-Aldrich, St Louis, Mo) was dissolved in saline (0.9%) solution.