Expression of $\alpha 4\beta 7$ Integrin on Eosinophils and Modulation of $\alpha 4$ -Integrin-Mediated Eosinophil Adhesion via CD4

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Key Words

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Introduction

Eosinophils, which comprise only 2-3% of circulating leukocytes, are normally tissue-dwelling cells localized in mucosal areas and associated with a restricted number of pathologic conditions. It has been shown that hematopoietic cytokines [granulocyte/macrophage-colony-stimulating factor, interleukin (IL)-3, and IL-5] elaborated by T cells regulate the production and function of eosinophils, but the mechanisms of eosinophil selective recruitment into tissues are not yet fully understood.

VLA-4 ($\alpha 4\beta 1$), a member of the integrin family, mediates cell interactions with fibronectin and with activated en-dothelial cells through binding to the fibronectin CS1 domain and to VCAM, respectively. Expression of VLA-4 on eosinophils, but not neutrophils, suggests one possible mechanism for the selective regulation of eosinophil recruitment and activation [1]. Another integrin, $\alpha 4\beta 7$, sharing the common $\alpha 4$ subunit and binding to the same ligands as VLA-4 [2], also recognizes mucosal addressin cell adhesion molecule (MAdCAM), which is expressed on mucosal high-endothelial venules [3], and is responsible for lymphocytes homing to the gut [4]. We have demonstrated that CD4 expressed on the eosinophil plasma membrane is the receptor for lymphocyte chemoattractant factor and transduces signals that cause eosinophils to migrate [5]. In this study, we demonstrate that human eosinophils express $\alpha 4\beta 7$, and further investigate the effect of CD4 ligation on $\alpha 4\beta 1/\alpha 4\beta 7$ mediated adhesion to CS1 and VCAM.

Materials and Methods

Eosinophil Isolation. Eosinophil-enriched granulocytes were isolated from normal donors or patients with idiopathic hypereosinophilic syndrome by dextran sedimentation and

centrifugation through Per-coll solution. After the hypotonic lysis of erythrocytes, eosinophils were isolated with a magnetic cell separation (MACS) system by depleting CD16⁺ neutrophils. Indirect Immunofluorescence Staining. Purified eosinophils were stained with monoclonal antibodies (mAbs) anti- α 4 (HP1/2), anti- β 1 (18D3), anti- $\alpha\beta$ 7 (ACT-1), and appropriate IgG subclass controls followed by phycoerythrin-conjugated goat antimouse antibodies, and analyzed on a FACStarR Plus.

RNA Extraction and RT-PCR. Total cellular RNA was first extracted from purified eosinophils with RNazol^{TM}B reagent, and used to synthesize first-strand cDNA with the Superscript Preamplification

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System. PCRs were then performed for 35 cycles at 94°C for 1 min, 57-60°C for 1 min, and 72°C for 2 min on a PTC-100 thermal controller. Adhesion Assay. Immuno2 strips of 12-flat-bottom wells were coated with bovine serum albumin (BSA)-conjugated CS1 (1 μ g/ml) or VCAM (1 μ g/ml) followed by 1% heat-inactivated BSA to block nonspecific binding.

Eosinophils pretreated with or without 10 μ g anti-CD4 (OKT4D) antibody were placed into ligand-coated wells for 30 min incubation at 37°C. After washing away the unattached cells, attached cells were lysed and quantified by a colorimetric method measuring the amount of released lactate dehydrogenase. For blocking studies, eosinophils were preincubated with mAbs anti- α 4 (HP1/2), anti- β 1 (P4C10), anti- $\alpha\beta$ 7 (ACT-1) or IgG1 antibody (MOPC21) as a negative control for 30 min before being placed in the ligand-coated plates.

preparation could be the source of β 7, total cellular RNA from peripheral mononuclear cells was tested and showed rare signals.

The function of eosinophil $\alpha\beta$ 7 was further examined by adhesion assays on fibronectin-CS1- and VCAM-coated plates, and the effect of signaling through eosinophil CD4 was studied with OKT4D (anti-CD4 mAb). CD4 ligation with OKT4D mAb promoted eosinophil adhesion, but the effect varied from donor to donor. Blocking the common α 4 subunit with mAb HP1/2 inhibited eosinophil adherence to CS1 and VCAM. However, anti- $\alpha\beta$ 7 mAb, ACT-1, which blocks lymphocyte binding to CS1 but not VCAM, only partially blocked CS1 binding of CD4-cross-linked eosinophils, but had no effect on unstimulated cells.

Results

Blood eosinophils freshly isolated from normal and eosinophilic donors, stained with the anti- $\alpha\beta$ 7 mAb, ACT-1, were analyzed by flow cytometry. All eosinophils, but not neutrophils, constitutively expressed $\alpha\beta$ 7, as is also found for VLA-4 expression. Eosinophils from hypereosinophilic patients showed higher levels of $\alpha\beta$ 7 than those from normal donors. The presence of mRNA for β 7 was also detected by RT-PCR. Regarding the possibility that a small portion of contaminated mononuclear cells in the eosinophil

Conclusion

Human peripheral blood eosinophils constitutively express $\alpha\beta$ 1/ $\alpha\beta$ 7 which are the receptors for the fibronectin CS1 domain and VCAM. Both $\alpha\beta$ 1 and $\alpha\beta$ 7 show multiple activation states regulated either in vivo or in vitro by CD4 ligation. Thus, regulation of two α 4 integrins expressed on eosinophils, $\alpha\beta$ 1 and $\alpha\beta$ 7, may be involved in recruitment of eosinophils into tissues.

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