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Research Article

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Definition of Immunoglobulin A Receptors on Eosinophils and Their Enhanced Expression in Allergic Individuals

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Abstract

Fcα receptors (FcαR), detected by the binding of IgA and by anti-FcαR antibodies, were found to be differentially expressed on eosinophils and neutrophils. Neutrophils were the major granulocyte population expressing FcαR, and they expressed much higher levels of FcαR than eosinophils. The expression of FcαR by eosinophils could be upregulated approximately threefold by Ca^{2+} ionophore treatment in a dose- and time-dependent manner. This effect, which was blocked by a chelating agent, was not duplicated by other cellular stimuli. Eosinophils in allergic individuals displayed enhanced FcαR expression, whereas neutrophils did not. The FcαR on eosinophils had a higher molecular mass (70–100 kD) than those identified on neutrophils (55–75 kD). However, removal of N-linked carbohydrates from FcαR of eosinophils and neutrophils revealed a major protein core of 32 kD for both cell types. The data indicate that expression of FcαR molecules with a characteristic glycosylation pattern is upregulated on eosinophils in allergic individuals. (*J. Clin. Invest.* 1993. 92:1681–1685.) Key words: Fcα receptor • IgA receptor • Fc receptor • eosinophil • allergy

Introduction

Eosinophils may play important roles in protection against parasitic diseases and in mediating inflammation in allergic individuals (1). These functions are linked to the eosinophil's ability to release biologically active factors following antigen activation. Several granule-derived proteins are released in the ensuing degranulation process. These include highly cytotoxic factors, such as eosinophil cationic protein, major basic protein, and eosinophil-derived neurotoxin (1).

One degranulation mode is initiated via Fc receptors (FcR)[1] in an antibody-dependent cell cytotoxic manner (2). Both IgG and IgE antibodies promote this reaction cascade by binding to specific FcγR and FcεR present on the cell surface (3, 4). However, IgA is the most abundant Ig isotype in the secretions (5) where eosinophils carry out many of their effector functions. It has been shown that IgA can bind to eosinophils (6, 7), which suggests they may possess IgA receptor(s), but FcαR on eosinophils has not been identified and characterized.

An FcαR on monocyte/macrophages and granulocytes has been defined as a variably glycosylated protein of 55–75 kD (8, 9) that can bind IgA1 and IgA2 antibodies via their Fc regions (9, 10). The open reading frame of the recently identified FcαR gene encodes a transmembrane protein of approximately 30 kD, which has six potential sites for N-linked glycosylation in its extracellular region (11). The selective expression of FcαR by myeloid lineage cells has been confirmed by analysis of FcαR mRNA expression (11) and FcαR molecules identified by monoclonal antibodies specific for native and recombinant FcαR protein (12–14).

In this report, we have used the natural IgA ligand and anti-FcαR mAbs to analyze the expression, regulation, and biochemical nature of FcαR on eosinophils. FcαR molecules were detected on eosinophils from all normal individuals following in vitro activation, whereas fresh eosinophils from allergic individuals frequently expressed FcαR. The FcαR molecules expressed by eosinophils differ from those on neutrophils and macrophages in that the former have a higher content of N-linked carbohydrate moieties.

Methods

Subjects. Heparinized blood samples were obtained from 45 adult individuals (27 male and 18 female). 22 had severe symptoms of allergic rhinitis and/or asthma. These allergic individuals were further selected on the basis of acute wheal and flare reactions in response to two or more allergens when tested by the prick method with extracts of house dust mite, grass pollen, ragweed pollen, tree pollen, and animal danders (15). The other 23 individuals had no history of allergy and were skin-test negative. None of the allergic subjects had received systemic corticosteroids or other medication at the time of blood collection.

Reagents. The following mouse antibodies were used: A3 ($\gamma1\kappa$), A59 ($\gamma1\kappa$), A62 ($\gamma1\kappa$), and A77 ($\gamma1\kappa$) mAbs specific for the FcαR (13), the 32.2 ($\gamma1\kappa$) mAb specific for the FcγR I (CD64) (Medarex, Inc., W. Lebanon, NH), the IV.3 ($\gamma2b$) mAb specific for the FcγRII (CD32w) (ATCC), the 3G8 ($\gamma1\kappa$) mAb specific for the FcγR III (CD16) (16), the W8E7 ($\gamma2a\kappa$) anti-CD10 mAb, the LeuM1 ($\mu\kappa$) anti-CD15 mAb, and the My4 ($\gamma1\kappa$) anti-CD14 mAb (Coulter Corp., Hialeah, FL). Control antibodies included an irrelevant mouse IgG2a (Becton Dickinson & Co., Mountain View, CA), JH3 ($\gamma1\kappa$) anti-Id (17), the Cla ($\mu\kappa$) anti-chicken Ia mAb (18), and the MOPC 141 ($\gamma2b$) myeloma. FITC-labeled goat anti-mouse Ig antibodies lacking cross-reactivity with human Ig were from Southern Biotechnology Associates (Birmingham, AL). The human IgA myeloma proteins and $F(ab')_2$ fragments of the corresponding goat anti-Id antibodies are described elsewhere (19).

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1. *Abbreviation used in this paper:* FcR, Fc receptors.

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Isolation of eosinophils and neutrophils. Red cells and granulocytes were first separated from mononuclear cells by centrifugation over Ficoll/Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) density gradients. Granulocytes were isolated from the red cell pellet by differential sedimentation in 1.5% dextran in PBS (9). All reagents used for granulocyte isolation were prepared in pyrogen-free distilled water. Neutrophils and eosinophils of normal density were separated by using a discontinuous metrizamide gradient (20). Purity of eosinophils varied from 60–90%, whereas neutrophils were > 99% pure as determined by morphological characteristics following Giemsa staining. In some experiments, the eosinophils were further enriched (> 99.5%) by fluorescence-activated cell sorting, where eosinophils were selected on the basis of their light scatter characteristics and nonreactivity with anti-CD16 mAb (21, 22). Eosinophils were cultured for 1–18 h in RPMI 1640 supplemented with 25% of autologous human serum (and penicillin and streptomycin) in the presence or absence of various concentrations of Ca^{2+} ionophore (Ionomycin; Calbiochem, San Diego, CA). Other stimuli included PMA (2–100 ng/ml), Con A (1–50 μg/ml; Sigma Chemical Co., St. Louis, MO), rIFNγ (100 U/ml; Amgen Biologicals, Thousand Oaks, CA), rIFNβ (100 U/ml; Kyowa Hakko, Tokyo Japan), G-CSF (10–10000 pM), and GM-CSF (10–1000 pM). IL-1α (Immunex, Seattle, WA), IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, and TGFβ (Amgen Biologicals) were all used at concentrations of 10–1000 U/ml over a 12-h period of stimulation.

Immunofluorescence analysis of cells. In order to mask FcγR, the cells ($1–2 \times 10^5$) were preincubated with 10 μl of aggregated human IgG (10 mg/ml) for 15 min at 4°C before incubation with 10 μl of test mAb (0.1 mg/ml) for 20 min at 4°C in PBS containing 10% FCS and 0.1% sodium azide. After extensive washing of the cells, FITC-labeled goat antibodies to mouse Ig (0.1 mg/ml) were used as the developing reagent. For detection of IgA binding, an indirect immunofluorescence assay (19) was employed. For this assay, cells were incubated with 10 μl of polymeric IgA (0.5 mg/ml) plus 10 μl of biotin-labeled $F(ab')_2$ fragments of the corresponding goat anti-Id antibodies (0.5 mg/ml) for 20 min at 4°C. Phycoerythrin-labeled streptavidin (Becton Dickinson & Co.) was used as a developing reagent. Unrelated IgA myelomas were used as negative controls. Cells were analyzed by flow cytometry using a FACScan® instrument (Becton Dickinson & Co.).

Immunoprecipitation analysis. Preparations of viable eosinophils (> 99.5% purity) and neutrophils ($3–4 \times 10^6$ cells each) were surface labeled with $Na^{125}I$ (1 mCi; Amersham Corp., Arlington Heights, IL) by the lactoperoxidase method (23). After washing, cells were lysed in 1 ml of 0.5% NP-40 in PBS containing 0.02% sodium azide, 1% aprotinin, 1 mM diisopropylfluorophosphate, 5 mM iodoacetamide, and 1 mM PMSF (9, 13). Cell lysates were cleared by four separate incubations with 20 μg of an isotype-matched control mAb (JH3) plus 30 μl of goat anti-mouse Ig antibody-coupled Sepharose 4B beads (3 mg/ml packed beads) for 2–4 h at 4°C with constant rotation, and by two additional incubations with the anti-mouse Ig-coupled beads only. The absorbed lysates were divided into three aliquots that were incubated with control (JH3) or test (A59 or A62) mAb in the presence of 3 μl of anti-mouse Ig-coupled beads for 4 h at 4°C with constant rotation. After washing extensively with lysis buffer, bound materials were dissociated by addition of 0.5% SDS–0.1 M 2-mercaptoethanol and subjected to *N*-glycanase digestion as described (9). Digested and undigested materials were resolved by SDS–PAGE analysis (24) using 10% acrylamide. Molecular weight markers were purchased from Bio-Rad Laboratories (Richmond, CA).

Statistical analysis. The results were analyzed with the independent sample two-tailed Student's *t* test, and evaluation for correlation was conducted by linear regression analysis.

Results

Expression of FcαR on eosinophils from normal and allergic individuals. The eosinophil population was discriminated from the neutrophil population by differences in forward light scatter and side light scatter characteristics determined by flow cytometric analysis (21, 22). A forward-side scatter population distinct from neutrophils was first observed when eosinophils were partially purified on metrizamide gradients. Sorting for this population resulted in an enrichment in eosinophils to > 98% purity as determined by microscopic evaluation of stained cells. These forward-side scatter characteristics were then used to establish analytical gates for immunofluorescence analysis of eosinophils and neutrophils. The eosinophils were also distinguishable from neutrophils by their lack of or relatively weak expression of cell surface CD10, CD14, CD15, CD16, and CD64 antigens, whereas both cell types expressed CDw32 and CD11b antigens.

To evaluate FcαR expression on blood granulocyte subpopulations, purified eosinophils and the purified neutrophils were examined for binding to either the polymeric IgA ligand or the anti-FcαR mAbs by indirect immunofluorescence. Whereas the eosinophils from an allergic individual expressed easily detectable levels of IgA binding and reactivity with the A59, A3, A62, and A77 anti-FcαR mAb, the eosinophils from a normal individual were noticeably less reactive in both assays (Fig. 1). The level of FcαR expression on normal eosinophils was approximately 10-fold lower than on neutrophils as estimated by mean fluorescence intensities (1.3±1.0 vs. 13±5; Fig. 2).

Because the FcαR was more easily identified on eosinophils from an allergic individual, a comparative analysis of anti-FcαR and anti-FcγR reactivity was performed using eosino-

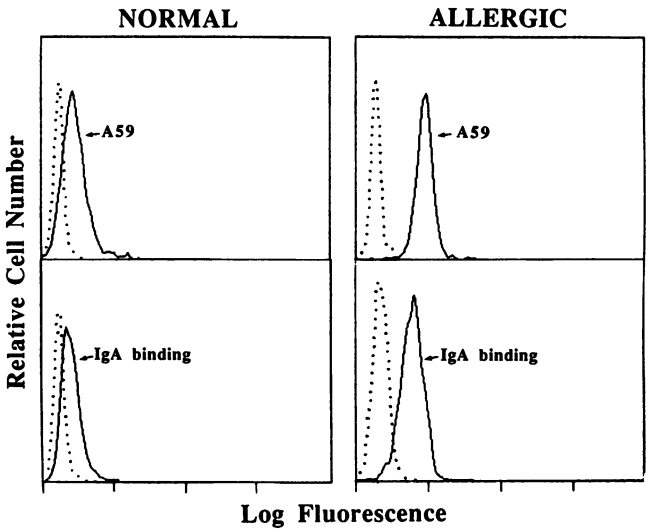

Figure 1. Immunofluorescence analysis of blood eosinophils from normal and allergic individuals using the A59 anti-FcαR monoclonal antibody and the IgA ligand. Blood eosinophils incubated first with aggregated human IgG to block FcγR binding sites were indirectly stained with the A59 mAb (0.1 mg/ml) or an IgG1κ mAb control with irrelevant specificity (JH-3) and FITC-conjugated goat antibodies to mouse IgG (*upper panels*). For IgA binding, eosinophils were first incubated with polymeric myeloma IgA plus biotin-labeled $F(ab')_2$ fragments of corresponding goat anti-Id antibodies, and then with phycoerythrin-labeled streptavidin as a developing reagent (*lower panels*). An unrelated IgA myeloma protein was used as the negative control. Immunofluorescence intensity was analyzed by flow immunocytometry using analytical gates based on eosinophil light scatter characteristics. Background fluorescence of control mAb or myeloma protein is indicated by the dotted histogram.

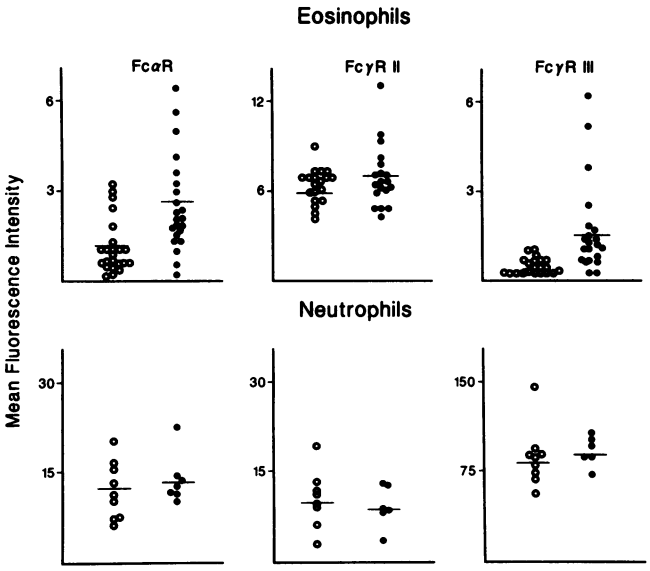

Figure 2. Expression levels of FcαR and FcγR on eosinophils and neutrophils from normal (○) and allergic (●) individuals. Purified eosinophils and neutrophils were stained with anti-FcαR (A59), anti-FcγRII (IV.3), anti-FcγRIII (3G8), or with isotype matched control mAbs as described in Fig. 1. FITC-conjugated goat anti-mouse Ig antibodies were used as a developing reagent. Immunofluorescence intensity was analyzed by flow cytometry using forward and side light scatter gates specific for each cell type. Results were expressed as the mean fluorescence intensity, which was estimated by: x of staining with anti-FcR mAbs − x of background control staining with their isotype matched controls, in which x indicates the computer-determined value for mean intensity for each fluorescence profile. The mean fluorescence intensities of FcαR and FcγRIII on eosinophils were statistically different in both groups of subjects ($P < 0.001$ and < 0.002, respectively). Note differences in the vertical scales for the panels.

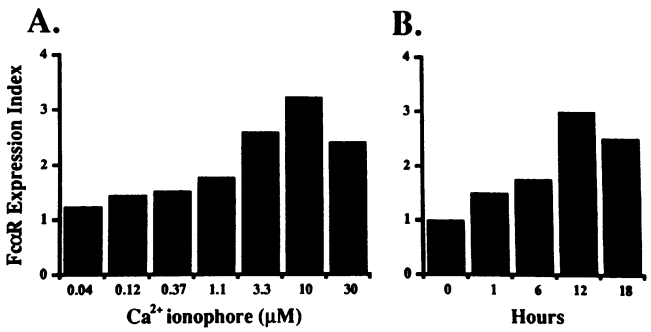

Figure 3. The effect of calcium ionophore stimulation on FcαR expression by normal eosinophils. FACS-purified eosinophils (> 99.5% pure) incubated for 12 h with various doses of Ca^{2+} ionophore (*A*) and for varying time intervals with 10 μM Ca^{2+} ionophore (*B*) were washed and stained with an anti-FcαR mAb (A59) or the IgG1κ control mAb (JH-3) as described in Fig. 1. The immunofluorescence intensity determined by flow cytometry was used to estimate an FcαR expression index (x of staining of Ca^{2+} ionophore-treated cells − x of background control staining of Ca^{2+} ionophore-treated cells)/(x of staining of nontreated cells − x of background control staining of nontreated cells); x indicates the computer-determined value for the mean fluorescence intensity of each FACS profile.

phils and neutrophils from both allergic and normal individuals (Fig. 2). Anti-FcαR mAb reactivity was significantly increased ($P < 0.001$) for eosinophils from 22 allergic individuals (2.9±1.9) as compared with eosinophils from 23 normal individuals (1.3±1.0). FcγRIII expression was also higher on eosinophils from allergic individuals ($P < 0.002$), albeit at low levels relative to that on neutrophils (Fig. 2). In contrast, FcγRII expression was similar for both cell types in normal and allergic individuals.

Levels of IgA, IgG, and IgM were found to be in the normal range for all of the allergic individuals (data not shown), whereas increased IgE levels of > 100 μg/ml were found in more than half of the allergic individuals (12/22) and none of the controls (0/23). Significant correlation was not observed between FcαR levels on either cell type and serum Ig isotype levels. In addition, no significant differences were observed in neutrophil expression of FcαR and FcγR for normal and allergic individuals.

Regulation of FcαR expression on normal eosinophils. Highly purified eosinophils (> 99.5%) were examined for FcαR and FcγR expression before and after treatment with various cellular stimuli. FcαR expression was upregulated by approximately threefold following Ca^{2+} ionophore stimulation (Fig. 3), whereas parallel experiments revealed no increase in the level of FcαR expression by cells of the U937 monocytic cell line (not shown). The increase in eosinophil FcαR expression was ionophore dose dependent with the maximal response occurring at a concentration of 10 μM. The increase in FcαR expression, which was maximal at 12 h after Ca^{2+} ionophore stimulation, was inhibited by the chelating agent EGTA (Fig. 4). These results were obtained in experiments employing both the A59 and A62 anti-FcαR mAbs and the IgA ligand itself (not shown). In contrast, eosinophil FcγRII and FcγRIII levels were unaffected by Ca^{2+} ionophore stimulation (data not shown).

FcαR expression by normal eosinophils was unaffected by similar treatment with a variety of other stimuli, including PMA, Con A, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, rIFN-α, -β, and -γ, TGFβ, G-CSF, and GM-CSF (data not shown). Changes were not observed even after exposure of the eosino-

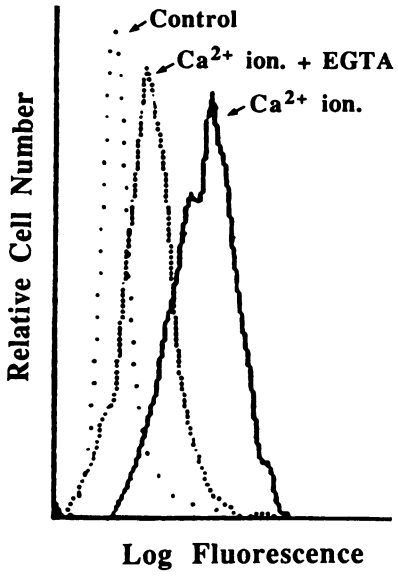

Figure 4. Calcium ionophore–induced upregulation of FcαR on eosinophils is blocked by the chelating agent EGTA. Blood eosinophils of 99.5% purity were stimulated with Ca^{2+} ionophore (10 μM) in the presence or absence of EGTA (5 μM) for a 12-h period, washed, and stained with an anti-FcαR mAb (A59) as described in Fig. 1. The dotted histogram represents background fluorescence with a nonreactive, isotype-matched control mAb.

phils to cytokine combinations, such as IL-3, IL-5, and GM-CSF, which have been shown to induce eosinophilic differentiation (25). FcαR expression reactivity was also unaffected when normal eosinophils were cultured in serum from allergic individuals.

Biochemical nature of eosinophil FcαR molecules. Eosinophils highly purified (> 99.5% pure) by fluorescence-activated cell sorting were used for these studies in order to avoid neutrophil contamination, since the neutrophils express relatively high levels of FcαR molecules. Eosinophils in sufficient numbers ($\sim 4 \times 10^6$) were obtained using blood samples from allergic individuals for high numbers of blood eosinophils expressing relatively high FcαR levels. When iodinated cell surface proteins from the eosinophils were immunoprecipitated with A59 and A62 mAb, a broad 70–100-kD band could be identified (Fig. 5, lanes *9* and *11*) whereas FcαR molecules isolated from neutrophils had a molecular mass of 55–75 kD (lanes *3* and *5*). Removal of N-linked carbohydrate moieties from the FcαR molecules on eosinophils yielded the same major 32-kD protein core as that found on neutrophils (lanes *10* and *12* vs. lanes *4* and *6*), whereas the minor 36-kD protein band seen in neutrophils was not observed in the *N*-glycanase-treated lysates of eosinophils. This result was confirmed using eosinophils from five other allergic individuals.

Discussion

The results of this study confirm that eosinophils can bind IgA (6, 7), and demonstrate their expression of FcαR, albeit in lower levels than on neutrophils. Eosinophil expression of FcαR was selectively enhanced by treatment with a Ca^{2+} ionophore, whereas FcγRII and FcγRIII expression were unaffected. Our results further suggest that FcαR expression may be differentially regulated depending on the myeloid cell lineage. Treatment of monocytic cell lines (U937 and PLB985) with phorbol ester enhanced their expression of FcαR (9, 13), but did not affect FcαR expression by eosinophils. Conversely, while a Ca^{2+} ionophore enhanced FcαR expression by eosinophils, this treatment did not alter FcαR expression on U937 monocytoid cells. The existence of differential regulatory elements for FcαR expression in different myeloid cell types is thus suggested by these results.

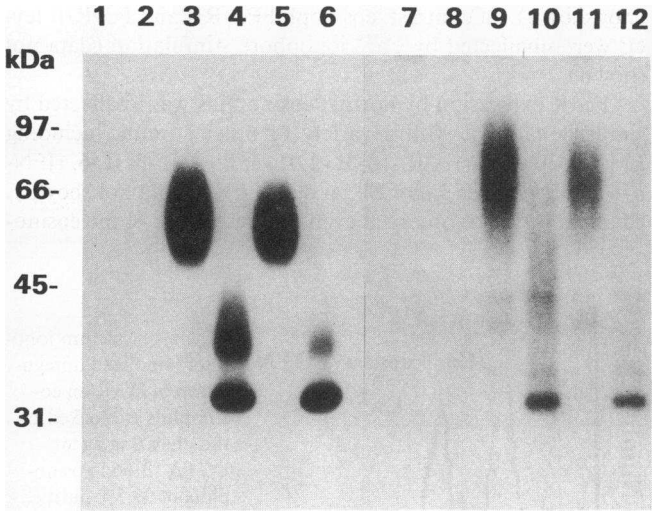

Figure 5. Comparative analysis of FcαR molecules on eosinophils and neutrophils. FACS-isolated eosinophils (> 99.5% pure) and neutrophils from the blood of an allergic individual were surface labeled with $Na^{125}I$ and the membrane proteins then solubilized using a 0.5% NP-40 lysis buffer as described in Methods. The lysates were precleared several times with an excess of a mouse IgG1κ mAb (JH-3) coupled to Sepharose 4B beads, then divided into three aliquots and incubated with mouse IgG1κ (lanes *1, 2, 7, 8*), A59 (lanes *3, 4, 9, 10*), or A62 mAb (lanes *5, 6, 11, 12*) coupled to Sepharose 4B beads. The mAb-reactive molecules isolated from the cell lysates were incubated in the presence (lanes *2, 4, 6, 8, 10, 12*) or absence (lanes *1, 3, 5, 7, 9, 11*) of *N*-glycanase. The acetone-precipitated samples were then resuspended and analyzed by SDS-10% PAGE.

Since enhanced FcαR expression by eosinophils can occur as a consequence of in vitro cell activation, the finding of enhanced FcαR expression on eosinophils in allergic individuals may reflect in vivo activation. IgA is the major Ig isotype in the secretions where eosinophils carry out some of their effector functions (1–5) implying functional relevance of the FcαR on eosinophils. Cytophilic IgA has been detected on the surface of intestinal eosinophils in parasite-infected mice (26). Moreover, treatment of eosinophils with secretory IgA antibodies resulted in the release of eosinophil-derived neurotoxin (7). In the latter study, secretory IgA was shown to initiate a signal for eosinophil degranulation that was two to three times more potent than that of IgG antibodies. FcαR on activated eosinophils could thus play an effector role in host defense against helminth infections and in the pathogenesis of the inflammation in hypersensitivity diseases, such as asthma (27).

Not all allergic individuals exhibit upregulated FcαR expression on the eosinophils. In approximately 25% of our patients with severe allergic rhinitis, asthma, or both, the eosinophils expressed FcαR below or near the normal mean level. Neither serum IgE levels nor clinical status were obvious correlates with the eosinophil FcαR levels, indicating further need for investigation into the basis for the FcαR upregulation in allergic individuals.

The FcαR molecules on eosinophils are closely related to the FcαR on neutrophils, as both are recognized by monoclonal anti-FcαR antibodies. However, we found that the FcαR molecules isolated from eosinophils have higher molecular mass (70–100 kD) than those from neutrophils (55–75 kD). A previous report had suggested that a 55–60-kD IgA receptor exists on eosinophils (28) and, in preliminary immunoprecipitations of surface molecules from eosinophil preparations obtained by metrizamide gradients, we also identified FcαR with molecular masses of 55–75 kD. In our studies, however, these FcαR molecules were found to originate from contaminating neutrophils (5–10%), which express relatively high levels of FcαR, and more rigorous eosinophil purification led to elimination of the 55–75-kD FcαR and its replacement by the 70–100-kD FcαR form.

The relative increase in FcαR size on the eosinophil was found to be due to differential glycosylation of a single protein core. Removal of N-linked carbohydrates by *N*-glycanase digestion of FcαR from eosinophils yielded a single protein band of 32 kD, whereas similar treatment of FcαR from neutrophils gave rise to both 32- and 36-kD bands. This result was confirmed using two monoclonal anti-FcαR antibodies, A59 and A62 (13), both of which are reactive with the recombinant product of the single FcαR gene (11, 14). Our results therefore provide further evidence for molecular heterogeneity of FcαR molecules on cells of neutrophil, monocyte/macrophage, and

eosinophil lineages, and suggest that differently glycosylated FcαR are created by posttranslation modification. Because only one FcαR gene can be identified with the available FcαR cDNA probe, the theoretical possibility of two closely related genes with different numbers of glycosylation sites seems unlikely. Elucidation of the precise molecular basis for the differential glycosylation of FcαR molecules on the different cell types is needed because differences in the carbohydrates moieties could influence ligand binding, thus affecting specific effector functions mediated by the different types of myeloid cells.

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