CELLULAR DISTRIBUTION, REGULATION, AND BIOCHEMICAL NATURE OF AN Fcα RECEPTOR IN HUMANS

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The cellular attachment of Ig molecules to cell surface Fc receptors (FcR)[1] elicits a variety of functional consequences, depending upon the Ig isotype specificity of the FcR and the cell type that expresses it (1). For example, the binding of IgG to FcγR on macrophages leads to the endocytosis and lysosomal degradation of soluble immune complexes (2), the phagocytosis of IgG-coated particles (3), and the secretion of potent inflammatory mediators such as prostaglandins, leukotrienes, oxygen intermediates, and neutral proteases (1, 4).

Three different types of IgG Fc receptors (FcγR) have been identified on human blood cells (reviewed in reference 5). FcγR I (CD64) is a 72-kD glycoprotein on monocytes and macrophages that serves as a high affinity receptor for monomeric IgG (5, 6). FcγR II (CDw32), a low affinity receptor of 40 kD, is found on a wide variety of cell types, including monocytes, platelets, neutrophils, and B cells (5–7). FcγR III (CD16) is a variably glycosylated, low affinity receptor of 50–70 kD that is present on neutrophils, eosinophils, NK cells, a subset of T cells, and cultured monocytes (5, 8, 9). Interestingly, the FcγR III on neutrophils is anchored to the membrane via a glycosyl-phosphatidylinositol (GPI) linkage (10–13), whereas a transmembrane form of FcγR III is identified on NK cells (12, 13). Two different types of IgE FcR have been described. The high affinity FcεR I, composed of a tetrameric complex (a 45-kD α chain, a 33-kD β chain, and two 9-kD γ chains), is present on mast cells and basophils (14, 15). The low affinity FcεR II (CD23), composed of a 45-kD glycoprotein, is present on monocytes, eosinophils, T cells, and B cells (16, 17). A glycoprotein of ~100 kD is involved in the transport of polymeric IgA and IgM across epithelial cells, and thus is denoted as the poly-Ig FcR (18). The genes encoding all of these FcR, except FcεR II, belong to the Ig gene superfamily (reviewed in references 15 and 19).

This work was supported by grants CA-16673, CA-13148, and AI-18745 from the National Institutes of Health. M. D. Cooper is a Howard Hughes Medical Institute investigator.

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[1] *Abbreviations used in this paper:* anti-Id, antiidiotype; DAF, decay-accelerating factor; FcR, Fc receptor; G-CSF, granulocyte CSF; GM-CSF, granulocyte/macrophage CSF; GPI, glycosyl-phosphatidylinositol; pI, isoelectric point; PI-PLC, phosphatidylinositol-specific phospholipase C.

IgA binding to various types of blood mononuclear cells in humans has been noted by several investigators (20 and see review in reference 21). IgA-binding cells were identified as monocytes and granulocytes in our previous studies (22), and in those of other laboratories (23, 24). The IgA receptors (IgA-R) on monocytes were shown to be constitutively expressed (22, 24) and to be involved in IgA-mediated phagocytosis (24). Granulocyte/macrophage CSF (GM-CSF) and granulocyte CSF (G-CSF) can induce a change from low to high affinity receptors for IgA on granulocytes and the acquisition of IgA-mediated phagocytic capability (25).

The present studies were designed primarily to examine the biochemical nature of IgA-R on monocytes and granulocytes. The results indicate that a heavily glycosylated protein with an M_r of ~60 kD is the FcR for IgA on both of these cell types and that this FcαR is distinct from the previously reported FcR.

Materials and Methods

Human Paraproteins. IgA myeloma proteins, IgA1κ and IgA1λ, were purified from patients' sera by salt fractionation, DEAE ion exchange cellulose, and Ultrogel AcA 22 gel filtration column chromatographies, as previously described (26). The polymeric IgA fraction was shown by immunoelectrophoresis and SDS-PAGE, under both reducing and nonreducing conditions, to be free of other contaminating serum proteins and to have the appropriate molecular size. Fabα and Fcα fragments were obtained by cleaving IgA1 with IgA1-specific protease (see review in reference 27). Briefly, polymeric IgA1λ (10 mg/ml in 0.05 M phosphate buffer, pH 7.0) was digested at 37°C for 15 h with crude IgA1 protease supernatant from *Hemophilus influenzae* (ATCC 35891) (HK 50) at a ratio (vol/vol) of 10:1 (kindly provided by Dr. J. Mestecky, University of Alabama at Birmingham). After dialyzing against 10 mM Tris-HCl buffer, pH 8.0, Fabα and Fcα fragments were separated by DEAE ion exchange column chromatography using a linear gradient to 0.3 M NaCl. Purity (≥98%) of the Fabα and polymeric Fcα fragments was confirmed by SDS-PAGE analysis under both reducing and nonreducing conditions. An IgG4λ paraprotein was purified as previously described (28), and shown by SDS-PAGE analysis to be uncontaminated with other Ig isotypes.

Preparation of Antiidiotype Antibodies. The purified IgA and IgG4λ myeloma proteins were used to raise polyclonal antiidiotype (anti-Id) antibodies in goats and rabbits. Removal of nonidiotypic antibodies by affinity columns, purification of anti-Id antibodies by the corresponding myeloma protein–coupled Sepharose 4B columns, and preparation of $F(ab')_2$ fragments of anti-Id antibodies have been described elsewhere (22, 26, 28). Complete digestion of IgG molecules into $F(ab')_2$ fragments was established by SDS-PAGE analysis. $F(ab')_2$ fragments of anti-Id antibodies against IgA or IgG4 myeloma proteins were coupled to biotin as described (22). The idiotype specificity of these antibodies was confirmed by immunofluorescent staining of the homologous myeloma cells and by lack of staining of nonhomologous myeloma cells and normal plasma cells.

Cells. Mononuclear cells isolated from normal blood by Ficoll-Hypaque density gradient centrifugation were subjected to rosette formation with 2-aminoethylisothiouronium bromide–treated SRBC (29). Monocytes were enriched from the T cell–depleted fraction by adherence to plastic tissue culture dishes for 1 h at 37°C and recovered by incubation with PBS containing 5% FCS for 30 min on ice and gentle scraping with a rubber policeman. Cells in these preparations were predominantly reactive (70–80%) with the Leu-M1 (CD15) mAb (30). Granulocytes were isolated from the RBC pellet by differential sedimentation in 1.5% dextran in PBS (31). All reagents for granulocyte preparation were made by using pyrogen-free distilled water containing soybean trypsin inhibitor (1 mg/ml; Sigma Chemical Co., St. Louis, MO) to avoid cell aggregation. This fraction contained >95% granulocytes by morphological criteria.

Two human myelomonocytic cell lines, U937 (32) and PLB 985 (33), were obtained from the American Type Culture Collection (Rockville, MD) and Dr. T. A. Rado (University of Alabama at Birmingham), respectively. For the induction of IgA-R expression, cells were

stimulated with varying concentrations of PMA (Sigma Chemical Co.), rIFN-γ (Amgen Biologicals, Thousand Oaks, CA), rIFN-α (Roche-Takeda Co., Tokyo, Japan), rIFN-β (Kyowa Hakko, Tokyo, Japan), rIL-1β (Amgen Biologicals), or rIL-4 (Immunex, Seattle, WA). Cells were also cultured with varying doses of polymeric IgA1κ for 0.5–18 h. In some experiments, after preincubation with polymeric IgA1κ for 18 h, the washed cells were cultured in the medium without IgA for various periods.

Immunofluorescence Analysis of Cells. For the detection of IgA-R, a previously established immunofluorescence assay was performed (22). Briefly, cells (5×10^5) were incubated for 15–60 min at 4°C with 10 μl of purified polymeric IgA (0.5 mg/ml) and 10 μl of biotin-labeled F(ab')$_2$ fragments of the appropriate goat anti-Id antibodies (0.5 mg/ml) in PBS containing 0.01% $CaCl_2$, 0.01% $MgCl_2$, 1% BSA, and 0.05% sodium azide. After washing, phycoerythrin-labeled streptavidin (Becton Dickinson & Co., Mountain View, CA) was used as a developing reagent. Unrelated IgA myelomas were used as controls. Cells were analyzed by flow immunocytofluorometry using a FACScan instrument (Becton Dickinson & Co.). In some experiments, the following mouse mAbs and their isotype-matched control mAbs were used: (*a*) 32.2 (γ1κ isotype) and IV.3 (γ2b) mAbs specific for FcγR I and II, respectively, kindly provided by Dr. M. Fanger (Dartmouth Medical School, Hanover, NH) and Dr. C. L. Anderson (Ohio State University, Columbus, OH) (34, 35); (*b*) 3G8 (γ1κ) mAb specific for FcγR III, a generous gift of Dr. J. C. Unkeless (Mt. Sinai Medical School, NY) (8); and (*c*) IA10 (γ2aκ) mAb specific for decay-accelerating factor (DAF; CD55), a kind gift of Dr. V. Nussenzweig (New York University School of Medicine, NY) (36). FITC-labeled goat antibodies specific for mouse Ig (Southern Biotechnology Associates, Birmingham, AL) were used as the developing reagent.

Immunoprecipitation of Iodinated Membrane Proteins. 2–3 $\times$ 10^7 viable cells were extensively washed in PBS, labeled with $Na^{125}I$ (1 mCi; Amersham Corp., Arlington Heights, IL) by the lactoperoxidase method (37), and lysed in the following solutions: (*a*) 0.5% NP-40 in PBS containing 0.01% $CaCl_2$, 0.01% $MgCl_2$, 0.02% sodium azide, 0.01% soybean trypsin inhibitor, leupeptin (10 μg/ml), pepstatin A (1 μg/ml), chymostatin (2 μg/ml), antipain (2 μg/ml), 10 mM benzamidine hydrochloride, 50 mM ϵ-amino-caproic acid, 20 mM iodoacetamide, and 1 mM PMSF, for monocytes and U937 cells; and (*b*) 0.5% NP-40 in PBS containing 0.01% $CaCl_2$, 0.01% $MgCl_2$, 0.02% sodium azide, 1% aprotinin, 1 mM diisopropylfluorophosphate, 5 mM iodoacetamide, and 1 mM PMSF for granulocytes. In some experiments, 0.3% CHAPS or 1% digitonin was used instead of NP-40. After centrifugation, the cell lysates were first incubated with Sepharose 4B coupled with normal human IgG and with normal goat IgG (5 mg/ml of gel) for 2 h at 4°C with rotation. This preclearance procedure was repeated seven more times, and lysates were then incubated with 10 μl of polymeric myeloma IgA (1 mg/ml) plus 20 μl of Sepharose 4B coupled with F(ab')$_2$ anti-Id antibody (2 mg/ml of gel) for 2 h (for granulocytes) or overnight (for monocytes and U937 cells) at 4°C. After washing extensively with lysis buffer, the bound molecules were dissociated by addition of Laemmli's sample buffer (38), and resolved by SDS-PAGE analysis using 10% acrylamide. Molecular weight markers are lysozyme, 14,400; soybean trypsin inhibitor, 21,500; carbonic anhydrase, 31,000; OVA, 42,699; BSA, 66,200; and phosphorylase b, 97,400 (Bio-Rad Laboratories, Richmond, CA). In some experiments, IgG4λ myeloma plus its rabbit F(ab')$_2$ anti-Id antibody-coupled Sepharose 4B and 3G8 mAb-coupled Sepharose 4B were used to detect FcγR I and III, respectively. For neuraminidase treatment, IgA-bound molecules were first eluted from beads with 0.5 M acetic acid containing 0.5% NP-40, the pH was adjusted to 7.0 by addition of 2 M Tris, and then the molecules were incubated at 37°C for 2 h in the presence of 1 mM PMSF, 1% aprotinin, and neuraminidase (50 U/ml; Gibco Laboratories, Grand Island, NY). For glycosidase digestion (39), the IgA-bound molecules were dissociated by incubation for 20 min at 80°C in the presence of 0.5% SDS-0.1 M 2-ME, diluted into 0.17% SDS, 33 mM 2-ME, 0.2 M sodium phosphate (pH 8.6), 10 mM 1,10-phenanthroline hydrate, and 1.25% NP-40, and incubated overnight at 37°C with *N*-glycanase (Genzyme, Boston, MA) at 10–40 U/ml. Digested and undigested control materials were precipitated in acetone at −20°C, washed in 70% ethanol, dried, and resuspended in Laemmli's sample buffer. For two-dimensional gel analysis, the IgA-bound molecules were resuspended in 9.5 M Urea/2% Triton X-100/5% 2-ME, and separated first in tube gels containing a 4:1

mixture of 5/7 ampholite and 3/10 ampholite, and then run in slabs of SDS-PAGE according to the method of O'Farrell et al. (40).

Enzyme Treatment of Cells. Granulocytes, monocytes, and PMA-activated U937 cells (5×10^6/ml) were incubated for 45 min at 37°C with HBSS containing various amounts of one of the following enzymes: trypsin-TPCK (Sigma Chemical Co.), pronase (Calbiochem-Behring Corp., La Jolla, CA), and neuraminidase (Gibco Laboratories). For GPI-anchor experiments, cells were resuspended in HBSS, pH 7.4, containing 10 mM Hepes and 0.1% BSA, and treated for 45 min at 37°C with phosphatidylinositol-specific phospholipase C (PI-PLC; from *Bacillus thuringiensis*; enzyme activity, 0.35 μM/min/ml), a generous gift of Dr. M. G. Low (Columbia University, NY) (41). After treatment, cells were washed with PBS containing 5% FCS, overlaid on Ficoll-Hypaque to eliminate dead cells, stained for surface IgA-R and other antigens, and analyzed by flow immunocytometry.

Results

Expression of IgA-R and IgG-R by Blood Monocytes, Granulocytes, and Related Cell Lines. We have previously demonstrated by immunofluorescence that freshly isolated blood monocytes constitutively express class-specific receptors for both IgA1 and IgA2 molecules (22). To determine whether the same or distinctive subpopulations of monocytes and granulocytes express IgG-R and IgA-R, two-color immunofluorescence analysis was performed. A combination of the polymeric IgA-myeloma protein plus the corresponding biotinylated anti-Id antibody was used for the detection of IgA-R, and mAbs specific for each FcγR (32.2, IV.3, or 3G8) for the simultaneous detection of FcγR I, II, or III. As shown in Fig. 1, monocytes expressed the IgA-R and the FcγR I and II, but not the FcγR III. Granulocytes expressed the IgA-R at a relatively lower level. All of the IgA-R-bearing granulocytes expressed FcγR II and III, but not FcγR I.

Two myelomonocytic cell lines (U937 and PLB 985) were also examined for the expression of IgA-R. Both the U937 (see below) and the PLB 985 cell lines exhibited IgA binding at relatively low levels. The IgA-R on these cell lines was not specific for a particular IgA myeloma protein, since binding assays using two different IgA1, as well as an IgA2, myelomas yielded the same results. Both cell lines displayed constitutive expression of both FcγR I and II, but not of FcγR III. The results indicate

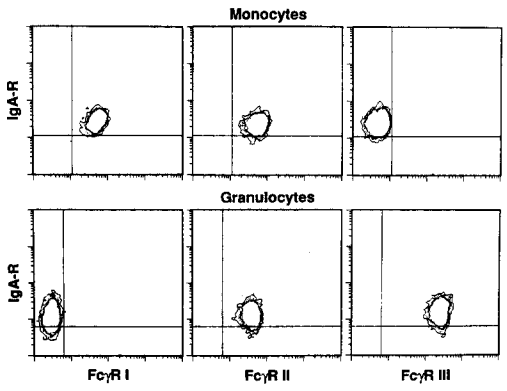

FIGURE 1. Immunofluorescence analysis of IgA-R and IgG-R expression by human monocytes and granulocytes. Cells were first incubated with an IgA immune complex (polymeric IgA1κ myeloma plus its biotinylated $F(ab')_2$ anti-Id antibody) and phycoerythrin-labeled streptavidin, as described in Materials and Methods. The cells were preincubated with human serum IgG (10 mg/ml) in order to mask the binding sites of FcγR, and then counterstained for the FcγR expression by using intact mAbs specific for a nonbinding site epitope of each FcγR (0.05 mg/ml): 32.2 mAb for FcγR I, IV.3 mAb for FcγR II, and 3G8 mAb for FcγR III. FITC-labeled goat anti-mouse Ig antibody without cross-reactivity to human Igs was used as a developing reagent. Nonhomologous IgA1 myeloma and isotype-matched mouse myeloma proteins were used as controls.

that both monocytes and granulocytes express IgA-R, while each expresses a distinctive array of FcγR.

Regulation of IgA-R Expression by U937 Cells. To examine for differences in the regulation of IgA-R and FcγR expression, U937 cells were examined before and after treatment with various stimuli. IgA-R expression was upregulated two- to three-fold after stimulation with PMA (Fig. 2). The increased expression was PMA dose dependent with the maximal response occurring at a concentration of $\sim 10^{-7}$ M. Increased IgA-R expression was observed as early as 12 h after PMA stimulation, and the maximal response was obtained at 18 h (Fig. 3). In contrast, the levels of FcγR I and FcγR II expression were unaffected by stimulation with PMA over a wide range of concentrations. PMA did not induce the expression of FcγR III (CD16) that could be detected by the 3G8 mAb. The same results were obtained with another myelomonocytic cell line, PLB 985 cells (data not shown).

rIFN-γ enhanced the expression of FcγR I in a dose-dependent manner, as noted previously (42), whereas rIFN-α, -β, and -γ had no effect on IgA-R expression by U937 cells (Fig. 4; the results with rIFN-α and -β are not shown). No rIFN-γ-induced change in the expression of FcγR II was observed, and FcγR III expression was not

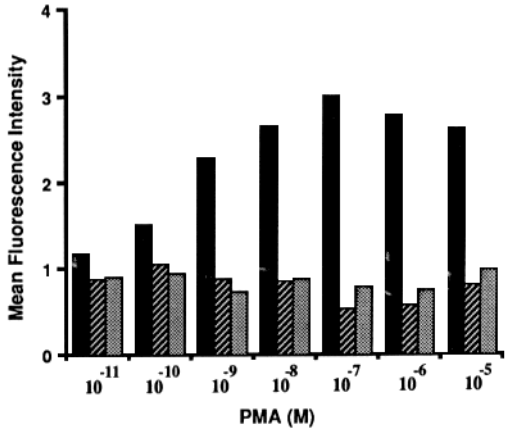

FIGURE 2. The effect of PMA stimulation on expression of IgA-R and FcγRs by U937 cells. Cells were incubated for 18 h with various doses of PMA, washed, and stained for IgA-R (■), FcγR I (▨), and FcγR II (▩), as described in Fig. 1. The stained cells were analyzed by flow immunocytometry. Results were expressed as the mean fluorescence intensity, which was estimated by: (x of staining of PMA-treated cells − x of background control staining of PMA-treated cells)/(x of staining of nontreated cells − x of background control staining of nontreated cells); in which x indicates the computer mean fluorescence intensity value of each FACS profile.

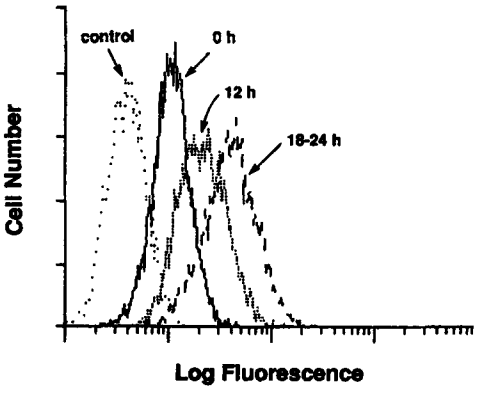

FIGURE 3. Time course of PMA-induced IgA-R expression by U937 cells. Cells were incubated for varying time intervals with PMA (10^{-7} M), washed, and stained for IgA-R, as described in Fig. 1. IgA-R expression was unaltered over the first 6-h period (0.5-, 1-, 2-, and 6-h profiles are not shown).

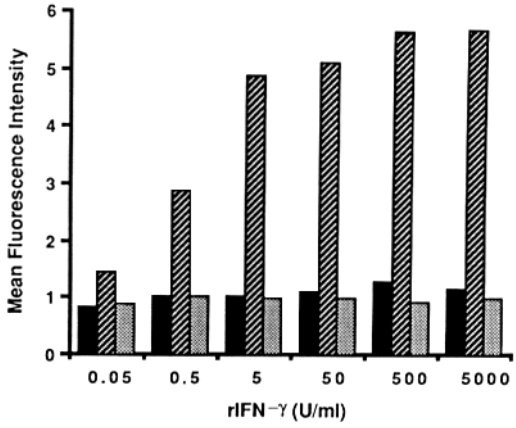

FIGURE 4. Recombinant IFN-γ stimulation enhances FcγR I, but not IgA-R, expression by U937 cells. Cells were incubated for 18 h with various doses of rIFN-γ, washed, and stained for IgA-R (■), FcγR I (▨), and FcγR II (▩), as described in Fig. 1. Mean fluorescence intensity was calculated as described in Fig. 2.

induced. Neither rIL-1β nor rIL-4 (10–1,000 U/ml) affected IgA-R expression (data not shown).

Polymeric IgA promptly induced increased IgA-R expression by U937 cells in a dose-dependent manner (Fig. 5). This upregulation was dependent on the continuous presence of IgA; removal of polymeric IgA resulted in a time-dependent decline of IgA-binding capability. The cellular expression of FcγR I and II did not change during the course of these experiments.

The results reveal that PMA and polymeric IgA induce enhanced expression of IgA-R by U937 cells, but do not affect FcγR expression. Conversely, IgA-R expression is unaffected by IFN-γ, which enhances FcγR I expression.

Characterization of IgA-R Molecules. To determine the molecular nature of the IgA-R, iodinated IgA-binding proteins from the cell surface of granulocytes, monocytes, and the U937 cell line were examined by SDS-PAGE. A broad band with an apparent M_r of 60 ± 3 kD (mean ± SD from 21 experiments) was specifically precipi-

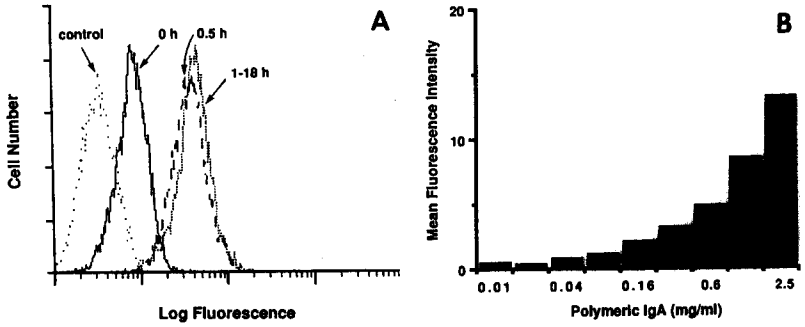

FIGURE 5. Polymeric IgA induces upregulation of IgA-R expression by U937 cells. (*A*) U937 cells were cultured for varying intervals (0.5, 1, 3, 6, 12, and 18 h) with polymeric IgA1κ at 0.5 mg/ml, washed, and stained with the corresponding anti-Id antibody. In this experiment, an anti-Id antibody specific for another IgA myeloma (IgA1λ) was used as a negative control. (*B*) Cells were incubated for 1 h with twofold serial dilutions of polymeric IgA1κ (2.5 mg/ml), washed, and stained with the corresponding anti-Id antibody. Mean fluorescence intensity was calculated as described in Fig. 2.

tated from all three cell sources by an IgA immune complex consisting of a polymeric IgA1κ myeloma and F(ab')$_2$ fragments of anti-Id antibodies (Fig. 6, lanes *4*, *6*, *8*, and *13*). In agreement with the immunofluorescence results, PMA-activated U937 cells yielded a more intense 60-kD band than did the unstimulated U937 cells (Fig. 6, lane *8* vs. *13*). The same molecular mass estimate was obtained under both reducing and nonreducing conditions. When the radiolabeled cells were solubilized with 0.3% CHAPS or 1% digitonin, mild detergents that can lyse cells without disrupting noncovalently associated complexes of membrane proteins, no additional bands were coprecipitated with the 60-kD protein (data not shown). The specificity of the 60-kD protein for IgA was indicated by the following observations: (*a*) F(ab')$_2$ fragments of anti-Id antibodies (goat and rabbit) plus the Id IgA1κ myeloma revealed the 60-kD protein, whereas anti-Id alone did not precipitate the molecule (Fig. 6; compare with Fig. 8); (*b*) another IgA myeloma (IgA1λ) and its corresponding anti-Id antibody also precipitated the same 60-kD molecule; (*c*) monomeric IgA1κ and an IgA2λ both precipitated the molecule; and (*d*) after removal of FcγRs by preclearance with normal IgG and IgG4 myeloma or with mAbs specific for FcγR I, II, and III, the distinctive 60-kD IgA-R was still identified (see Fig. 6, lanes *1* and *2* vs. *4*, and lanes *9–11* vs. *13*).

In two-dimensional gel analysis, the IgA-R isolated from PMA-activated U937 cells was resolved into a series of spots with isoelectric points (pI) ranging from 4.5 to 5.6 (Fig. 7 *A*). Neuraminidase treatment reduced the size of the IgA-R to 54 kD, which was distributed in a two-dimensional gel as five major spots with pI between 5.8 and 6.6 (Fig. 7 *B*). This pattern was very different from the 72-kD FcγR I, which was resolved into ~10 spots with pI between 6.0 and 7.0 before, and into basic end spots with pI >7.0 after neuraminidase treatment (data not shown), consistent with the results reported by others (6, 34).

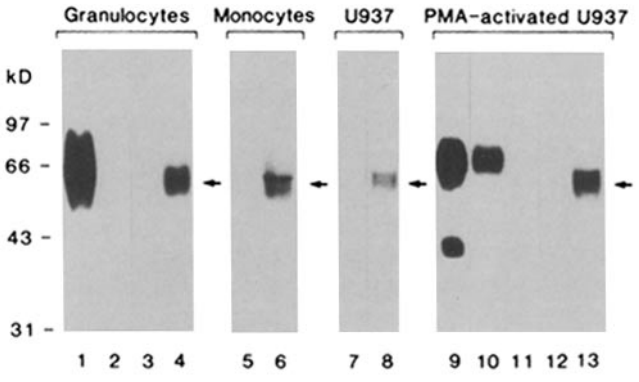

FIGURE 6. SDS-PAGE analysis of IgA-R molecules. Granulocytes and monocytes freshly purified from peripheral blood, and U937 cells activated without or with 10^{-7} M PMA (~3×10^7 cells each) were labeled with 1 mCi of $Na^{125}I$ and solubilized with 0.5% NP-40 buffer, as described in Materials and Methods. The radiolabeled membrane lysates were precleared several times with Sepharose 4B beads to which 3G8 anti-FcγR III mAb (lane *1*), normal human IgG (lane *9*), or rabbit F(ab')$_2$ anti-Id antibody plus its corresponding Id, IgG4λ myeloma protein (lane *10*) were coupled. After removal of FcγRs (see lanes *2* and *11*), the precleared lysates were divided into two portions. One was used as a negative control by incubating with goat F(ab')$_2$ anti-Id-coupled beads (lanes *3*, *5*, *7*, and *12*). The other portion was used to assay for IgA-R by incubating with the IgA immune complex: goat F(ab')$_2$ anti-Id-coupled beads plus its homologous polymeric IgA1κ (lanes *4*, *6*, *8*, and *13*). The bound proteins were dissociated by Laemmli's sample buffer and analyzed by SDS-10% PAGE under both nonreducing (data not shown) and reducing conditions. Mobilities and sizes (kD) of standard proteins are indicated. The arrows indicate the 60-kD IgA-R molecule.

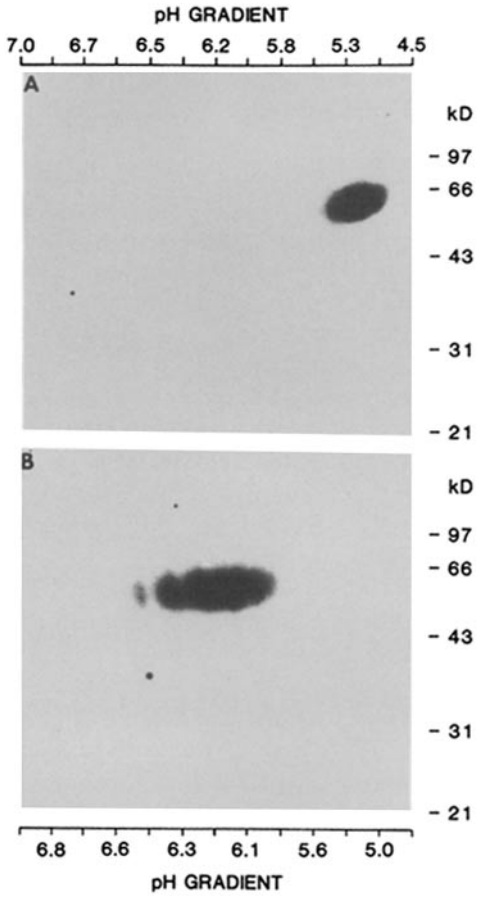

FIGURE 7. Two-dimensional (IEF and SDS) gel electrophoresis analysis of IgA-R. IgA-R isolated from radiolabeled membrane lysate of PMA-activated U937 cells (5×10^7 cells) was eluted from beads and divided into two portions. One (*A*) received buffer, and the other (*B*) received neuraminidase (50 U/ml), as described in Materials and Methods. The samples were precipitated with acetone, resuspended, and separated first by IEF, and then by SDS-10% PAGE. Direct pH measurements were made with sliced control gels resuspended in distilled water. Mobilities and sizes (kD) of standard proteins are indicated.

When the purified IgA-R from granulocytes and activated U937 cells was digested with an excess of *N*-glycanase (40 U/ml; reference 43), the 60-kD IgA-R from both cell sources was resolved into two bands with apparent M_r of 32 and 36 kD (Fig. 8, lanes *2* and *5* vs. *3* and *6*). The relative intensities of these two bands were constant after digestion with varying concentrations of *N*-glycanase (10–40 U/ml), suggesting that the larger 36-kD band was not the result of incomplete deglycosylation. In agreement with previous reports (9, 13, 43–46), *N*-glycanase treatment of granulocyte FcγR III and U937 FcγR I resolved into two bands of 23 and 28 kD (Fig. 8, lane *8* vs. *9*) and into a single band of ~40 kD, respectively (data not shown).

These findings demonstrate that the IgA-R on granulocytes, monocytes, and U937 cells: (*a*) migrates as a broad band of ~60 kD under both reducing and nonreducing conditions; (*b*) appears identical in size on both cell types; (*c*) is distinctive from IgG-R (FcγR I, II, and III); (*d*) exhibits heterogenous charge (pI, 4.5–5.6); and (*e*) is composed of two protein cores (32 and 36 kD) with multiple *N*-linked carbohydrate moieties.

Determination of Regions Involved in IgA Binding. A polymeric IgA1λ molecule, its purified Fabα and Fcα fragments, and other classes of Ig were used as inhibitors

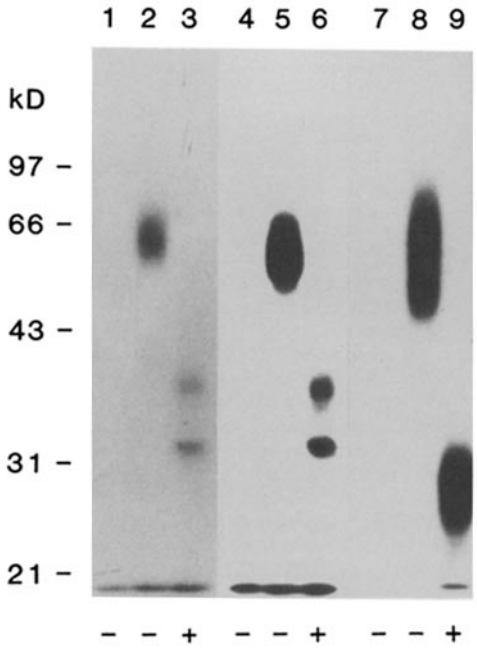

FIGURE 8. Glycoprotein nature of IgA-R molecules. 3×10^7 PMA-activated U937 cells (lanes 1-3) and freshly isolated granulocytes (lanes *4–9*) were labeled and solubilized as described in Fig. 6. The IgA-R and FcγR III were isolated from the radiolabeled membrane lysates and incubated in the presence (+) or absence (−) of *N*-glycanase (40 U/ml), as described in Materials and Methods. The samples were precipitated with acetone, resuspended, and analyzed by SDS-10% PAGE. Lanes *1* and *4*, rabbit $F(ab')_2$ anti-Id alone; lanes *2*, *3*, *5*, and *6*, polymeric IgA1 plus rabbit $F(ab')_2$ anti-Id; lane *7*, control mouse IgG1κ; lanes *8* and *9*, 3G8 anti-FcγR III mAb. Mobilities and size (kD) of standard proteins are indicated.

in the immunofluorescence assay for IgA binding. Both the intact IgA1λ and the polymeric Fcα fragments inhibited binding of the IgA1κ myeloma to activated U937 cells in a dose-dependent manner, whereas Fabα fragments, other classes of myelomas (IgG, IgM, IgD), and normal serum IgG did not (Fig. 9 *A*). The minimal inhibition noted with the highest dose of Fabα (10 mg/ml) probably reflects the minor contamination of Fcα fragments (1–2%) detected by SDS-PAGE densitometry analysis.

To determine if the Fc portion of the IgA molecule is the specific ligand for the 60-kD cell surface molecule, the same panel of inhibitor proteins was used in the immunoisolation assay for IgA-R molecules on activated U937 cells. Addition of a 100-fold excess of nonhomologous IgA1λ or its polymeric Fcα fragments blocked completely the binding of the 60-kD IgA-R molecules to beads coated with the IgA1κ/anti-Id complex (Fig. 9 B, lanes *2* vs. *3* and *5*). In contrast, Fabα fragments did not affect the IgA-R binding (Fig. 9, lane *4*). More direct evidence was obtained by incubation of the 60-kD protein bound to the IgA1κ/anti-Id immune complex with or without *H. influenzae*–derived IgA1 protease, which cuts the hinge region of α1 H chains. The IgA1-protease treatment released the 60-kD molecule from the beads into the supernatant (Fig. 9 B, lanes *7* and *9*), whereas the untreated control did not (lanes *6* and *8*). These results demonstrate that the IgA binding to the 60-kD glycoprotein is via the Fcα portion.

Enzyme Susceptibility of the Cell Surface IgA-R. To determine whether the IgA-R molecule is anchored to the cell membrane through a GPI linkage, as is the case for the FcγR III on neutrophils (10–13, 46), IgA-R expression on granulocytes, monocytes, and activated U937 cells was examined before and after treatment with PI-PLC. Both DAF (47) and FcγR III were removed from the cell surface of granulocytes by PI-PLC treatment as expected, but the IgA-R and FcγR II were unaffected

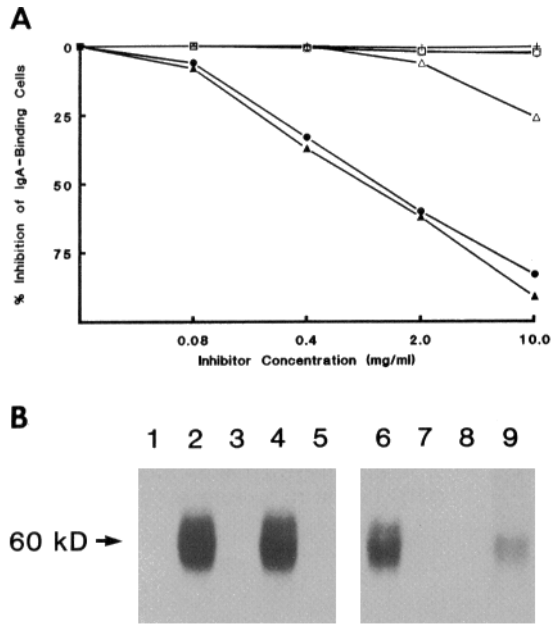

FIGURE 9. Specificity of IgA receptors and determination of the regions of IgA molecule involved in binding. (*A*) PMA-activated U937 cells (5×10^5) were incubated for 30 min at 4°C with various inhibitor proteins: nonhomologous polymeric IgA1λ (●), its polymeric Fcα fragment (▲), its Fabα fragment (Δ), IgMκ myeloma (+), IgDλ myeloma (O), or IgG4λ myeloma plus normal serum IgG (□). The cells were then incubated with polymeric IgA1κ (0.25 mg/ml) and its corresponding biotinylated $F(ab')_{2\gamma}$ anti-Id antibody (0.25 mg/ml) for an additional 30 min at 4°C. The cell-bound IgA was identified by phycoerythrin-labeled streptavidin. By calculating the mean fluorescence intensity of IgA binding over background, a numerical estimate of the relative efficiency of inhibition could be obtained for the different preparations, as described elsewhere (22). (*B*) After preclearing FcγRs, the radiolabeled membrane lysates from PMA-activated U937 cells (5×10^7 cells) were divided into two portions. One portion was further aliquoted into five tubes that contained goat $F(ab')_2$ anti-Id-coupled beads without (lane *1*) or with 10 μg of the corresponding polymeric IgA1κ (lanes *2–5*) in the presence of 100-fold excess of inhibitors: polymeric IgA1λ (lane *3*), its Fabα fragment (lane *4*), and its polymeric Fcα fragment (lane *5*). The other portion was incubated with IgA1κ/ anti-Id complex beads, washed, and incubated without (lane *6*) or with *H. influenzae*-derived IgA1 protease for 1 h at 37°C (lane *7*). The supernatants were recovered from both aliquots (lanes *8* and *9*, respectively), followed by acetone precipitation. All bound or released materials were dissolved in Laemmli's sample buffer and analyzed by SDS- 10% PAGE. The arrow indicates the 60-kD IgA-R molecule.

by this treatment (Table I). Similar results were obtained using freshly purified monocytes and activated U937 cells: the IgA-R, FcγR I, and FcγR II were unaffected by PI-PLC treatment, whereas the DAF was removed. Thus, unlike FcγR III, the IgA-R is not GPI anchored on either granulocytes or monocyte/macrophages.

The effects of proteolytic and glycolytic enzymes on IgA-R expression were examined by treatment of the cells with trypsin-TPCK, pronase, or neuraminidase. Unlike DAF, the IgA-R appeared to be trypsin resistant on all cell sources (Table I). This was true even with a high dose of trypsin (5 mg/ml; data not shown). All FcγRs, regardless of cellular origin, were also trypsin resistant, as has been noted previously (44, 45). By contrast, the IgA-R, FcγR III, and DAF were pronase sensitive, while the FcγR I and II were relatively pronase resistant. Interestingly, neuraminidase-mediated removal of sialic acid residues consistently resulted in a three- to fivefold increase in IgA binding, whereas the expression of FcγR and DAF was either only slightly increased or unaltered.

Discussion

While much has been learned about the biological and molecular characteristics of FcγR and FcεR during the last decade, relatively little is known about the molec-

TABLE I
Enzyme Treatment of Cell Surface Receptors

Cell type and receptors	Mean fluorescence intensity after treatment with:			
	PI-PLC	Trypsin	Pronase	Neuraminidase
Granulocytes				
IgA-R	1.5	0.9	0.3	5.2
FcγR II	1.3	1.2	0.7	2.3
FcγR III	0.2	1.3	0.1	2.2
DAF	0.2	0.3	0.05	1.4
Monocytes				
IgA-R	1.0	1.0	0.2	3.6
FcγR I	1.5	0.8	0.8	2.2
FcγR II	1.0	0.8	0.7	1.4
DAF	0.15	0.4	0.3	1.0
PMA-activated U937 cells				
IgA-R	1.1	1.0	0.3	5.1
FcγR I	0.9	0.8	0.6	1.4
FcγR II	0.9	0.8	0.7	1.3
DAF	0.3	0.3	0.05	0.9

Cells (5×10^6 each) were treated with 1 ml of 0.35 nM PI-PLC, 0.1% trypsin-TPCK, 0.1% pronase, or neuraminidase (50 U/ml) for 45 min at 37°C. Treated and untreated cells were stained for IgA-R (polymeric IgA myeloma plus anti-Id), FcγR I (32.2 mAb), FcγR II (IV.3 mAb), FcγR III (3G8 mAb), and DAF (IA10 mAb), as described in Materials and Methods. The mean fluorescence intensity was estimated as described in Fig. 2.

ular nature of the IgA-R that has been identified by immunofluorescence on human monocytes and granulocytes (22–24). The results of the present experiments indicate that freshly isolated monocytes and granulocytes express both IgA-R and FcγR, as do their corresponding cell lines (U937 and PLB 985). The IgA-R appears to be a glycoprotein of ~60 kD on both cell types. The binding of IgA to the 60-kD molecule is mediated specifically by the Fcα portion of IgA molecules, thus identifying this molecule as an FcαR on both granulocytes and monocyte/macrophages.

The simultaneous expression of both FcαR and FcγR by these myeloid lineage cells is consistent with other evidence indicating that a given cell can express more than one type of FcR (see reviews in references 5 and 21). Monocytes, thus, can express the FcγR I and II, FcεR II, and FcαR. However, our results indicate that each FcR may behave differently in response to the same stimulus, suggesting unique regulatory elements for each receptor. Treatment of U937 cells with PMA or polymeric IgA enhanced the expression of IgA-R, but not of FcγR I and II, whereas rIFN-γ selectively induced FcγR I upregulation. The kinetics of FcαR upregulation induced by PMA and polymeric IgA stimulation differ. Enhanced FcαR expression occurred ~12 h after PMA stimulation and within 30 min after exposure to polymeric IgA, implying different induction mechanisms for these two stimuli. Moreover, removal of the polymeric IgA stimulus resulted in an immediate decline in IgA-R expression, as is characteristic of ligand-induced FcR expression (21, 48–50). However, it should be noted that FcαR expression on monocytes appears to be constitutive even in IgA-deficient individuals (22).

Weisbart et al. (25) have reported that hematopoietic factors, GM-CSF and G-CSF, can affect granulocytes to reduce the numbers of IgA-binding sites while increasing the affinity of the remaining receptors. It remains unresolved whether this change from low to high affinity IgA-R on granulocytes is due to a modification of a preexisting, low affinity IgA-R or to the appearance of another receptor with high affinity for IgA. It will thus be of interest to determine whether the 60-kD FcαR described here is altered by CSF stimulation.

Several reports indicate that the Fc portion of α H chains can bind either noncovalently or covalently to several plasma proteins, including albumin (67 kD), amylase (55 kD), α1-antitrypsin (58 kD), aspartate aminotransferase (~40 kD), lactate dehydrogenase (34 kD), and α1-microglobulin or protein HC (31 and 90 kD) (51–59). The preparations of IgA myeloma proteins used in this study did not contain detectable amounts of these proteins, and the α H chains were of normal size by SDS-PAGE analysis, further indicating the absence of such binding factors. The possibility that plasma proteins may bind first to the cell surface and then to the IgA myeloma proteins is excluded by the finding that the IgA-R in CHAPS-, digitonin-, and NP-40-solubilized membrane preparations exhibited the same M_r with no evidence of associated proteins under both reducing and nonreducing conditions.

Other types of proteins are involved in IgA binding by certain cell types. The poly-Ig-R of ~100 kD is expressed at the basolateral surface of intestinal and glandular epithelial cells and functions in the transcellular transport of polymeric IgA and IgM (18). Antisera against secretory component, the released protein of the poly-Ig-R, appear to inhibit the binding of IgA-coated erythrocytes by murine spleen cells, suggesting the existence of poly-Ig-R on spleen cells (60). However, it has been reported recently that no poly-Ig-R mRNA is expressed by human spleen cells (61). The ~45-kD asialoglycoprotein receptor expressed by hepatocytes can mediate the endocytosis of desialylated plasma glycoproteins (62). Recent studies indicate that polymeric IgA1 can bind to this receptor via the *O*-linked oligosaccharides present on the hinge region of IgA1 molecules (63). However, the FcαR described here is clearly different from either of these receptors.

While IgA-binding has been demonstrated for certain blood cells in both mice and humans (see reviews in references 21 and 64), the biochemical nature of a possible IgA-R on blood cells has been elusive in both species. Albrechtsen et al. (65) have recently reported an IgA-binding molecule on human granulocytes that is resolved as a broad band ranging from 55 to 60 kD. They were unable to ascertain whether this IgA-binding molecule differed from the FcγR III, which has a similar molecular size. The present results extend this previous analysis by showing that the ~60-kD IgA-binding molecule present on both granulocytes and monocyte/macrophages is an FcαR that is distinct from the other types of FcR that have been described. The class-specific binding of IgA to the 60-kD receptor is mediated by the Fc portion of IgA, suggesting that CH2 and/or CH3 is involved in binding to the FcαR.

The FcαR molecules were found to be heterogenously charged with pI of 4.5–5.6. Neuraminidase treatment reduced the FcαR size to ~54 kD and increased the pI to between 5.8 and 6.6. The acidic charge of the IgA-R thus could be due in part to the post-translational addition of sialic acid (~20 residues) to the molecule. Removal of *N*-linked oligosaccharides from the 60-kD FcαR consistently yielded two bands of 32 and 36 kD on both cell types in experiments using different concentrations of *N*-glycanase. The fact that resistant *N*-linked oligosaccharides have not been

reported (66) argues against a partial deglycosylation. Rather, the appearance of two deglycosylated IgA-R bands with a 4-kD difference raises two interesting possibilities: (*a*) an allelic polymorphism of this FcαR; or (*b*) two different glycosylated FcαR isoforms with similar electrophoretic mobilities. An analogous situation has been reported for the FcγR III in which two bands are obtained after complete deglycosylation (9, 13, 43, 45, 46; see Fig. 8). Polymorphic FcγR III are observed on granulocytes, where two allelic forms, NA-1 and NA-2, can be distinguished by human alloantisera and specific mAbs (67). Two single nucleotide changes in the extracellular domain of the granulocyte FcγR III gene account for allelic forms of the molecule, with the loss of an *O*-linked glycosylation site in the NA-1 allele (13). Thus a two-band pattern occurs in SDS-PAGE analysis after *N*-glycanase treatment of the granulocyte FcγR III from donors heterozygous for NA-1 and NA-2 alleles (13, 46). The similarity between the two-band patterns observed for the 60-kD FcαR and for the FcγR III after *N*-glycanase digestion may suggest that these molecules share the same type of gene complexity. However, it is noteworthy that there is a difference between FcαR and FcγR III with regard to their attachment to the cell membrane. The FcαR described here is PI-PLC resistant on all cell sources examined, suggesting the presence of transmembrane segments, whereas both GPI-linked and transmembrane forms of FcγR III have been identified on granulocytes and NK cells, respectively (12, 13). The possibility for two FcαR isoforms could be explained by alternative RNA splicing (e.g., murine FcγR II β gene) (68), by post-transcriptional changes in mRNA processing, as described for apolipoprotein B (69), or by two distinct genes.

We conclude that monocyte/macrophages and granulocytes express a novel FcαR. The production of mAbs specific for this FcαR and identification of the encoding gene(s) will allow further definition of this receptor and exploration of the tissue-specific nature of this receptor molecule.

Summary

In these studies, we characterize an Fc receptor (FcR) for IgA that is present on human granulocytes, monocyte/macrophages, and their corresponding cell lines. Receptor expression appears to be constitutive but can be selectively upregulated on monocyte cell lines by stimulation with a phorbol ester and polymeric IgA. Both the induction requirements and ligand specificity of the IgA receptor differ from the IgG receptors, FcγR I, II, and III, that are also expressed on monocytes and granulocytes. IgA binding to the cell surface receptor is mediated via the Fcα region. The FcαR is a heterogenously charged, ~60-kD molecule with an isoelectric point of 4.5–5.6 that binds monomeric or polymeric IgA1 and IgA2 molecules. This transmembrane glycoprotein appears to be composed of 32- and 36-kD protein cores with multiple *N*-linked carbohydrate moieties. We conclude that this FcαR represents a novel member of the FcR family that may have a distinctive role in host defense.

We thank Dr. A. Chevailler for his help in this study; Dr. J. Mestecky for the gift of IgA protease; Dr. M. G. Low for the gift of PI-PLC enzyme; Drs. M. Fanger, C. L. Anderson, J. C. Unkeless, and V. Nussenzweig for providing mAbs samples; and Drs. J. Zikan, J. Mestecky, J. R. McGhee, K. McNagny, J. M. Pickel, T. Ohno, and P. Burrows for reading this manuscript.

Received for publication 3 October 1989.

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