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 isotypespecificity 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 (4, 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 FceR 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 FceR 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 FceR II, belong to the Ig gene superfamily (reviewed in references 15 and 19).

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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; pl, isoelectric point; PI-PLC, phosphatidylinositol-specific phospholipase C.

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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, 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, IgAlk and IgAlα, were purified from patients' sera by salt fractionation, DEAE ion exchange cellulose, and Ultrogel AcA 24 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 IgAl with IgAl-specific protease (see review in reference 27). Briefly, polymeric IgAlα (10 mg/ml in 0.05M phosphate buffer, pH 7.0) was digested at 37°C for 15 h with crude IgAl 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 IgG4α paraprotein was purified as previously described (28), and shown by SDS-PAGE analysis to be uncontaminated with other Ig isotypes.

Preparation of Antidiotypic Antibodies. The purified IgA and IgG4α myeloma proteins were used to raise polyclonal antidiotypic (anti-Id) antibodies in goats and rabbits. Removal of nondiotopic 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 idiotypic 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 rosetting 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 IgA for 0.5-18 h. In some experiments, after preincubation with polymeric IgA 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% CaCl₂, 0.01% MgCl₂, 1% BSA, and 0.05% sodium azide. After washing, phycoerythrin-labeled streptavidin (Becton Dickinson & Co., Mountain View, CA) was used as the 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 (γ1k isotype) and IV.3 (γ2b) mAbs specific for FcyR 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 (γ1k) mAb specific for FcyR III, a generous gift of Dr. J. C. Unkeless (Mt. Sinai Medical School, NY) (8); and (c) IA10 (γ2a) 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 × 10^7 viable cells were extensively washed in PBS, labeled with Na^251I (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% CaCl₂, 0.01% MgCl₂, 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% CaCl₂, 0.01% MgCl₂, 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 preclearence 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, IgG4A myeloma plus its rabbit F(ab')2 anti-id antibody–coupled Sepharose 4B and 3G8 mAb–coupled Sepharose 4B were used to detect FcyR 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 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 x 10⁶/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 non-binding 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.](image)
that both monocytes and granulocytes express IgA-R, while each expresses a distinctive array of FcyR.

Regulation of IgA-R Expression by U937 Cells. To examine for differences in the regulation of IgA-R and FcyR 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 FcyR I and FcyR II expression were unaffected by stimulation with PMA over a wide range of concentrations. PMA did not induce the expression of FcyR 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 FcyR 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 FcyR II was observed, and FcyR III expression was not
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 FcγRI 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 FcγRI expression. Conversely, IgA-R expression is unaffected by IFN-γ, which enhances FcγRI 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-
ated 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 IgA2κ 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. 7A). 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. 7B). 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 × 10⁷ cells each) were labeled with 1 mCi of Na¹²⁵I and solubilized with 0.5% NP-40 buffer, as described in Materials and Methods. The radiolabeled membrane lysates were precleared several times with Sepha-rose 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 x 10⁷ cells) was eluted from beads and divided into two portions. One (4) received buffer, and the other (5) 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. Motilities 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 Mr 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 (FcγRI, II, and III); (d) exhibits heterogeneous 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 IgA1 molecule, its purified Fabα and Fcα fragments, and other classes of Ig were used as inhibitors
FIGURE 8. Glycoprotein nature of IgA-R molecules. $3 \times 10^7$ 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 IgA 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 × 10⁵) were incubated for 30 min at 4°C with various inhibitor proteins: nonhomologous polymeric IgA (●), its polymeric Fcα fragment (△), its Fabα fragment (△), IgM myeloma (+), IgG myeloma (O), or IgG myeloma plus normal serum IgG (□). The cells were then incubated with polymeric IgA (0.25 mg/ml) and its corresponding biotinylated F(ab')₂ 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 × 10⁶ 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 IgA (lanes 2–5) in the presence of 100-fold excess of inhibitors: polymeric IgA (lane 2), its Fabα fragment (lane 4), and its polymeric Fcα fragment (lane 5). The other portion was incubated with IgA anti-Id complex beads, washed, and incubated without (lane 6) or with H. influenzae-derived IgA 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.

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-
The table below shows the mean fluorescence intensity after treatment with PI-PLC, trypsin, pronase, or neuraminidase for granulocytes, monocytes, and PMA-activated U937 cells.

| 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          |
| FcγRII                  | 1.3      | 1.2     | 0.7     | 2.3          |
| FcγRIII                 | 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          |
| FcγRII                  | 1.5      | 0.8     | 0.8     | 2.2          |
| FcγRIII                 | 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          |
| FcγRII                  | 0.9      | 0.8     | 0.6     | 1.4          |
| FcγRIII                 | 0.9      | 0.8     | 0.7     | 1.3          |
| DAF                     | 0.3      | 0.3     | 0.05    | 0.9          |

Cells (5 x 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-IgA), FcγRI (32.2 mAb), FcγRII (IV.3 mAb), FcγRIII (3G8 mAb), and DAF (IA10 mAb), as described in Materials and Methods. The mean fluorescence intensity was estimated as described in Fig. 2.
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 pre-existing, 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 α2-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 FcyR 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 FcaR; or (b) two different glycosylated FcaR 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, 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 FcaR 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 FcaR and FcγR III with regard to their attachment to the cell membrane. The FcaR 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 FcaR 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 FcaR. The production of mAbs specific for this FcaR 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 Fca region. The FcaR 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 FcaR represents a novel member of the FcR family that may have a distinctive role in host defense.

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