CD16

Developmentally Regulated IgG Fc Receptors On Cultured Human Monocytes

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Receptors for the Fc portion of IgG (FcRs)[1] play a role in mediating both normal and pathologic immunophagocytosis. One FcR on human cells (termed CD16 by the International Workshop on Human Leukocyte Differentiation Antigens) (1) is present on the surfaces of neutrophils, NK cells, and resident macrophages, but is absent from the surfaces of peripheral blood monocytes (2). CD16, recognized by mAbs 3G8 (2), Leu 11 (3), and B73.1 (4), binds immune complexes efficiently, but does not bind monomeric IgG. CD16 on neutrophils exhibits broad electrophoretic mobility (50,000–73,000 daltons) after SDS-PAGE, which suggests that it contains abundant carbohydrate. Previous studies in chimpanzees demonstrated that this receptor plays an important role in removal of model immune complexes from the circulation (5). Moreover, infusion of anti–CD16 mAbs into patients with refractory immune thrombocytopenic purpura transiently raised platelet counts, suggesting a role for CD16-mediated phagocytosis in the pathogenesis of this disease (6). However, very little is known about this receptor on normal human macrophages. In this study, we investigated the expression, structure, and function of CD16 on monocytes during their differentiation in culture to macrophage-like cells.

Materials and Methods

Preparation of Peripheral Blood Monocytes and Neutrophils. Blood was obtained from normal human volunteers, anticoagulated with acid/citrate/dextrose, and then subjected to centrifugation on Ficoll-Hypaque to separate mononuclear cells and neutrophils (7). Monocytes were subsequently separated from lymphocytes by the method of Recalde (8). Briefly, mononuclear cells were incubated in hypertonic saline and then centrifuged for 30 min at 600 *g* over Ficoll-Hypaque (density of 1.078) to which had been added 2.8 mg/ml of NaCl. Monocytes remained buoyant and lymphocytes pelleted to the bottom of the tube. Monocytes were 80–95% pure as judged by Wright staining and nonspecific esterase staining. Neutrophils were further purified by dextran sedimentation. Preparations typically contained >95% neutrophils as judged by Wright staining.

Cultivation and Stimulation of Monocytes. Monocytes were seeded into Teflon dishes (Savillex Corp., Minnetonka, MN) in RPMI 1640 (Flow Laboratories, Inc., McLean, VA)

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[1] *Abbreviations used in this paper:* Endo D, endoglycosidase D; Endo H, endoglycosidase H; FcR, Fc receptor.

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containing 10% human serum and 2% FCS at 1 × 10^6 cells/ml. Cell numbers decreased with time in culture, ranging from 2.5 to 7.3 × 10^5/ml (mean 3.9 × 10^5/ml) at 14 d. To stimulate expression of FcRs, human rIFN-γ (a generous gift of Dr. Michael Shepard, Genentech Inc., So. San Francisco, CA) was added to freshly isolated monocytes in the amounts indicated in Results.

Monoclonal Antibodies. The cell line producing mAb 3G8 (IgG1) (2), was a generous gift of Dr. Jay C. Unkeless, Mt. Sinai Medical School, New York. Ascites containing mAb IB4 (IgG2a) (9) was a generous gift of Dr. Samuel D. Wright, The Rockefeller University, New York. Ascites containing 32.2 (20) was a generous gift of Dr. Michael Fanger, Dartmouth School of Medicine, Hanover, VT. Purified mAb 3C10 (IgG2b) (10) was a generous gift of Dr. Ralph Steinman, The Rockefeller University, New York. IV3 culture supernatant (IgG2b) (11) was a generous gift of Dr. John Looney, University of Rochester Medical School, Rochester, NY. Cell lines producing MOPC-21 (murine IgG1 myeloma), 4F2C13 (IgG1) (12), and 63D3 (IgG1) (13) were obtained from American Type Culture Collection, Bethesda, MD. Purified RPC-5 (murine IgG2a myeloma) was obtained from Litton Bionetics, Kensington, MD. mAbs were purified from ascites by precipitation with 45% saturated ammonium sulfate, followed by precipitation with caprylic acid (1.5%, vol/vol) (Sigma Chemical Co., St. Louis, MO) (14). Fab fragments were prepared by digestion with immobilized papain (Sigma Chemical Co.), and then purified by passage over protein A–Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) at pH 8.3. No contamination with Ig heavy chain was detected by SDS-PAGE.

Immunoassays. Cultured monocytes (0.5–1.0 × 10^6 cells/tube) were harvested from Teflon dishes, washed in PBS, and then incubated in PBS for 1 h at room temperature to remove cell surface IgG adsorbed from culture media. Subsequent examination by immunofluorescence microscopy using fluorescein-conjugated goat anti–human Ig (Jackson Immunoresearch, Avondale, PA) revealed no evidence of surface-bound Ig. Cells were then incubated for 30 min at 4°C with either purified Fab fragments of mAbs (10 μg/ml) or 30 μl culture supernatants (as indicated in Results). After washing, cells were incubated for 30 min at 4°C with $F(ab')_2$ goat anti-mouse Ig conjugated to FITC (Jackson Immunoresearch). After washing again, cells either were allowed to settle onto poly-L-lysine (100 μg/ml)–coated masked slides (Carlson Scientific, Inc., Peotone, IL) and examined by fluorescence microscopy, or were examined by flow cytometry using a cytofluorograph II (Ortho Diagnostic Systems, Inc., Westwood, MA). The cytofluorometer was calibrated each day with Fluorosphere Fullbright Gr. II fluorescent beads (Coulter Diagnostics, Hialeah, FL). Monocyte fractions were selected on the basis of size and granularity, and contaminating lymphocytes were gated out. Peak channels of fluorescence varied $<10\%$ between days.

For binding assays with radiolabeled mAbs, 0.5–1.0 × 10^6 cells were incubated at 4°C with equal volumes of ^{125}I-labeled mAbs (2.5 μg/ml) and either PBS containing 1.0 mg/ml BSA (PBS/BSA) or unlabeled mAb (1.0 mg/ml). After 30 min, cells were suspended in 0.5 ml of cold PBS/BSA and layered over 0.5 ml Versilube F50 silicon oil (General Electric, Waterford, NY) contained in 1.5 ml polypropylene microfuge tubes, and centrifuged for 5 min at 10,000 *g*. After removing the aqueous and oil phases by aspiration, tips of tubes containing cell pellets were excised and subjected to gamma counting. Specific binding was determined by subtracting radioactivity that became cell associated in the presence of excess unlabeled antibody from total radioactivity.

Immunoprecipitation. 2–4 × 10^7 cultured monocytes or freshly isolated neutrophils were surface labeled with ^{125}I using Iodogen (25 μg/tube) (Pierce Chemical Co., Rockford, IL), and then lysed in PBS containing 10 mM octyl-thioglucoside, 1.0 mM diisopropylfluorophosphate, 1.0 mM PMSF, and 0.02% (wt/vol) sodium azide. Postnuclear supernatants of lysed cells were then incubated sequentially at 4°C with BSA coupled to Sepharose 4B (Pharmacia Fine Chemicals) and mAbs coupled to Sepharose 4B (each for 2 h). Sepharose beads were washed extensively, resuspended in sample buffer containing 10% (vol/vol) glycerol, 1.0% (vol/vol) 2-ME, 2.0% (wt/vol) SDS, 0.001% (wt/vol) bromphenol blue, and 62.5 mM Tris, pH 6.5, boiled for 3 min, and then subjected to SDS-PAGE followed by autoradiography.

Treatment with Glycosidases. For digestion with glycosidases, immunoprecipitates were eluted from resuspended Sepharose beads by boiling for 3 min. Treatment with *N*-glycanase (Genzyme, Boston, MA) was carried out in 0.25 M sodium borate (pH 8.6) containing 0.5% SDS, 10 mM 1,10 phenanthroline (Sigma Chemical Co.), and 1.0% NP-40 (vol/vol). Treatment with endoglycosidase H (Endo H; Boehringer Mannheim Biochemicals, Indianapolis, IN) was carried out in 0.1 M sodium citrate (pH 5.5) containing 0.1% SDS (wt/vol). Treatment with endoglycosidase D (Endo D; Boehringer Mannheim Biochemicals) was carried out in 0.1 M sodium phosphate (pH 6.5) containing 0.1% SDS (wt/vol). Enzymes were added and mixtures were incubated overnight at 37°C. After incubation with glycosidases, reaction mixtures were boiled for 3 min to inactivate enzymes, and then subjected to SDS-PAGE followed by autoradiography. 1 U of Endo D and Endo H is defined as the amount of enzyme required to release 1.0 μmol of carbohydrate from appropriate substrates. 1 U of *N*-glycanase is defined as the amount of enzyme required to release 1.0 nmol of carbohydrate from appropriate substrates. Enzymatic activity of glycosidases was confirmed by digestion of fetuin (100 μg/ml) by Endo H (30 mU/ml) and Endo D (30 mU/ml); of DNAase (100 μg/ml) by Endo H; and of RNase (100 μg/ml) by Endo D. Digestion was confirmed by subjecting glycoproteins to SDS-PAGE. Decreases in apparent M_r were interpreted as being due to deglycosylation.

Attachment and Phagocytosis of IgG-coated Erythrocytes. Inhibition studies performed with antibodies in solution were performed as follows. Freshly isolated monocytes and monocytes that had been cultured for 14 d (1×10^6 cells/ml) were applied to polystyrene plates coated with poly-L-lysine (100 μg/ml) (Sigma Chemical Co.) and allowed to adhere at room temperature. RPMI with 5% FCS was added to monolayers for 30 min at 4°C. Sheep erythrocytes (RBCs) that had been previously coated with rabbit anti–sheep RBC IgG (final concentration, 0.25%; Cordis Laboratories Inc., Miami, FL) and mAbs (10 μg/ml) were then added and incubated for 1 h at 4°C. Attachment of IgG-coated RBCs to monocytes was scored by phase-contrast microscopy using a 40× water immersion lens (Nikon, Japan). Monolayers were then incubated at 37°C for 1 h, after which noningested RBCs were lysed by brief exposure to distilled water. Phagocytosis was then measured as above. Results are reported as attachment index or phagocytic index; i.e., the number of RBCs bound or ingested per 100 monocytes.

Inhibition studies also were performed with antibodies in the solid phase as described above with the following modification. Terasaki plates were coated with mAbs (50 μg/ml) or media for 2 h at room temperature. After washing, monocytes were added and allowed to adhere for 1 h at 37°C, before adding IgG-coated RBCs.

Results

Monocytes Change Both Morphologically and Phenotypically as a Function of Length of Time in Culture. Human peripheral blood monocytes appeared to mature in culture and to develop features characteristic of macrophages. By day 7, cultured cells gradually lost the morphology, cell size (12–16 μm), and cytoplasm/nucleus ratio typical of freshly isolated monocytes (15), and developed features of mature macrophages with more-rounded nuclei, larger cell size (40–80 μm), and cytoplasm/nucleus ratios of ~3:1. Giant cells (1–4%) began to appear at day 7 and increased in number with time in culture to 4–12% by day 18. Phenotypically, freshly isolated monocytes expressed epitopes recognized by mAb IB4 (complement receptor type 3, or CR 3) (9), as well as epitopes recognized by mAb 4F2C13 and 3C10, which are found on both monocytes and macrophages (10, 12). Freshly isolated monocytes also expressed the epitope recognized by mAb 63D3, which is found on monocytes, but not on macrophages (13). Over a period of 18 d in culture, monocytes exhibited no changes in the level of expression of CR 3 or reactivity with 4F2C13 and 3C10, but gradually lost reactivity with

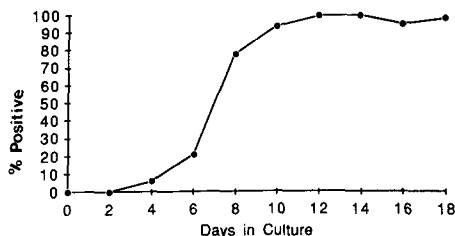

FIGURE 1. Binding of mAb 3G8 Fab to monocytes cultured for 18 d. Monocytes cultures for 18 d were tested at the indicated time points for reactivity with mAb 3G8 Fab (10 μg/ml) by indirect immunofluorescence. Reactivity gradually increased until day 10 when ~100% of cells were positive. Similar results were obtained by flow cytometry. Results represent mean values obtained in experiments using cells from 10 different donors tested sequentially over 18 d.

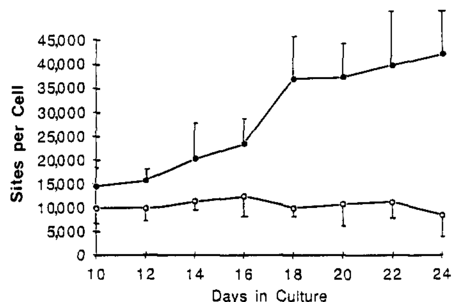

FIGURE 2. Cell surface density of CD16 and FcR_{p72} on cultured monocytes. Monocytes were cultured for 10 d, and then binding assays were performed at 2-d intervals for 14 d. ^{125}I-labeled 3G8 Fab was used for determinations of CD16 and ^{125}I-labeled murine IgG2a was used for determinations of FcR_{p72}. Cell surface density of CD16 (*closed circles*) increased as a function of length of time in culture, while cell-surface density of FcR_{p72} (*open circles*) remained approximately the same. Results represent mean values obtained in six separate experiments.

mAb 63D3. Therefore, monocytes appeared to differentiate in culture to macrophage-like cells both morphologically and phenotypically.

Expression of CD16, but not Other Types of FcRs, Increases with Cellular Differentiation. CD16 is not expressed on the surface of peripheral blood monocytes (2). Nonetheless, immunocytochemical staining of sections of both human liver and human spleen has revealed reactivity with mAb 3G8 in a pattern consistent with the distribution of resident macrophages (6). Previous work revealed that 15% of cultured monocytes react with anti-CD16 mAb 3G8 on day 6 (16). We further investigated expression of CD16 on cultured monocytes. With increasing time in culture, both the percentage of cells expressing CD16 and the number of sites per cell increased. Immunofluorescence assays revealed that the percentage of cells recognized by mAb 3G8 increased to ~100% by day 10 (Fig. 1). Therefore, expression of CD16 increases as monocytes differentiate into macrophages both in vivo and in vitro.

FcRs for IgG can be classified according to their ability to bind monomeric IgG. Although all FcRs bind immune complexes, only FcRs with an apparent M_r of 72,000 (FcR_{p72}) (2, 17) can bind monomeric IgG. CD16 and an FcR with an apparent M_r of 40,000 (CDw32) (1, 11) bind only immune complexes. We measured cell surface expression of these three different FcRs (FcR_{p72}, CDw32, and CD16) on cultured human monocytes. Radioimmunoassays using ^{125}I-labeled mAb 3G8 demonstrated that the cell surface density of CD16 increased from a mean of 14,820 sites per cell on day 10 to a mean of 43,680 sites per cell on day 24 (Fig. 2). (Assays for CD16 were not performed until ~100% of cells were positive by immunofluorescence.) When assessed by binding of ^{125}I-labeled monomeric IgG2a, the level of expression of FcR_{p72} on monocytes did not change with differentiation in culture. Freshly isolated monocytes (data not shown), as well as monocytes examined at 2-d intervals from day 10 to day 24, possessed ~10,000 sites per cell.

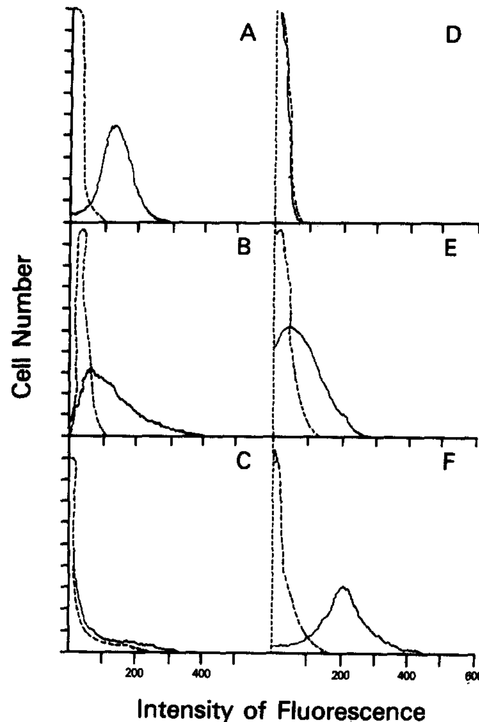

FIGURE 3. Expression of CDw32 compared to expression of CD16 on cultured monocytes. Monocytes were isolated from blood, and then tested for reactivity by indirect immunofluorescence and flow cytometry with control-irrelevant myeloma protein (*dotted lines*) or with mAbs (*solid lines*). *A–C* demonstrate reactivity with anti-CDw32 mAb IV3 on days 0, 8, and 18, respectively. *D–F* demonstrate reactivity with mAb 3G8 on days 0, 8, and 18, respectively. Expression of CDw32 appeared to decrease with respect to the percentage of positive cells and in the relative intensity of fluorescence. The number of positive cells decreased from 91.4% (freshly isolated monocytes) to 50.1% (day 8) to 22.2% (day 14) with a progressively decreasing peak channel of fluorescence. In contrast, the level of expression of CD16 progressively increased from 0% positive (day 0), to 64% (day 8), to 97% (day 18). Results shown were obtained with monocytes from a single donor, and are representative of results obtained in two other experiments using cells from two other donors.

Surface expression of CDw32 on monocytes actually decreased with time in culture. Fig. 3 demonstrates reactivity of anti-CDw32 mAb IV3 and anti-CD16 mAb 3G8 with freshly isolated monocytes, as well as with monocytes cultured for 8 d and 18 d. Expression of CDw32 decreased with respect to both percentage of positive cells and cell surface density, while expression of CD16 increased. It appears, therefore, that CD16 is the predominant FcR on differentiated monocytes.

Agents That Promote Expression of FcR_{p72} *Do Not Promote Expression of CD16.* IFN-γ can induce new expression of certain surface antigens (e.g., class II histocompatibility antigens) (18) and can increase surface expression of others. The surface expression of FCR_{p72} on monocytes and U937 cells increases 8–10-fold after treatment of cells with IFN-γ (19). This increase correlates with an increase in the ability of U937 cells to generate superoxide anion radicals after stimulation with either immune complexes or anti-FcR_{p72} mAbs (20). We were interested in determining whether expression of CD16 also is regulated by IFN-γ. Freshly isolated monocytes were incubated with human rIFN-γ (1–1,000 U/ml), and then assayed for expression of CD16 and FcR_{p72}. Cells from 35 of 35 normal blood donors demonstrated increases in numbers of sites per cell of at least fivefold in expression of FcR_{p72} after treatment with greater than 10 U/ml of IFN-γ, increasing from 11,438 (q 2,478) to 82,734 (q 10,456). In contrast, cells from only 2 of 35 donors demonstrated measurable numbers of CD16 with comparable doses. Monocytes from these two individuals, however, were reproducibly induced to express CD16 after 24 h in culture with IFN-γ.

TABLE I
Effect of mAbs in Solution on Binding and Internalization of IgG-coated RBCs by Freshly Isolated and 14-d-old Cultured Monocytes

mAbs	Freshly isolated monocytes		14-d-old monocytes	
	Attachment index*	Phagocytic index‡	Attachment index	Phagocytic index
Media	543§	310	988	472
MOPC 21 (IgG 1)	598	305	983	501
4F2C13	512	217	1,042	512
3G8 Fab	601	343	542	103
RPC-5 (IgG 2a)	444	195	288	301
IV.3 IgG	561	348	937	444
3G8 Fab and RPC-5	382	343	107	55
3G8 Fab and IV.3	528	352	357	120
RPC-5 and IV.3	324	202	298	301
RPC-5, 3G8 Fab, and IV.3	277	204	98	40

Freshly isolated and 14-d-old monocytes were plated at 1×10^6 cells/ml on Terasaki plates that had been previously coated with poly-L-lysine (100 μg/ml) as described in Materials and Methods. Cells were allowed to adhere for 1 h at 37°C, before antibodies (10 μg/ml) and sheep RBCs coated with rabbit anti-sheep RBCs were added. After 1 h at 4°C, nonadherent cells were removed and rosettes counted. Plates were then incubated at 37°C for 1 h, and RBCs that had not been internalized were removed by hypotonic lysis. Internalized RBCs were then counted.
* Number of RBCs bound per 100 cells.
‡ Number of RBCs ingested per 100 cells.
§ Results represent means of triplicate wells in experiments using cells from two separate donors.

Nearly 100% of monocytes from both individuals were positive as determined by immunofluorescence, and for the one individual tested, there were 10,400 sites/cell. However, IFN-γ failed to induce expression of CD16 on most normal donors.

CD16 on Cultured Monocytes Can Mediate Phagocytosis. Very little information is available concerning the individual contribution of each type of FcR to ligand binding and internalization by human mononuclear phagocytes. Consequently, we performed the following experiments to determine whether both CD16 and FcR_{p72} could independently mediate attachment and phagocytosis of IgG-coated RBCs. Studies of inhibition of ligand binding and phagocytosis by mABs were performed in two ways, with mAbs in solution and with mAbs in the solid phase (coated onto culture dishes). When mAbs and IgG-coated RBCs were added simultaneously to freshly isolated monocytes on poly-L-lysine–coated culture dishes, IgG2a (10 μg/ml) had a small inhibitory effect on ligand attachment and phagocytosis, while mAbs 3G8 and IV.3 had no effect (Table I). When 14-d-old monocytes were studied, 3G8 Fab had a marked inhibitory effect on phagocytosis. While neither 3G8 nor IgG2a added as single agents were able to completely inhibit binding or phagocytosis, the combination of the two antibodies was completely inhibitory.

When freshly isolated and 14-d-old monocytes were plated onto dishes coated with mAbs, similar results were obtained (Table II). In these experiments, cell surface receptors migrate to, and are bound by, the mAbs and become unavailable for ligand binding. Again, binding and phagocytosis of IgG-coated RBCs

TABLE II
Effect of Solid Phase mAbs on Binding and Internalization of IgG-coated RBCs by Freshly Isolated, and 14-d-old Monocytes

mAbs	Freshly isolated monocytes		14-d-old monocytes	
	Attachment index*	Phagocytic index‡	Attachment index	Phagocytic index
Media	404§	214	734	412
MOPC 21 (IgG 1)	402	237	741	452
4F2C13	482	274	794	453
3G8 Fab	399	219	452	104
RPC-5 (IgG 2a)	201	101	471	252
IV.3 IgG	449	333	798	454
3G8 Fab and RPC-5	243	140	45	18
3G8 Fab and IV.3	490	255	404	89
RPC-5 and IV.3	298	113	428	204
RPC-5, 3G8 Fab, and IV.3	280	102	62	10

Freshly isolated and 14-d-old monocytes were plated at 1×10^6 cells/ml on Terasaki plates that had been previously coated with antibodies (50 μg/ml) as described in Materials and Methods. Cells were allowed to adhere for 1 h at 37°C, before sheep RBCs coated with rabbit anti-sheep RBCs were added. After 1 h at 4°C, nonadherent RBCs were removed and rosettes counted. Plates were then incubated at 37°C for 1 h, and RBCs that had not been internalized were removed by hypotonic lysis. Internalized RBCs were then counted.
* Number of RBCs bound per 100 cells.
‡ Number of RBCs ingested per 100 cells.
§ Results represent means of triplicate wells of two separate donors.

were not significantly inhibited when freshly isolated monocytes were added to surface-bound IgG2a, IV.3, or 3G8. Although both 3G8 Fab and IgG2a inhibited ligand binding to a moderate degree by 14-d-old monocytes, only mAb 3G8 markedly inhibited phagocytosis. These findings suggest that whereas both FcR_{p72} and CD16 are capable of mediating attachment of IgG-coated RBCs, CD16 may play a more important role than do FcR_{p72} or CDw32 in mediating internalization.

CD16 on Cultured Monocytes Differs from CD16 on Neutrophils. Various murine cell types express FcRs that share antigenic determinants but differ in apparent M_r (Pure, E., personal communication). Consequently, we compared the structure of CD16 on human neutrophils with the structure of CD16 on cultured monocytes. Immunoprecipitates prepared from surface-labeled, freshly isolated neutrophils and from surface-labeled, 10-d-old monocytes had the typical heterogeneous appearance of CD16 on autoradiograms after SDS-PAGE (Fig. 4). Neutrophils CD16 appeared as a broad band from ~50–73 kD. However, CD16 on cultured monocytes was represented by a predominant band at ~53 kD with less heterogeneity. In some experiments, other bands at 40 and 31 kD were also immunoprecipitated, but these were not consistently present. Therefore, as in the murine system, antigenically similar FcRs from human cells also appear to be structurally heterogeneous.

To determine whether differences in glycosylation accounted for this heterogeneity, immunoprecipitates prepared from lysates of surface-labeled neutrophils and cultured monocytes were treated with glycosidases, and then analyzed by

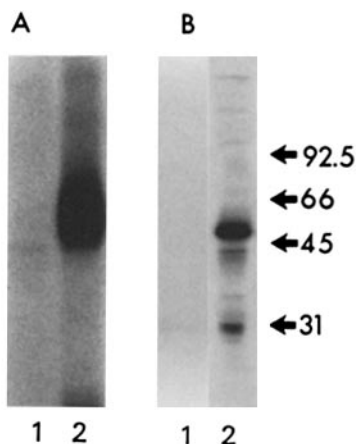

FIGURE 4. Immunoprecipitation of CD16 from neutrophils and cultured monocytes. Neutrophils (*A*) and monocytes cultured for 10 d (*B*) were surface labeled with ^{125}I, and then lysed. Lysates were incubated with either MOPC 21-Sepharose (lane *1*) or 3G8 Fab-Sepharose (lane *2*), and then subjected to SDS-PAGE on a 10% gel, followed by autoradiography. 5,000 cpm was applied to lane *2* in both *A* and *B*. CD16 from neutrophils appeared to be more heterogeneous in size than CD16 from cultured monocytes, which was represented by a predominant band at ~53 kD.

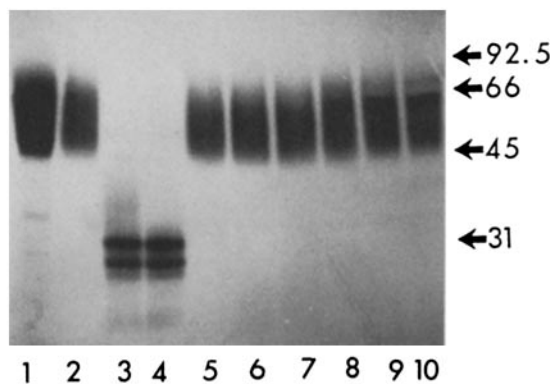

FIGURE 5. Neutrophil CD16 is sensitive to digestion with *N*-glycanase. ^{125}I surface-labeled neutrophils were lysed, and lysates immunoprecipitated with 3G8 Fab-Sepharose. CD16 was treated with sample buffer (lane *1*); *N*-glycanase, 1, 10, or 30 U/ml (lanes *2–4*, respectively); Endo H, 10, 30 or 100 mU/ml (lanes *5–7*, respectively); and Endo D, 15, 40, or 150 mU/ml (lanes *8–10*, respectively). Reaction mixtures were then subjected to SDS-PAGE as in Fig. 4. Digestion with *N*-glycanase, but not Endo H or Endo D effected a decrease in the M_r of CD16.

SDS-PAGE. Neither Endo D (15–150 mU/ml) nor Endo H (10–100 mU/ml) altered the apparent M_r of CD16 from neutrophils (Fig. 5). This suggests that both high mannose (Endo H–sensitive) (21) and more complex (Endo D–sensitive) (22) oligosaccharides either are not attached to neutrophil CD16 or are not accessible for cleavage by these enzymes. Control glycoproteins were successfully digested by both enzymes in parallel experiments (see Materials and Methods). However, after treatment of neutrophil CD16 with *N*-glycanase (as little as 10 U/ml), which hydrolyzes glycosidic links at asparagine residues (23), protein bands with apparent M_r's of 31 and 29 kD appeared. CD16 on cultured monocytes was similarly unaffected by treatment with Endo D and Endo H (Fig. 6). Treatment with *N*-glycanase (1–100 U/ml), however, also did not affect the appearance of the predominant 53-kD band. To confirm that *N*-glycanase was enzymatically active, CD16 immunoprecipitated from neutrophils was treated in parallel with CD16 immunoprecipitated from monocytes. Again, only CD16 from neutrophils was digested to the molecular mass species described above. To confirm that *N*-glycanase is capable of digesting carbohydrates on monocyte surface proteins, FcR_{p72} was immunoprecipitated from cultured monocytes using mAb 32.2 (20), then treated with *N*-glycanase (Fig. 7). FcR_{p72} reproducibly

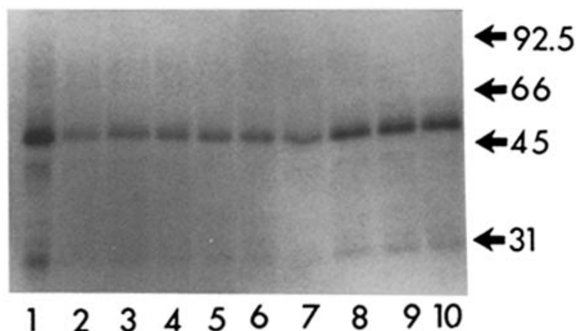

FIGURE 6. Treatment of CD16 from cultured monocytes with glycosidases. Immunoprecipitates of CD16 from monocytes cultured for 10 d were prepared as in Fig. 5. CD16 was treated with sample buffer (lane *1*); *N*-glycanase, 1, 10, or 30 U/ml (lanes *2–4*, respectively); Endo H, 10, 30, or 100 mU/ml (lanes *5–7*, respectively); and Endo D, 15, 40, or 150 mU/ml (lanes 8–10, respectively). None of the glycosidases selected caused a decrease in the M_r of CD16 from cultured monocytes. Reaction mixtures were then subjected to SDS-PAGE as in Fig. 5.

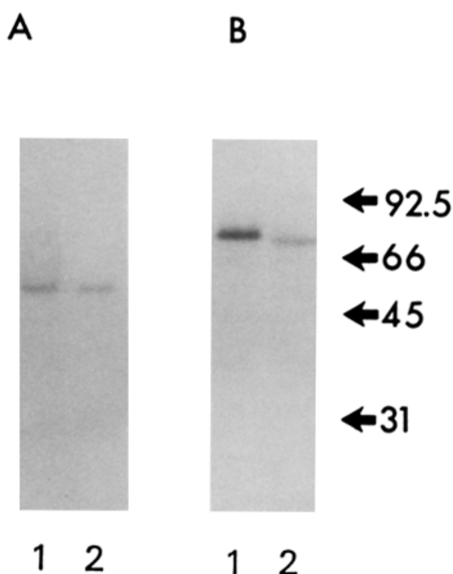

FIGURE 7. FcR_{p72} from cultured monocytes is sensitive to treatment with *N*-glycanase. Immunoprecipitates of CD16 (*A*) and FcR_{p72} (*B*) from monocytes cultured for 10 d were prepared using 3G8 Fab-Sepharose and 32 IgG-Sepharose, respectively, and then prepared as in Fig. 5. CD16 and FcR_{p72} were treated with buffer (lane *1*) or *N*-glycanase, 50 U/ml (lane *2*). Reaction mixtures were then subjected to SDS-PAGE as in Fig. 4.

decreased ~3 kD with treatment with *N*-glycanase. Although neutrophil CD16 appears to be heavily glycosylated, monocyte CD16 appears to have little, if any, carbohydrate. Therefore, differential glycosylation may account for the differences between CD16 from neutrophils and CD16 from monocytes apparent on SDS-PAGE.

Discussion

We have studied the surface expression, functions, and structure of CD16 on cultured monocytes, and have confirmed that expression of CD16 is developmentally regulated. In contrast to two other FcRs, FcR_{p72} and CDw32, CD16 is absent from peripheral blood monocytes and increases with respect to both the percentage of positive cells and cell surface density with length of time in culture. In fact, by ~2 wk in culture, CD16 is the predominant FcR on cell surfaces of monocytes. Unlike expression of FcR_{p72}, expression of CD16 usually does not

increase on cells incubated with IFN-γ. This is similar to the regulation of low density lipoprotein receptors, which also increase in number when human monocytes differentiate in culture, but which actually decrease in number when cells are incubated with LPS or IFN (24). Thus, like the low density lipoprotein receptor, CD16 may be a marker for normal cellular differentiation.

We currently cannot explain our observation that monocytes from two individuals could be induced to express CD16 with IFN-γ. One explanation may relate to a genetic polymorphism noted previously by Perussia et al. (4) in expression of the antigenic determinant recognized by anti-CD16 mAb B73.1. Although this mAb recognizes NK cells from all individuals, only ~50% of individuals express the epitope recognized by B73.1 on their neutrophils. We are currently attempting to correlate reactivity of neutrophils with B73.1 and induction of CD16 on cultured monocytes by IFN-γ.

The relative importance of the three different FcRs in mediating immunophagocytosis is not known. All three mediate rosetting with IgG-opsonized erythrocytes in vitro, as determined by blockade using anti-CD16 and anti-CDw32 mAbs (2, 25) or by blockade using high concentrations of monomeric IgG1 (in the case of FcR_{p72}) (26). In previous studies, Fleit et al. (16) found that rosetting with IgG-opsonized erythrocytes was inhibited by 70% when monocytes were attached to surfaces coated with mAb 3G8. Jungi and Hafner (27) also found that cultured monocytes become better able to phagocytose IgG-opsonized particles with increasing length of time in culture. This phenomenon paralleled development of low affinity IgG binding sites determined by Scatchard analysis. Moreover, Wright et al. (28) found that stimulation of cultured monocytes with IFN-γ, which increases expression of FcR_{p72} (19), actually decreased phagocytosis of IgG-opsonized particles by cultured monocytes, suggesting that FcR_{p72} is not critical for the ingestion of such particles. Based on the relative abundance of CD16 on cultured monocytes, results of previous studies demonstrating a role for CD16 in rosetting, and the relation between increased ability of cultured monocytes to phagocytose IgG-opsonized particles and surface expression of CD16, it is attractive to hypothesize that CD16 is the most important FcR on macrophages for mediating immunophagocytosis. Our results support this hypothesis, and suggest that while all three FcRs can mediate ligand attachment, CD16 appears to play a more important role in mediating phagocytosis.

We also have investigated the structure of CD16 on both cultured monocytes and neutrophils. Neutrophil CD16 appears to contain abundant *N*-linked carbohydrate. CD16 on cultured monocytes, however, appears to be glycosylated to a lesser extent (if at all). Differential glycosylation has also been proposed to explain apparent differences in M_r between otherwise identical surface glycoproteins on different cell types, presumably because of cell-specific expression of glycosyltransferases (29). For example, Thy-1 from rat brain tissue and Thy-1 from rat lymphocytes are composed of nearly identical protein moieties, but differ considerably in carbohydrate content (30). Differences in the core proteins may also account for these differences as has been noted with murine FcRs. cDNA probes for murine FcRs for IgG2b/1 detect at least three different transcripts in murine macrophages (31). Two of these transcripts encode proteins that differ slightly in length, but that react with anti-FcR2g/1 mAb 2.4G2.

Therefore, the differences noted between CD16 on neutrophils on CD16 on cultured monocytes may be due to either differential posttranslational modification or differences at the level of transcription.

Summary

We have demonstrated that one Fc receptor for IgG (FcR) (CD16) on cultured human monocytes appears to be a developmentally regulated membrane protein. This receptor appears to contain less carbohydrate (if any) than does its counterpart on human neutrophils. Expression of CD16 on cultured monocytes increases with respect to both percentage of positive cells and numbers of sites per cell with length of time in culture. This was in contrast to expression of other types of FcRs that either decreased (CDw32) or did not change (FcR_{p72}). Unlike an FcR that binds monomeric IgG (FcR_{p72}), expression of CD16 on monocytes from most normal individuals was not influenced by IFN-γ. After 14 d in culture, CD16 appeared to be the predominant FcR on cultured monocytes, and was capable of mediating both ligand attachment and phagocytosis. These findings support the hypothesis that CD16 plays an important role in mediating immunophagocytosis.

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