PHAGOCYTOSIS MEDIATED BY THREE DISTINCT Fcγ RECEPTOR CLASSES ON HUMAN LEUKOCYTES

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The mononuclear and polymorphonuclear cells of the body have the capacity to bind, phagocytose, and ultimately destroy antibody-coated particles such as bacteria by a series of linked molecular processes that initially involve the plasma membrane Fc receptors for IgG (FcγR). Our understanding of these FcγR-mediated processes is rudimentary. We know that first the Ig-coated particles bind to the surface of the cell by interaction with FcγR. By poorly understood means, crosslinking of these receptors signals the movement of lamellar membrane and underlying cytoplasm around the particle until it is eventually engulfed, whereupon the particle is transported intracellularly to lysosomes for eventual degradation (1, 2).

Attempts to define the molecular details of these processes have in recent years focused on the structure of the FcγR. Considerable structural information is now available. We now know that phagocytic cells of man bear three distinct classes of FcγR. Although they are all members of a group of closely related molecules within the Ig gene superfamily, these receptors can be distinguished from one another on the basis of size, primary structure, affinity for ligand, to some extent specificity for ligand, and mAb reactivity. Within each of the three classes of receptors, it is clear that subclasses exist, as evidenced by studies of the cDNA sequences (recently reviewed in references 2, 3).

Whether all of these classes of FcγR, two or three of which are expressed on all phagocytic cells, are capable of mediating phagocytosis has been the focus of much recent attention (4–6). Our strategy has been to design immune complex probes specific for each of the receptor classes. Attempts using immune complexes of individual IgG subclasses have met with only partial success because the specificity of these receptor classes for subclasses of IgG ligand is relative rather than absolute. Likewise, approaches using FcγR class-specific mAbs to inhibit the binding of IgG immune complexes to one or more receptor classes have not been definitive, presumably because the avidity of a large immune complex for a cell surface may outweigh the affinity of mAbs for the FcγR under study. Instead, we have evaluated FcγR class-specific phagocytosis by presenting adherent phagocytic cells with erythro-
cytes coated with anti-FcγR mAbs, thereby attempting to crosslink FcγR and stimulate phagocytosis.

We find that FcγRI and FcγRII on all cell types tested, and FcγRIII on mononuclear phagocytes, are capable of mediating phagocytosis. However, FcγRIII on polymorphonuclear leukocytes (PMN) under the conditions of these experiments appears unable to generate a phagocytic signal. FcγRIII on PMNs, it should be noted, is not a membrane-spanning molecule but rather is linked to the outer leaflet of the plasma membrane by a glycosyl-phosphatidylinositol moiety (7-9).

Materials and Methods

Chemicals and Reagents. Percoll was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden; Ficoll-Hypaque (Histopaque-1077), BSA, fraction V, human serum albumin (HSA), and aprotinin from Sigma Chemical Co., St. Louis, MO; Mono-Poly Resolving Medium from Flow Laboratories, McLean, VA; IFN-γ from Genentech, South San Francisco, CA; ox erythrocytes (OE) and FCS from Colorado Serum Co., Denver, CO; N-succinimidyl-3-(2 pyridyldithiol) propionate (SPDP) from Pharmacia Fine Chemicals; N-succinimidyl-S-acetylthioacetate (SATA) from Calbiochem-Behring Corp., La Jolla, CA; HBSS and RPMI 1640 medium from Whittaker M. A. Bioproducts, Inc., Walkersville, MD; Macrophage colony-stimulating factor (M-CSF) (10) from Cetus Corp., Emeryville, CA; Penicillin, streptomycin, and Medium 199 from Gibco Laboratories, Grand Island, NY; dimethyl formamide (DMF) from Pierce Chemical Co., Rockford, IL; and Diff-Quik from American Scientific Products, McGaw Park, IL. A balanced salt solution (BSS) was prepared as described (11). Terasaki plates were obtained as Microtest Plates from Walter Sarstedt, Inc., Princeton, NJ. FITC anti-murine Ig was obtained from Cappel Laboratories, West Chester, PA. PBS contained 8.0 mM phosphate, 137 mM NaCl, 2.7 mM KCl, 0.5 mM MgCl2, and 0.9 mM CaCl2. PBS/BSA/Az consisted of PBS plus 1 mg/ml BSA and 0.2% NaN3. IgG and Fab or F(ab')2 fragments of the following mAbs were obtained from Medarex, Inc., West Lebanon, NH; mIgG1 anti-FcγRI mAb 32, mIgG2b anti-FcγRII mAb IV3, mIgG1 anti-FcγRIII mAb 3G8. The fragments contained <1% intact IgG by gel filtration analysis. Murine myeloma proteins of the IgG1 (MOPC 21) and IgG2b (MOPC 141) subclasses and mIgG2b anti-β2 microglobulin mAb BM1, mIgG2b anti-complement receptor 3 mAb OKM1 were purified by ion-exchange chromatography from ascites fluid obtained from American Type Culture Collection, Rockville, MD. Human IgG was purified from pooled human serum by salt precipitation and ion-exchange chromatography. Rabbit anti-OE IgG was obtained from Organon Teknika Corp., Westchester, PA. Fab or F(ab')2 fragments of anti-OE were prepared by pepsin cleavage, were separated by gel filtration, and were adsorbed with protein A-agarose to remove residual IgG.

Preparation of Heteroantibodies. Heteroantibodies of F(ab')2; 32, Fab IV3, F(ab'); 3G8, Fab OKM1, or Fab BM1 covalently linked to F(ab'); anti-OE were made by the method of Kar-powsky et al. (12). In brief, Fab or F(ab'); fragments of mAbs at 1-2 mg/ml in PBS containing 5 mM EDTA were incubated with a threefold molar excess of SPDP for 2 h at 18°C. F(ab')2 anti-OE at 6 mg/ml in PBS/EDTA were incubated with a threefold molar excess of SATA in DMF for 1 h at 18°C and were gel filtered over a G25 Sephadex column equilibrated with PBS/EDTA. Antibodies thus derivatized were dialyzed overnight in degassed PBS/EDTA under N2. SATA-F(ab')2 anti-OE was deacetylated using 50 mM hydroxylamine for 1 h at 18°C, and was gel filtered through a a PBS/EDTA-equilibrated G25 Sephadex column. Equimolar amounts of SATA-F(ab')2 anti-OE and SPDP-Fab or -F(ab'); fragments of mAb were immedi-

Abbreviations used in this paper: AI, adherence index; BSS, balanced salt solution; CR3, complement receptor 2; FcγR, Fc receptor(s) for IgG; HSA, human serum albumin; M-CSF, macrophage CSF; OE, ox erythrocytes; PI, phagocytic index; PMN, polymorphonuclear leukocytes; SATA, N-succinimidyl-S-acetylthioacetate; SPDP, N-succinimidyl-3-(2-pyridyldithiol).
ately mixed and incubated at 18°C for 4 h, after which crosslinking was terminated with iodoacetamide, added to a 10% molar excess over thiol groups. Heteroantibodies, dialyzed into PBS, were used at a concentration of 0.7–1.45 OD$_{280}$ U/ml.

**Cells.** Peripheral blood, obtained by venipuncture from normal human volunteers, was drawn into heparin-filled syringes and was separated into its cellular components on Mono-Poly Resolving Medium. PMN were harvested as a discrete band. In some experiments PMN were cultured overnight at 2 × 10$^6$/ml in Medium 199, 20% heat-inactivated FCS, L-glutamine, with IFN-γ (300 U/ml). Before use PMN were suspended at 2 × 10$^6$/ml in PBS supplemented with dextrose (3 mM), HSA (0.5 mg/ml), and aprotinin (0.3 U/ml) (PBS-HAG). Monocytes were prepared by the method of Wright (13). Briefly, mononuclear cells from buffy coats of cytophoresis packs were centrifuged on Ficoll-Hypaque and the interface layer was collected. The cell concentration was adjusted to 2–4 × 10$^6$/ml in RPMI 1640 medium containing 10% normal human serum. 5 ml of mononuclear cells were layered onto 37.7 ml of Percoll adjusted to isotonicity and the gradients were promptly centrifuged in a swinging bucket rotor at 1,000 g for 20 min at 5°C. The top of two thick hazy bands contained monocytes. Purified monocytes were washed in RPMI 1640, were suspended at 10$^6$/ml in RPMI 1640 containing 14% normal human serum, and were either used fresh or were cultured in teflon beakers with M-CSF (1,000 U/ml). In some experiments monocytes were purified by adherence to fibronectin-coated tissue culture flasks as described (14). Human alveolar macrophages (>90 pure) from nonsmoking normal volunteers were obtained by bronchoalveolar lavage (BAL) using standard methods (15). Before use the monocytes and macrophages were suspended at 2 × 10$^6$/ml in BSS containing 5 mM Mg$^{2+}$ and 5% FCS.

**Adherence and Phagocytosis Assays.** OE were coated with antibodies (EHA) by incubating heteroantibodies for 2 h at 21°C with an equal volume of a 50% suspension of OE in PBS. Mock-coated erythrocytes were carried through the entire procedure without the addition of antibody. OE were incubated in similar fashion with a subagglutinating concentration of rabbit IgG anti-OE to prepare IgG-opsonized OE (EA). The coated erythrocytes were then washed three times and resuspended at 1% (vol/vol) in PBS. Phagocytosis and adherence assays using OE targets were performed by the method of Wright (13). Wells of Terasaki plates were coated with HSA (10 µl of 1 mg/ml PBS solution) by incubation for 60 min at 21°C and were washed with PBS. Phagocytes were allowed to adhere to the well bottoms by incubating 10 µl of a phagocyte suspension in the wells for 60 min at 37°C. Nonadherent cells were washed away and 10 µl of an ox erythrocyte suspension were added to wells. In the inhibition experiments mAbs (intact IgG, Fab, or F(ab')$_2$ fragments) were added along with phagocytic targets. Plates were incubated 30 min at 37°C in a humidified incubator, were again washed in PBS, and were then fixed with 2% glutaraldehyde for analysis of adherence. In separate plates phagocytosis was analyzed after dipping plates in water to lyse adherent OE before fixation with glutaraldehyde.

Phagocytosis and adherence were scored by evaluating 200 phagocytes microscopically for the number of internalized or adherent erythrocytes at ×600 magnification using a phase contrast water immersion lens. The data are expressed as a phagocytic index (PI) or an adherence index (AI), the number, respectively, of phagocytosed or adherent erythrocytes per 100 leukocytes. Unless stated otherwise, the sample mean and standard error of the mean for three or more similar experiments are expressed.

In some experiments preparations of heteroantibody-coated ox erythrocytes used in Terasaki (adherent) plate assays were tested simultaneously in an assay that involved centrifugation of the erythrocytes and phagocytes into a pellet before the 37°C incubation (16). Briefly, 10$^6$ erythrocytes and 10$^6$ PMN in 200 µl RPMI 1640 containing 20% FCS were centrifuged into a pellet at 44 g and were incubated for 1 h at 37°C. Cytocentrifuge preparations were stained with Diff-Quik and PMNs were scored microscopically for internalized OE.

**Flow Cytometry.** To quantify and compare the amounts of murine mAbs bound to the heteroantibody-coated erythrocytes, 1.5 µl of antibody-coated erythrocytes prepared as above were pelleted and resuspended in 20 µl FITC-anti-mIgG. The mixtures were incubated 2 h at 4°C, were washed three times in PBS/BSA/Az, and were fixed in 1% paraformaldehyde. Single cell fluorescence was analyzed on an EPICS C flow cytometer (Coulter EPICS Division, Hialeah, FL) as previously described (17).
Receptor Specificity of Antibody-coated Erythrocytes. Our method for assessing the capacity of each of the three classes of FcγR on phagocytic cells to mediate phagocytosis was briefly the following: OE were coated with anti-FcγR mAbs by incubating them with heteroantibodies consisting of Fab or F(ab')2 fragments of anti-OE covalently linked to Fab or F(ab')2 fragments of anti-FcγR mAb specific for each of the three FcγR classes, FcγRI (mAb 32), FcγRII (mAb IV3), or FcγRIII (mAb 3G8). These antibody-coated erythrocytes were then incubated with phagocytes adherent to albumin-coated Terasaki wells and the extent of phagocytosis was scored microscopically and expressed as a phagocytic index (PI), largely by the method of Wright.

We first evaluated whether each of the anti-FcγR-coated erythrocytes (referred to as E32, EIV3, and E3G8) was a specific probe for the appropriate FcγR by performing the phagocytosis assay with cultured monocytes in the presence of saturating concentrations of various anti-FcγR mAbs. These cells, which express all three classes of FcγR, were observed to bind (not shown) and to phagocytose vigorously exclusively with mAb directed against the homologous anti-FcγR mAb, at saturating concentrations, was phagocytosis inhibited, as seen in Fig. 1, with mAb 32 inhibiting E32 phagocytosis 81% ± 5, mAb IV3 inhibiting EIV3 phagocytosis 95% ± 3, and mAb 3G8 inhibiting E3G8 phagocytosis 97% ± 2 in a series of repetitive experiments. mAbs directed against different classes of FcγR or murine IgG of the same subclasses as the anti-FcγR mAbs, or human IgG, all at concentrations of 50 μg/ml, were unable to inhibit phagocytosis (Fig. 1).

Specificity of these targets is also indicated by the observations that antibody-coated targets failed to bind to cells that did not express the appropriate receptor. For example, E32 was not capable of binding to PMNs that have been shown to be devoid of significant numbers of FcγRI. Further, E3G8 did not bind to freshly purified monocytes that bear little or no FcγRIII (19, 20; Anderson, C. L., R. J. Looney, D. J. Culp, D. H. Ryan, H. B. Fleit, M. J. Utell, M. W. Frampton, P. Manganello, and P. M. Guyre, manuscript submitted for publication).

Phagocytic Capacity of FcγR Classes Expressed by Several Cell Types. The ability of five different types of phagocytic leukocytes to phagocytose by means of the three distinct FcγR classes was evaluated by incubating adherent phagocytes with erythrocytes coated with anti-FcγR mAbs. The phagocytic cells evaluated were freshly isolated monocytes that express FcγRI and FcγRII (21); cultured monocytes, alveolar macrophages, and PMNs cultured in IFN-γ that express all three classes (20, 22; Anderson et al., manuscript submitted for publication); and freshly isolated PMNs that display FcγRII and FcγRIII (17). Fig. 2 presents a summary of the results. Freshly isolated monocytes that bear FcγRI and FcγRII were capable of binding and phagocytosing E32 and EIV3, whereas E3G8 was neither bound nor phagocytosed. Monocytes that had been cultured for several days such that they expressed FcγRIII in addition to FcγRI and FcγRII were capable of binding and phagocytosing all three anti-FcγR-coated erythrocytes. Likewise, alveolar macrophages that bore all three FcγR classes bound and phagocytosed the erythrocyte probes for all three classes of receptor. In contrast, polymorphonuclear leukocytes behaved somewhat differently: freshly purified PMNs that bear FcγRII and FcγRIII were capable of binding
Figure 1. Specificity controls for phagocytosis of anti-FcγR mAb-coated erythrocytes. Cultured monocytes adherent to albumin-coated Terasaki wells were incubated for 60 min with anti-FcγR mAb-coated erythrocytes in the presence of anti-FcγR mAbs and antibodies of the same IgG subclass (all at 50 μg/ml). Unphagocytosed erythrocytes were lysed, and the extent of phagocytosis, enumerated by phase contrast microscopy, was expressed as a phagocytic index (PI). The percent inhibition of phagocytosis was calculated by the formula \[1 - \frac{I}{U}\times 100\] where \(I\) is the PI observed in the presence of inhibitory protein and \(U\) is the PI observed in the presence only of albumin-containing medium. The mean and SEM of three to four observations in most cases are plotted. mAbs 32, IV3, and 3G8 recognize monocyte FcγRI, II, and III, respectively.

Both EIV3 and E3G8, but only the probe for FcγRII was phagocytosed; E3G8 were not phagocytosed. Likewise, PMNs cultured overnight in IFN-γ such that they express all three FcγR classes bound the probes for all three FcγR but were capable of phagocytosing only those for FcγRI and FcγRII. As with fresh PMNs, IFN-cultured PMNs failed to phagocytose E3G8. Erythrocytes coated with mAbs against either β2 microglobulin (mAb BBM1) or complement receptor 3 (CR3) (mAb OKM1) were bound by all of these cell types but were not phagocytosed, while mock-coated erythrocytes were neither bound nor phagocytosed.

Simultaneous Analysis of E3G8 Phagocytosis by PMN and Cultured Monocytes. Considering the possibility that different lots of E3G8 had coincidentally led to the observations noted in Fig. 2, we performed two experiments in which IFN-PMN and cultured monocytes adherent to wells of the same Terasaki plate were incubated with the same preparation of E3G8. As seen in Fig. 3, erythrocytes coated with mAbs to all three FcγR classes bound to both cell types as did the erythrocytes coated with a mAb against an irrelevant antigen (either β2 microglobulin or CR3). However, E3G8 was phagocytosed only by cultured monocytes and not by interferon-cultured PMNs. Again, neither cell type phagocytosed erythrocytes coated with BBM1 or OKM1, and mock-coated erythrocytes were neither bound nor phagocytosed.

Relative Amounts of anti-FcγR mAb on Erythrocytes. Despite the fact that E3G8 was phagocytosed by mononuclear phagocytes, it seemed possible that the failure of E3G8
to be phagocytosed by PMNs could be due to inadequate quantities of mAbs bound to the erythrocyte. We therefore compared in two experiments not shown the amounts of murine antibody bound to the surface of E32, EIV3, E3G8 by indirect immunofluorescence using FITC-anti-murine Ig and flow cytometry. The amount of murine antibody on the E3G8 cells was appreciably greater than on any of the other probes, two of which (E32 and EIV3) were vigorously phagocytosed. This finding would indicate that the failure of E3G8 phagocytosis by PMNs was not a consequence of inadequate coating of mAb 3G8 on the erythrocyte surface.

**Relative Expression of FcγR on Phagocytes.** Even though FcγRIII appears to be more highly expressed on PMN and IFN-PMN than either FcγRI or FcγRII (5, 17, 20), we considered the possibility that under the conditions of our experimental protocol FcγRIII had been lost from the cell surface of PMN or IFN-PMN such that binding and subsequent phagocytosis of E3G8 were impeded. However, as can be seen in Fig. 2, the AI of E3G8 to PMN and IFN-PMN equaled or exceeded the AI of all of the other antibody-coated erythrocytes including EIV3, which were phagocytosed by both of these cells and E32 which were phagocytosed by IFN-PMN. Moreover, the AI of E3G8 exceeded or was equivalent to the AI seen with EOKM1, which also were not phagocytosed. These data would suggest that the expression of FcγRIII on these cells was sufficiently preserved such that the binding of E3G8 was not com-

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**Figure 2.** Adherence and phagocytosis of anti-FcγR mAb-coated erythrocytes. Freshly purified peripheral blood monocytes (Monos), monocytes cultured for several days in vitro (Cult Monos), freshly isolated polymorphonuclear leukocytes (PMN), PMN cultured overnight in IFN-γ (PMN-IFN), and alveolar macrophages (Alv Macs) that were adherent to albumin-coated Terasaki wells were incubated for 30 min with erythrocytes coated with heteroantibodies prepared from anti-FcγR mAbs (those used in Fig. 1, designated E32, EIV3, and E3G8), with erythrocytes coated with heteroantibodies prepared from mAbs recognizing determinants that were likely not to mediate phagocytosis (OKM1 anti-CR3 and BBM1 anti-β2 microglobulin, both designated EC, C for "control"), and with mock-coated erythrocytes (E). Unphagocytosed erythrocytes were lysed by hypotonic shock, and the extent of phagocytosis, enumerated by phase contrast microscopy, was expressed as a phagocytic index (PI). In separate Terasaki plates containing cells that were not subjected to hypotonic lysis, the adherence index (AI) was enumerated. The means and SEM of four to five observations for each cell type are plotted.
promised and could not be responsible for the failure of a phagocytic response to this target.

**Magnitude of Phagocytosis of Anti-FcγR-coated Erythrocytes.** We attempted to quantify the magnitude of the phagocytic response toward anti-FcγR-coated erythrocytes by measuring simultaneously the phagocytic index of erythrocytes coated with the more conventional ligand, rabbit IgG anti-OE antibody (EA) (Table I). In a series of experiments the mean phagocytic index for EA ranged from 125 with monocytes to 426 with alveolar macrophages. The vigor of the phagocytic response to anti-FcγR-

**Table I**

| EHA* | Monocytes | IFN-PMN | Alveolar macrophages |
|------|-----------|---------|----------------------|
|      | PI^1      |         |                      |
| E32  | 54 ± 4    | 128     | 285                  |
| EIV3 | 34 ± 7    | 107 ± 2 | 174                  |
| EA   | 125 ± 26  | 209 ± 43| 426                  |
| E    | 0 ± 0     | 0 ± 0   | 0                    |

* Erythrocytes were coated with anti-FcγR mAbs 32 or IV3 (E32 and EIV3) or with rabbit IgG anti-OE (EA) or they were mock coated (E).

^1 Phagocytosis by monocytes, IFN-cultured PMNs (IFN-PMN), and alveolar macrophages was measured as described in the legend to Fig. 2, and phagocytic indices (PI) were scored. For monocytes and IFN-PMN the data are expressed as the mean ± the deviation from the mean of two experiments.
coated erythrocytes never reached this magnitude, but was nevertheless substantial, ranging from 27% (for EIV3 phagocytosis by monocytes) to 67% (for E32 phagocytosis by alveolar macrophages) of the PI seen with EA.

**Phagocytosis by Adherent Cells and by Pelleted Cells.** Considering the possibility that PMN phagocytosis of E3G8 may depend on the manner of physical interaction between the target and the phagocyte, or on the state of adherence of the phagocyte, we compared two methods of evaluating phagocytosis, the one described above in Fig. 2 and another method in which opsonized erythrocytes mixed with phagocytes were centrifuged into a pellet before a 37°C incubation (16). Three such experiments are summarized in Table II. E3G8 were not phagocytosed by PMN using either of the two methods, whereas these target cells were fully capable of binding vigorously to PMN, the AI for E3G8 in the adherence assay being 700 ± 0, whereas the AI for E was 17 ± 1. EIV3 and EA by both methods were seen to be phagocytosed by PMN, although the extent of phagocytosis was more vigorous when the phagocytes were adherent to the Terasaki wells. Little or no phagocytosis, as expected, was seen with EOKM1 and E.

**Discussion**

As part of a larger attempt to correlate structure of FcyR with their functions we have asked in this study whether the receptors of all three FcyR classes are capable of generating a phagocytic signal upon presentation of erythrocytes coated with anti-FcyR mAbs. We found that FcγRI and FcγRII on whatever phagocytic cell they were expressed were capable of mediating phagocytosis. E32 that binds only to FcγRI was vigorously internalized by monocytes, cultured monocytes, and IFN-PMN, all of which express this FcγR class. Likewise, EIV3 that bind FcγRII were ingested after presentation to all of the five cell types used in this study. It is noteworthy that the two FcγR that are not constitutively expressed but rather require induction, FcγRI on IFN-PMN and FcγRIII on cultured monocytes, also were capable of mediating phagocytosis.

We have considered the possibility that small amounts of contaminating intact IgG in the heteroantibody preparations were responsible for the phagocytic signals produced by the antibody-coated erythrocytes. One might argue that antireceptor mAb might ligate the erythrocyte to the phagocyte surface while contaminating quantities of intact IgG interacted with FcγR to generate the internalization signal. However, were this scenario possible, one would have anticipated that the erythrocytes coated with the several other mAbs with specificities for β2-microglobulin, CR3, and FcγRIII (for PMN) would also have been phagocytosed because these heteroantibodies were prepared by the identical procedure. In fact, these targets were bound but not phagocytosed (Fig. 2).

We found as well that FcγRIII was capable of mediating a phagocytic signal to E3G8, but only by the form of FcγRIII expressed on cultured monocytes or macrophages. These two cell types readily phagocytosed E3G8. However, FcγRIII expressed on PMN or IFN-PMN was incapable of generating a phagocytic signal upon presentation of E3G8 even though E3G8 targets were bound vigorously. We were unable to attribute the phagocytic failure of FcγRIII on PMNs to insufficient coating of the 3G8 heteroantibody to target erythrocytes or to inadequate expression of FcγRIII on PMNs (Fig. 2). Furthermore, the very same E3G8 targets which were phagocy-
Phagocytosis by PMN was assessed in two ways, in the conventional manner described in Fig. 1 in which phagocytes were adherent to Terasaki wells (adherent cells), and by a different assay described in the text that entails centrifugation of phagocytes and erythrocytes into a pellet (pelleted cells). Erythrocytes were coated with anti-FcyR mAbs IV3 or 3G8 (EIV3 or E3G8), with rabbit IgG anti-OE (EA), with anti-CR3 mAb OKM1 (EOKM1), or they were mock coated (E).

For the adherent cells, phagocytosis and adherence were measured as described in the legend to Fig. 2, and phagocytic and adherence indices (PI and AI) were scored. For pelleted cells, the PI was scored as described in the text. The data are expressed as the mean ± SEM of three separate experiments. E3G8 were capable of binding to PMN as evidenced by an adherence index on Terasaki wells of 700 ± 0, while the adherence index for E was 17 ± 1.

It is important to note that two forms of FcyRIII have been described, the products of two distinct genes (9). The form on PMNs is attached to the outer leaflet of the plasma membrane by a phosphatidylinositol-glycan moiety, while the form on natural killer cells and on macrophages is a more conventional integral membrane protein consisting of a membrane spanning portion and a cytoplasmic domain (7). One might anticipate, therefore, that the PMN form of FcyRIII, bearing no cytoplasmic extension, might not be able to generate a transmembrane signal that would initiate a phagocytic response. The FcyRIII expressed on the mononuclear phagocytes having a cytoplasmic tail more clearly has the potential to interact with a phagocytic signal-generating mechanism in the cell interior.

Our observation that FcyRIII on PMNs is incapable of mediating E3G8 phagocytosis lends support to the general notion that the PMN FcyRIII is severely limited in its capacity to mediate the variety of biological signals stimulated by immune complexes. There is considerable doubt that FcyRIII on PMNs is capable of signalling the production of superoxide (5, 6). Furthermore, FcyRIII on PMNs mediates an antibody-dependent cytotoxic (ADCC) signal only with chicken erythrocyte targets, not with nucleated cell targets (23, 24). By contrast, FcyRI and FcyRII, regardless

**Table II**

| EHA* | Pelleted cells | Adherent cells |
|------|---------------|----------------|
|      | PI mean ± SEM |                |
| EIV3 | 51 ± 24       | 162 ± 12       |
| E3G8 | 1 ± 1         | 1 ± 1          |
| EOKM1| 1 ± 1         | 1 ± 0          |
| EA   | 67 ± 14       | 271 ± 13       |
| E    | 0 ± 0         | 0 ± 0          |

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‡ For the adherent cells, phagocytosis and adherence were measured as described in the legend to Fig. 2, and phagocytic and adherence indices (PI and AI) were scored. For pelleted cells, the PI was scored as described in the text. The data are expressed as the mean ± SEM of three separate experiments. E3G8 were capable of binding to PMN as evidenced by an adherence index on Terasaki wells of 700 ± 0, while the adherence index for E was 17 ± 1.
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of the cell type on which they are expressed, are capable of all immune complex-mediated functions that have been tested, including superoxide generation (6, 17, 25, 26), ADCC against both erythrocyte and nucleated targets (23, 24), and phagocytosis (presented herein).

Our observation that crosslinking of FcγRIII on PMNs fails to mediate phagocytosis is in apparent conflict with the findings of Salmon et al. (16) who find that PMN phagocytosis but not adherence of Con A-coated erythrocytes (EconA) is inhibited by Fab fragments of anti-FcγRIII (mAb 3G8). Their experimental method was somewhat different than ours in that PMNs in our studies were adherent to albumin-coated plastic, whereas in their experiments EconA and PMNs were centrifuged into a pellet before incubation at 37°C. However, duplicating their procedure using our reagents, we were nevertheless unable to demonstrate phagocytosis of E3G8 by PMNs whereas EIV3 and EA were obviously phagocytosed (Table II). Other reasons for the disparity must be sought.

Kimberly's studies suggest that crosslinking of either FcγRIII or FcγRIII and an ancillary molecule is necessary for generation of a phagocytic signal. In light of our data presented herein Kimberly's observations might be interpreted as indicating that FcγRIII crosslinking is a necessary but insufficient stimulus for the phagocytosis of EconA by PMNs. What further stimuli are required beyond FcγRIII crosslinking needs further definition.

The work of others, however, is consistent with our finding that PMN FcγRIII is limited in its capacity to signal phagocytosis. Tosi and Berger have shown that elastase-incubated PMN have lost 85% of their capacity to bind 3G8 yet endocytose IgG-coated paraffin particles normally (5).

The studies described herein ask only whether the three FcγR studied are capable of mediating a phagocytic signal when crosslinked; they do not ask whether these three FcγR mediate phagocytosis when presented with physiologic ligand such as IgG immune complexes. One might postulate that FcγRIII on PMNs is indeed capable of generating a phagocytic signal when presented with physiologic ligand. This possibility is difficult to study for the reasons pointed out in the Introduction, but it might perhaps be analyzed by downmodulating PMN FcγRII on mAb IV3-coated surfaces and evaluating the phagocytosis of EA by FcγRII alone. Our preliminary attempts at these experiments have been inconclusive.

One might also propose that a different anti-FcγRIII mAb, recognizing a different epitope on FcγRIII than does mAb 3G8, would be capable of generating a phagocytic signal upon crosslinking of FcγRIII on PMNs. Precedence for such phenomena exist for mAbs against other cell surface molecules (27-29); i.e., different mAbs against the same molecule may produce different biological effects upon binding. This possibility seems unlikely given the observation (Fig. 2) that FcγRIII on mononuclear phagocytes were capable of mediating phagocytosis of E3G8. However, were adequate quantities of F(ab')2 or Fab fragments of other anti-FcγRIII mAbs available, one could test this hypothesis.

Because the FcγR studied herein have all been shown to be structurally pleomorphic by recent work at the mRNA level (2, 3), our data here do not implicate FcγR of unique molecular structure as being mediators of phagocytosis. Three distinct cDNA have been defined for FcγR1, all presumably recognizable by E32, although two differ by only two amino acids in the extracellular portion while the third has a unique
cytoplasmic domain (2, 3). Which of these is expressed in the cells studied herein is not clear. Six cDNAs, the products of three distinct genes, have been identified for FcγRII. Only three of the cDNAs, products of the IIa and IIa' genes, are found in PMNs, but the principal differences appear in the signal and polyadenylation sequences (2). In addition to the IIa and IIa' transcripts, mononuclear phagocytes express the three transcripts of the IIB gene, one of which has a unique cytoplasmic domain and a second differs only in the signal sequence (30). So the actual number of unique receptor proteins expressed in phagocytes may not be as great as the number of cDNAs would indicate. Whether polymorphisms of the phagocytosis-mediating FcγRIII gene expressed in mononuclear cells exist is not yet known. Nevertheless, it is clear from the cDNA data beginning to appear that the antireceptor mAbs used in the experiments described herein may recognize more than a single protein.

Summary

We have evaluated the capacity of the three major classes of human FcγR to mediate phagocytosis by measuring the ability of adherent phagocytes to internalize erythrocytes coated with anti-FcγR mAb. Five different cell types were studied, freshly purified monocytes, cultured monocytes, alveolar macrophages, freshly purified polymorphonuclear neutrophilic leukocytes, and PMNs cultured in IFN-γ. FcγRI and FcγRII on whichever cells they were expressed were capable of phagocytosing anti-FcγR mAb-coated erythrocytes. Furthermore, FcγRIII on mononuclear phagocytes, which appears to be a conventional integral membrane protein that spans the lipid bilayer, was capable of phagocytosing anti-FcγRIII-coated erythrocytes. However, FcγRIII on neutrophils, a molecule linked to the membrane by a phosphatidylinositol-glycan moiety, although binding anti-FcγRIII-coated erythrocytes vigorously was incapable of mounting a phagocytic response. This deficiency correlates with the limited capacity of FcγRIII on neutrophils to mediate superoxide generation and antibody-dependent cell-mediated cytotoxicity and it may be related to the unique structural features of FcγRIII.

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