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Ira S. Mellman

Helen Plutner

Ralph M. Steinman

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Internalization and Degradation of Macrophage Fc Receptors during Receptor-mediated Phagocytosis

IRA S. MELLMAN, HELEN PLUTNER, RALPH M. STEINMAN, JAY C. UNKELESS, and ZANVIL A. COHN
Section of Cell Biology, Yale University School of Medicine, New Haven, Connecticut 06510; and The Rockefeller University, New York 10021

ABSTRACT Macrophage receptors for the Fc domain of immunoglobulin G (IgG) can mediate the efficient binding and phagocytosis of IgG-coated particles. After internalization, phagocytic vacuoles fuse with lysosomes, initiating the degradation of their contents. Using specific monoclonal and polyclonal antireceptor antibodies, we have now analyzed the internalization and fate of Fc receptors during the uptake of IgG-coated erythrocytes and erythrocyte ghosts by mouse peritoneal macrophages. Receptor-mediated phagocytosis led to the selective and largely irreversible removal of Fc receptors (>50%) from the macrophage plasma membrane. The expression of several other plasma membrane proteins (including a receptor for complement), recognized by a series of antimacrophage monoclonal antibodies, was affected only slightly. Interiorized Fc receptors were rapidly and selectively degraded. This was demonstrated by a series of turnover studies in which Fc receptor was immunoprecipitated from lysates of labeled macrophages. These experiments were made possible by the development of a polyclonal rabbit antiserum, raised against isolated Fc receptor, which recognized the receptor even in the presence of bound ligand. In control cells, the receptor turned over with a $t_{1/2}$ of ~10 h; after phagocytosis, >50% of the receptors were degraded with a $t_{1/2}$ of <2 h. The turnover of other unrelated plasma membrane proteins was unaffected ($t_{1/2}$ of 18-23 h) under these conditions.

Macrophages and many lymphocytes have on their plasma membranes (PM) receptors for the Fc domain of immunoglobulin G (IgG) (for review, see reference 1). IgG in concert with Fc receptors (FcR) regulate several aspects of inflammation and immunity. For example, the binding of IgG to macrophage FcR may result in: (a) the endocytosis of soluble immune complexes or opsonized particles (2–5), (b) the synthesis or release of a variety of potent inflammatory agents (e.g., proteases, prostaglandins, leukotrienes) (4, 6–8), and (c) the release of active oxygen intermediates that can serve cytolytic or microbicidal functions (9).

The study of FcR function has recently been aided by the development of highly specific monoclonal antibodies directed against mouse (10) and human (11) FcR. These antibodies can distinguish distinct receptors that recognize different IgG subclasses. For example, mouse macrophages express at least two FcR: a trypsin-resistant receptor for mouse IgG1/IgG2b-containing immune complexes, and a trypsin-sensitive receptor specific for monomeric IgG2a (1). The monoclonal anti-FcR antibody 2.4G2 recognizes only the protease-resistant immune complex receptor (10, 12). We have used an Fab fragment of 2.4G2 as an immunoaffinity reagent to purify this FcR from J774 macrophages (12). The isolated receptor was found to be a glycosylated intrinsic membrane protein of 45,000–60,000 mol wt which retained specific FcR activity in vitro (1, 12).

Monoclonal antibodies can also be used to study receptor dynamics and metabolism. In previous work, we have examined the internalization of FcR during fluid-phase pinocytosis. Pinocytosed lactoperoxidase was used to catalyze the iodination of pinocytic vesicle (or endosome) membrane within intact cells (13). FcR was identified on internalized membrane by immune precipitation, and the amount of $^{125}$I-FcR relative to other iodinated membrane proteins was the same as on the cell surface (13). In these experiments, the receptor was internalized at the same rate as many other PM polypeptides. Since iodinated endosome membrane proteins turn over with kinetics similar to those of their PM progenitors (3), it seems likely that these internalized proteins, including the FcR, are...
recycled in concert to the PM (4, 5). In this paper, we analyze the internalization and fate of the receptor during the phagocytic uptake of a large multivalent ligand, the IgG-coated erythrocyte ghost. These studies were facilitated by a new polyclonal anti-FcR antiserum which, unlike the monoclonal antibody 2.4G2, is equally capable of detecting free receptor and receptor-ligand complexes. We have found that, relative to other PM antigens, FcR are selectively removed from the macrophage cell surface during receptor-mediated phagocytosis. Selectively internalized receptors are not recycled but are rapidly degraded in secondary lysosomes.

MATERIALS AND METHODS

Cells: Thioglycolate-elicited peritoneal macrophages were isolated from female CD-1 (BALB/c X DBA) mice 4 d after an intraperitoneal injection of 1 ml of thioglycolate broth (14). Exudate cells were collected by centrifugation (500 g) and were washed two times with phosphate-buffered saline (PBS) containing 1 mM EDTA. Cells were plated in 16-mm or 35-mm dishes (at densities of 4 X 10^3 and 3 X 10^5 cells/dish, respectively) in Dulbecco's modified Eagle's medium (DME) supplemented with 10% heat-inactivated fetal bovine serum and antibiotics. After incubation at 37°C for 2 h, monolayers were rinsed four times with PBS to remove nonadherent cells and re-fed with fresh medium. Typically, 50-80% of the exudate cells and >95% of the adherent cells were macrophages.

Iodination: Macrophages were radioiodinated using the lactoperoxidase-glucose oxidase (LPO-GO) method of Hubbard and Cohn (15) as described in reference 13. Total peritoneal exudate cells were labeled in suspension (3 X 10^6 cells/ml) in DME at 12°C with carrier-free Na^125I (Amersham/Seapure, Arlington Heights, IL) at 1.5 mCi/ml. The reaction was terminated by diluting the cells with 10 ml cold DME. The cells were then pelleted by centrifugation at 4°C (500 g, 2.5 min) and washed three times with cold DME to remove unincorporated iodine before plating. Cell viability, determined by trypan blue exclusion, was unaffected by the iodination procedure (>94% viable cells). Incorporated ^125I was estimated by lysing cells in 0.5% NP40, 100-fold excess of unlabeled antibody to the reaction mixture. All determinations were performed in duplicate or triplicate, with parallel wells varying <5%. Cell protein was estimated by prelabeling cultures with [3H]leucine before assay. Cell loss was thus found not to occur at any of the timepoints employed.

Rosette Formation: Sheep erythrocytes (E) (Alsever's; Scott Laboratories, Fiskeville, RI), were washed three times by centrifugation (1,000 g, 10 min) in cold PBS. A 10% (vol/vol) solution was then opsonized (37°C, 30 min) using a sub-hemagglutinating concentration of a purified IgG fraction of a rabbit anti-E serum (usually ~0.25 mg/ml, Cerdiz Laboratories Inc., Miami, FL). E-bound IgG was routinely quantified by including ^125I-labeled antibody at high and low specific activities (the latter to determine nonspecific binding). Rosette formation was accomplished by adding washed, opsonized E (EigG) to plated macrophages for 1 h at 0°C in DME with 10% FCS. Unbound EigG were removed by several washes with cold PBS. Bound EigG were lysed hypotonically to form erythrocyte ghosts by immersing monolayers in cold distilled water for 15 s. While this treatment did not affect macrophage viability or endocytic activity, routinely both rosetted and control cultures were treated.

Immune Precipitation: Cells were lysed in PBS containing 0.5% NP40, 1 mM phenylmethylsulfonyl fluoride and 1 U/ml aprotinin (Sigma Chemical Co., St. Louis, MO). After centrifugation (47,000 g, 15 min, 4°C), ^125I-labeled antigens were immune precipitated using rat monoclonal antibodies and affinity-purified Fab' fragments of rabbit anti-rat IgG coupled to Sepharose 4B as described (13). 1 ml of rabbit anti-Fc serum was used per 0.2 ml of cell lysate. After 1 h at 0°C, immune complexes were isolated by use of protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ). Precipitates were analyzed by SDS PAGE (4-11% gradients) (20). Gel autoradiographs were used as templates to excise and then to quantitate radioactive proteins.

RESULTS

EigG Binding to Fc Receptors

FcR on mouse peritoneal macrophages were studied both before and after the phagocytosis of IgG-coated erythrocytes and erythrocyte ghosts. In the first series of experiments, we estimated the number of PM FcR involved in the attachment wells (24-well cluster dish, Costar, Cambridge, MA), washed three times with cold PBS, and then incubated for 1 h on ice in 0.2 ml of an ^125I-monoclonal antibody at 1 g/ml in PBS containing 1 mg/ml BSA and 0.02% NaN3. This antibody concentration was saturating for all antibodies used except B25-1 (3 mg/ ml). Specific activities employed ranged from 1 to 5 x 10^6 cpm/g of antibody. Cells were harvested after four washes in cold PBS by wiping out the wells with cotton-tipped swabs that were then assayed for ^125I in a gamma scintillation spectrometer. Background or nonspecific binding (usually <1%) was estimated by adding a 100-fold excess of unlabeled antibody to the reaction mixture. All determinations were performed in duplicate or triplicate, with parallel wells varying <5%. Cell protein was estimated by prelabeling cultures with [3H]leucine before assay. Cell loss was thus found not to occur at any of the timepoints employed.

To quantify the amount of surface antigen on intact cells, we determined the binding of ^125I-monoclonal antibodies to macrophages at 0°C. Macrophages (4 x 10^5) were plated in 16-mm wells (24-well cluster dish, Costar, Cambridge, MA), washed three times with cold PBS, and then incubated for 1 h on ice in 0.2 ml of an ^125I-monoclonal antibody at 1 g/ml in PBS containing 1 mg/ml BSA and 0.02% NaN3. This antibody concentration was saturating for all antibodies used except B25-1 (3 mg/ml). Specific activities employed ranged from 1 to 5 x 10^6 cpm/g of antibody. Cells were harvested after four washes in cold PBS by wiping out the wells with cotton-tipped swabs that were then assayed for ^125I in a gamma scintillation spectrometer. Background or nonspecific binding (usually <1%) was estimated by adding a 100-fold excess of unlabeled antibody to the reaction mixture. All determinations were performed in duplicate or triplicate, with parallel wells varying <5%. Cell protein was estimated by prelabeling cultures with [3H]leucine before assay. Cell loss was thus found not to occur at any of the timepoints employed.

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of the opsonized erythrocytes to the macrophages at 0°C. This was accomplished using ¹²⁵I-labeled Fab fragments of the anti-FcR monoclonal antibody 2.4G2. At the concentration used (1 μg/ml), this antibody does not bind to ligand-occupied FcR nor does it displace bound ElgG. Thus, 2.4G2 Fab binding gives an estimate of unoccupied FcR on rosetted macrophages. In subsequent experiments, we followed the metabolism of FcR after phagocytosis using a specific polyclonal antireceptor antibody that recognizes both occupied and unoccupied FcR.

The erythrocytes used in these experiments were coated with an average of 9 × 10⁵ IgG/cell (see Materials and Methods). The thioglycolate-elicited macrophages had ~2 × 10⁵ receptors/cell, estimated from the number of ¹²⁵I-2.4G2 Fab binding sites (12). Accordingly, each erythrocyte was a highly multivalent particle that contained several times the amount of potential ligand needed to saturate the complete cell surface complement of macrophage FcR. First, we determined the fraction of receptors involved in the binding of ElgG at various erythrocyte:macrophage ratios. Increasing concentrations of ElgG were added to parallel cultures of adherent macrophages at 0°C. After 1 h, unbound erythrocytes were washed away and the fraction of unoccupied Fc receptors was determined by use of ¹²⁵I-2.4G2 Fab. As shown in Fig. 1, the percentage of FcR accessible to 2.4G2 Fab decreased sharply as a function of the number of ElgG added. At the lowest doses (20 ElgG added per cell, corresponding to ~2 ElgG per macrophage "rosette") less than half of the macrophage FcR were occupied. The fraction of unoccupied receptors could not be decreased to <30-35%, even at the highest doses of ElgG (>500 added per cell, >25 ElgG bound per rosette). Moreover, the higher concentrations usually resulted in significant agglutination of macrophage-associated ElgG. The arrow (Fig. 1) indicates the dose of erythrocytes employed in subsequent studies (100 ElgG per cell). Under these conditions, ~50% of the receptors were occupied and an average of 10–13 erythrocytes were bound per macrophage with little or no hemagglutination.

Internalization of Bound Erythrocytes and Erythrocyte Ghosts

As illustrated in Fig. 2A and B, ElgG rosetted as described above were quickly internalized by the macrophages after...
warming to 37°C. Phagocytosis was virtually complete within 45–60 min and was monitored by phase-contrast microscopy (Fig. 2) and by electron microscopy (not shown). In addition, erythrocytes associated with the macrophages were not hemolyzed when cultures were incubated in hypotonic medium or distilled water, further proving their intracellular localization.

While the macrophages had little difficulty in ingesting 10–12 ElgG per cell, this large load of erythrocytes had adverse effects on macrophage viability as a function of time in culture. Within 4 h at 37°C after phagocytosis, the number of viable macrophages (assessed by trypan blue exclusion) decreased from >98% to <90%. As many as 50% of the cells were nonviable after 24 h. In addition, a variable number of cells developed large plasma membrane blebs that were not observed in control cultures. These difficulties were avoided by hypotonically lysing the rosetted erythrocytes at 0°C before phagocytosis. The erythrocyte ghosts were very rapidly internalized, with ingestion being complete within 15 min (Fig. 2 C and D). Endocytosis of the ghosts was also confirmed by electron microscopy. After uptake, macrophages contained a number of phase-lucent vacuoles that appeared to condense and then to disappear entirely. 1 h after phagocytosis, the cells had regained normal morphology by light microscopy (Fig. 2F). These cultures could be maintained indefinitely (48–72 h) without any detectable decrease in cell viability.

**Fate of IgG and Erythrocyte Membrane Proteins**

Degradation of both IgG and ghost membrane proteins began shortly after internalization, indicating the rapid fusion of incoming phagocytic vacuoles with macrophage lysosomes (21). When erythrocytes coated with 125I-IgG were employed, digestion of the antibody proceeded with first-order kinetics, with a $t_{1/2}$ of ~2.5 h (Fig. 3A). Radiolabel lost from the cells was recovered quantitatively in the medium as TCA-soluble 125I and could be detected within 15 min after warming the cultures to 37°C. Although degradation followed uniphasic kinetics, the proteolysis of the heavy and light chains of individual IgG molecules did not occur simultaneously (Fig. 3B). Instead, heavy chain disappeared more rapidly. Within 24 h after phagocytosis, neither IgG nor IgG fragments was detected.

We also examined the degradation of ghost membrane proteins, using erythrocytes iodinated with LPO-GO before opsonization (Fig. 4). Iodinated proteins were digested synchronously and at approximately the same rate as 125I-IgG. Degradation was essentially complete within 24 h. The lipid components of the erythrocyte membrane also appear to be degraded with similar kinetics (22).

**Selective Internalization of Fc Receptors**

To determine whether the endocytosis of IgG coated erythrocyte ghosts resulted in a selective internalization of FcR, we measured the amount of receptor and that of several other PM polypeptides remaining on the cell surface. Macrophages were rosetted with ElgG or IgG-coated ghosts at 0°C and then warmed to 37°C to initiate phagocytosis. At various times thereafter, triplicate cultures were placed on ice and PM antigens quantified by measuring the binding of a series of 125I-labeled anti-PM monoclonal antibodies (13). The results from these experiments are summarized in Fig. 5 (IgG-coated ghosts) and Fig. 6 (intact erythrocytes), which give the number of antibody binding sites in phagocytizing cells as a percentage of that measured in parallel control (nonrosetted) cells.

At time 0, macrophages rosetted (but not warmed) with IgG-

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**FIGURE 3** Degradation of 125I-antieythrocyte IgG ghosts after phagocytosis. Macrophages were plated on 35-mm dishes and rosetted with erythrocytes coated with 125I-IgG. After hypotonic lysis, cultures were warmed to 37°C and harvested at the indicated time intervals by scraping the monolayers into 4 ml of cold PBS and then collecting the cells by centrifugation (1,000 g, 10 min, 4°C). (A) Cell pellets were lysed in 0.5% NP-40 and analyzed by SDS PAGE under reducing conditions. IgG molecules were thus separated into heavy chains (50 kdaltons, 50K) and light chains (25 kdaltons, 25K). (B) Cell-associated TCA-precipitable 125I was also determined and expressed as the fraction of that measured in cultures that were not warmed after hypotonic lysis.
cultured overnight in 24-well plates, washed, and rosetted with and the cultures incubated at 37°C. At the indicated time intervals, expressed as a percent of that measured on control cells (i.e., the expression of several macrophage surface antigens was quantitated in parallel wells using 125I-labeled monoclonal antibodies (see Materials and Methods). Antibody binding on rosetted cells is expressed as a percent of that measured on control cells (i.e., cultures treated identically except that EIgG were not added at the initial rosetting step). Monoclonal antibody 2D2C (13) recognizes a 90-kdalton glycoprotein; 1.21J (13, 17) recognizes an oligomeric complex of two glycoproteins, 94 and 180 kdaltons; the major histocompatibility antigen H-2D\(^{d}\) (13, 18) was recognized by a monoclonal antibody designated B25-1. FcR was determined using 2.4G2 Fab (10). Identical results were obtained using macrophages cultured for only 1 h before rosetting.

| Hours at 37° after rosetting | 1 | 2 | 3 | 4 | 5 | 6 |
|------------------------------|---|---|---|---|---|---|
| Percent of nonrosetted control | 100 | 80 | 60 | 40 | 20 | 0 |

**FIGURE 5** Expression of macrophage plasma membrane antigens after the phagocytosis of IgG-coated ghosts. Macrophages were cultured overnight in 24-well plates, washed, and rosetted with ElgG. After rosetting at 0°C, erythrocytes were lysed hypotonically and the cultures incubated at 37°C. At the indicated time intervals, plates were washed three times with cold PBS, placed on ice, and the expression of several macrophage surface antigens was quantitated in parallel wells using 125I-labeled monoclonal antibodies (see Materials and Methods). Antibody binding on rosetted cells is expressed as a percent of that measured on control cells (i.e., cultures treated identically except that ElgG were not added at the initial rosetting step). Monoclonal antibody 2D2C (13) recognizes a 90-kdalton glycoprotein; 1.21J (13, 17) recognizes an oligomeric complex of two glycoproteins, 94 and 180 kdaltons; the major histocompatibility antigen H-2D\(^{d}\) (13, 18) was recognized by a monoclonal antibody designated B25-1. FcR was determined using 2.4G2 Fab (10). Identical results were obtained using macrophages cultured for only 1 h before rosetting.

| Hours at 37° after rosetting | 0 | 1 | 2 |
|------------------------------|---|---|---|
| Percent of nonrosetted control | 100 | 80 | 60 |

**FIGURE 6** Expression of macrophage plasma membrane antigens after the phagocytosis of intact IgG-coated erythrocytes. Experiments performed as described in legend to Fig. 5, except that rosetted erythrocytes were not lysed hypotonically before phagocytosis. Times after 3 h were not examined due to decreased macrophage viability (see text).

ghosts bound ~50% of the anti-Fc receptor antibody 2.4G2 Fab as did controls (Fig. 5). As discussed earlier, this indicates that at least half of the FcR on the cell surface were occupied by erythrocyte-bound IgG. The binding of three other antimacrophage monoclonal antibodies, 1.21J (which recognizes the oligomeric 180–kdalton and 94-kdalton subunits of glycoprotein, believed to be receptors for complement) (19), 2D2C (90-kdalton glycoprotein), and B25-1 (against H-2D\(^{d}\)), was not inhibited by the bound ghosts. After warming, the PM levels of each of these antigens decreased transiently by ~20%, including an additional 20% decrease in detectable FcR (2.4G2 Fab binding sites). These decreases were maximal at 15 min, at which time phagocytosis was complete (Fig. 2), and were restored to their time 0 values within 45 min. However, the amount of surface FcR remained significantly diminished relative to control cells for at least 24 h after warming.

Largely similar results were obtained using EIgG instead of IgG ghosts (Fig. 6). The one difference was that the additional 20% decrease in the number of surface FcR, and 2D2C and 1.21J antigens which occurred concomitant with phagocytosis was not reversible. Receptor number diminished to <40% of control where it remained for the duration of the experiment (3 h). No selective loss of FcR from the PM occurred during the phagocytosis of "nonspecifically" internalized particles such as 1-\(\mu\)m polystyrene latex beads or zymosan (not shown).

**Production of Polyclonal Rabbit anti-FcR Antibodies**

Since the erythrocytes and bound IgG were phagocytosed and degraded (Figs. 2–4), it seemed likely that the removal of receptors from the PM also resulted from the rapid degradation of internalized receptors. Therefore we compared the rates of FcR turnover by immunoprecipitation of receptor from lysates of radiolabeled cells that had, or had not, been exposed to IgG-erythrocyte ghosts. The anti-FcR monoclonal antibody 2.4G2 could not be used for this purpose since it is directed against a domain of the receptor at or near its ligand binding site (10, 12). Thus, intact ligand-receptor complexes would not be quantitatively detected since 2.4G2 binding is competitive with the binding of IgG immune complexes.

We circumvented this problem by producing an additional anti-FcR reagent raised against detergent-denatured, isolated receptor (see Materials and Methods). As shown in Fig. 7, the

**FIGURE 7** Characterization of a rabbit anti-mouse Fc receptor antiserum. **Left:** Immunoprecipitation of 125I-FcR from NP-40 lysates of LPO-iodinated J774 macrophages (13) using anti-FcR (lane 1), 2.4G2 Fab (lane 2), and preimmune rabbit serum (lane 3). The rabbit antiserum precipitates approximately two times more 125I than does 2.4G2 under the conditions used and competes for the same molecules recognized by the monoclonal (not shown). Two polypeptides (60 and 47 kdaltons, 60K and 47K) are recognized by both reagents. **Right:** Inhibition of ElgG rosetting by J774 cells (on coverslips) by 1:1,600 dilution of the rabbit antiserum (heat inactivated, 56°C, 30 min) (panel A). 1:40 dilution of preimmune rabbit serum did not detectably inhibit rosetting (panel A). X 460.
rabbit anti-FcR serum immunoprecipitated the same 60- and 47-kdalan glycoproteins from NP40 lysates of 125I-labeled J774 macrophages as did 2.4G2 Fab. Somewhat more 125I-receptor (approximately twofold) was precipitated by the polyclonal antibody than by 2.4G2; the receptor was not precipitated by preimmune sera. The antiserum was similar to 2.4G2 in its ability to inhibit the rosetting of ElgG by macrophages, even at high dilution (1:1,600) (Fig. 7). However, in addition to recognizing an antigenic domain of the receptor at or near its ligand binding site, the rabbit antiserum contained antigenic specificities for other portions of detergent-solubilized FcR. This was illustrated by the ability of the antiserum to mediate the immunoprecipitation of FcR from rosetted and nonrosetted 125I-labeled macrophages with indistinguishable efficiencies (averaged from seven separate experiments). Precipitation was not due to the displacement of bound IgG from the receptor since the antiserum has also been found to precipitate 125I-IgG immune complexes (3) and 125I-2.4G2 Fab (23, 24) when the latter were bound to unlabeled FcR in detergent solution.

**Fate of Internalized FcR**

We then employed the rabbit antiserum to determine the fate of FcRs selectively internalized during the phagocytosis of ElgG ghosts. Macrophages were iodinated at 4°C using LPO-GO, plated, and then the cells rosetted again at 4°C. At various time intervals after warming, cultures were harvested, lysed in NP40, and subjected to immune precipitation. An example of the immunoprecipitates of FcR from thiglycollate-elicted mouse peritoneal macrophages is shown in Fig. 8. In agreement with previous studies using 2.4G2 Fab (12), the rabbit antireceptor antiserum isolated a single, broad band from these cells with an average molecular weight of 47,000. For the turnover studies, radiolabeled bands were excised from dried gels and counted. This procedure was also carried out for three other unrelated membrane proteins for comparison (the antigens recognized by the monoclonal antiamacrophage antibodies 2D2C and 1.21J).

The data from eight experiments are combined in Fig. 9. In control cells, 125I-PM proteins were degraded according to uniphasic, log-linear kinetics. Turnover rates were relatively long (t½ = 18–23 h) for 2D2C (90-kdalan glycoprotein), 1.21Ja (180 kdaltons) and 1.21Jb (94 kdaltons) as well as for the FcR (t½ ~ 10 h). In cells that had bound and internalized IgG-coated ghosts, however, this situation was quite different. While there was little if any effect on the turnover of 2D2C, 1.21Ja, and 1.21Jb, the turnover of 125I-FcR now followed biphasic kinetics, with at least 50% of the labeled receptor being degraded at a greatly accelerated rate, t½ <2.0 h. The remainder of the iodinated receptor turned over at the control rate. The fraction of FcR that was rapidly degraded was equivalent to the fraction of FcR involved in the binding and internalization of the IgG-coated ghosts (Figs. 1 and 5).

To confirm that the uptake of IgG-coated ghosts did not induce major alterations in the turnover of other PM proteins, we analyzed the turnover of total iodinated PM polypeptides on one-dimensional SDS polycrylamide gels. Labeled membrane proteins in both control and phagocytizing cells were degraded at qualitatively similar rates (not shown). Moreover, it is unlikely that LPO-catalyzed iodination induced greatly aberrant rates of degradation since the turnover rate of membrane proteins (e.g., 2D2C) labeled metabolically with [35S]-methionine was similar (t½ = 19.5 h) to that obtained after radiiodination.

**DISCUSSION**

**Selective Internalization and Degradation of FcR**

In this paper we have demonstrated that the FcR-mediated phagocytosis of IgG-coated erythrocyte ghosts results in the selective and largely irreversible removal of receptors from the macrophage PM. This selectivity was manifested at three levels:

(a) Binding. The rosetting of ElgG at 0°C inhibited only the binding of the anti-FcR monoclonal antibody 2.4G2 Fab (Fig. 5). Each of the other macrophage PM antigens examined remained fully accessible to their respective 125I-1-monoclonal antibodies. Since 2.4G2 Fab competes with IgG for binding to FcR, the 50% reduction in the number of 125I-2.4G2 Fab binding sites on rosetted cells indicates that at least half of the macrophage's surface receptors were occupied by ligand. It seems likely that these occupied receptors were at least partially clustered at sites of ElgG attachment, given the multivalency of the particle and the ability of FcR to diffuse laterally in the plane of the membrane (25).

(b) Endocytosis. Concomitant with internalization, there is selective removal of FcR from the macrophage cell surface (Fig. 5). That intact receptor-ligand complexes were actually cleared from the PM is strongly suggested by the fact that the ligand (IgG) was immobilized on a large particle whose internalization was easily followed morphologically. The kinetics of receptor loss correlated temporally with IgG-coated ghost internalization; the extent of receptor loss correlated quantitatively with the number of receptors involved in particle binding at the PM. Thus, we presume that ligand binding caused the selective inclusion of FcR (relative to other PM antigens) in the membrane of the nascent phagocytic vacuole.

(c) Degradation. Finally, the turnover experiments demonstrated selectivity at the degradation step. IgG-coated ghost phagocytosis resulted in the rapid (t½ <2 h) degradation of ~50% of all PM-derived (surface iodinatable) FcR. In contrast, the rates of degradation of other, macrophage PM polypeptides were unaffected (Fig. 9). The fraction of rapidly degraded receptors must have been derived from the same 50% that were involved in the binding and/or uptake of the ghosts; and
accordingly the 50% of the receptors that turned over at the control rate (t_{1/2} \approx 10 \text{ h}) were presumably the ones that were not selectively internalized and/or bound to ligand. It is likely that the rapid phase of receptor degradation occurred in lysosomes because the ligand (IgG) and the erythrocyte ghost membrane proteins were degraded with similar kinetics (t_{1/2} \approx 2.5 \text{ h}) after internalization. Phagocytic vesicles are known to begin fusion with typical lysosomes within several minutes of entry (21).

In summary, the data indicate that FcR are clustered by ligand at the PM, selectively internalized with the particle, and degraded within phagolysosomes. It is clear that, at least in these respects, IgG-coated ghost phagocytosis differs from the "nonspecific" phagocytosis of particles such as polystyrene latex beads. Whereas latex uptake results in the internalization of a largely representative sample of PM proteins (26-28), FcR are neither selectively removed from the cell surface nor selectively enriched in phagocytic vacuole membrane (see Results and reference 28). Presumably, the phagocytosis of IgG-coated particles also involves the internalization of many PM polypeptides; however, by virtue of their interaction with ligand, FcR are preferentially internalized and degraded. Previous study (29) has indicated that the PM activity of another receptor, that for the third component of complement, is not diminished after FcR-mediated phagocytosis in a macrophage cell line. Insofar as the C3bi receptor is now thought to be recognized by the anti-"Mac-1" antibody 1.21J (19), the present results demonstrate directly and quantitatively that IgG-coated-ghost phagocytosis does not cause the concomitant removal and degradation of at least this complement receptor.

### Endocytosis and FcR Recycling

Several mechanisms may contribute to the rapid degradation of FcR during the phagocytosis of IgG-coated ghosts. First, since phagocytosis clearly involves the interiorization of PM FcR, it is conceivable that receptor internalization alone may trigger receptor degradation. However, this possibility does not seem tenable in view of our earlier observations, using LPO-catalyzed intracellular iodination (13), that the FcR—along with many other PM polypeptides—is normally included, albeit nonselectively, in PM internalized during pinocytosis. Macrophages continuously internalize two to three cell surface equivalents of PM as pinocytic vesicles per hour (13, 30); nevertheless, the FcR maintains a relatively long half-life. Moreover, the major iodinatable polypeptides of pinocytic vesicle membrane also exhibit long half-lives (t_{1/2} \approx 20 \text{ h}) similar to those of the corresponding proteins labeled on the cell surface (3). Thus, most PM proteins, presumably including the FcR, avoid degradation not by avoiding internalization but by continuously recycling to the cell surface. More direct evidence for FcR recycling has come from recent experiments that have demonstrated the rapid return to the PM of intact 2.4G2 Fab-FcR complexes after their internalization (31). Accordingly, it is clear that FcR internalization alone cannot account for the rapid degradation of receptors observed here.
Instead, degradation was a result of the internalization of FcR bound to specific ligand, a situation that in effect removes the receptor from its constitutive recycling pathway.

That phagocytosis of IgG-coated ghosts results in the internalization of a large segment of FcR-enriched membrane, however, may contribute to rapid receptor degradation. Incoming phagocytic vesicles apparently fuse with macrophage lysosomes shortly after internalization (see above). Consequently, a large fraction of the surface FcR pool would be delivered to a hydrolytic compartment rapidly and synchronously. The observed selective degradation of FcR might thus have been a consequence of the selective enrichment of receptors relative to other PM proteins in internalized membrane. A rapid phase in the degradation of other PM proteins may also have occurred, but was undetected (Fig. 9), since a much smaller fraction of the cell surface pool of these proteins was interiorized than in the case of the FcR.

A third possible explanation for the rapid degradation of FcR involves a direct effect of ligand on the intracellular fate of the receptor. Conceivably, IgG binding may increase the FcR's susceptibility to lysosomal proteases.

A fourth possibility is that the interiorization of FcR during IgG-coated-ghost phagocytosis alters the route or kinetics of recycling normally followed by receptors during pinocytosis. For example, phagocytosis may bypass a compartment, e.g., prelysosomal vacuoles or endosomes, from which recycling usually occurs. Alternatively, the presence of bound IgG and/or the clustering of receptor-ligand complexes may interfere with the inclusion of receptors into "recycling vesicles," even after lysosomal fusion. This may effectively increase the duration of exposure of FcR to lysosomal hydrolases relative to other membrane components. Consistent with this possibility are the observations of Muller et al. (27), which indicate that membrane recycling can occur from phagolysosomes. Intact FcR-IgG complexes could persist in these vacuoles since the receptor-ligand interaction is not sensitive to acidic pH (unpublished observations). POSSIBLY, portions of membrane enriched in receptors vesiculate into the lumen of the phagocytic vacuole to form multivesicular bodies before degradation.

Phagocytosis and Membrane Economy

Implicit in much of this discussion is the assumption that, unlike FcR, most PM proteins are free to recycle after IgG-coated-ghost phagocytosis. This assumption is supported by earlier studies that used intracellular iodination to show that this representative sample of PM polypeptides labeled at 0°C after the uptake of LPO-modified polystyrene latex beads returned to the cell surface upon reculture (27). While the present study has not directly addressed the recycling of these PM components, of possible interest is the small, transient decrease in the expression of PM antigens that occurred within the first 15 min after the internalization of IgG-coated ghosts (Fig. 5). Conceivably, the magnitude of this decrease reflects the area of membrane internalized. Similarly, its recovery may be representative of the recycling of much of this membrane as the size of the ghost-containing phagolysosome compartment decreases during the first hour after uptake (Fig. 2). Consistent with this possibility is the observation that this decrease is not rapidly recovered after the phagocytosis of intact erythrocytes (Fig. 6), which should be less "compressible" than erythrocyte ghosts.

Functions of Receptor Turnover

The ligand used in these studies was advantageous in that its internalization could be readily demonstrated and correlated with both the removal of FcR from the PM and receptor degradation. Thus, it is clear that the ligand-induced degradation of FcR occurred intracellularly. Conceivably, other receptors, which are removed from the cell surface after ligand binding, e.g., those for insulin (32) and epidermal growth factor (33), are also selectively degraded after endocytosis. Why should the macrophage FcR be subject to such metabolic control? Certainly, one of the major physiologic functions of macrophages is to serve as scavengers that clear (and/or kill) opsonized bacteria, viruses, and tumor cells from the circulation and interstitial spaces. It would seem advantageous for these cells to maintain maximal expression of surface FcR. However, these receptors may also have significant effector functions, especially in mediating inflammatory responses. For example, IgG binding has recently been found to generate the release of prostaglandins (7) and leukotrienes (6), both potent inflammatory agents. Thus, the type of regulation of FcR expression documented here may be important in controlling other aspects of macrophage function.

The authors thank Linda Herzman and Katherine Potter for assistance with the manuscript, and Pam Ossorio and Judy Adams for photographic work.

This work was supported by the following grants from the National Institutes of Health: RR05558 and GM29075 (to I. S. Mellman), AI13013 (to R. M. Steinman), AI14603 (to J. C. Unkeless), and A107012 (to Z. A. Cohn). R. M. Steinman is an Established Investigator of the American Heart Association. I. S. Mellman is a Junior Faculty Research awardee of the American Cancer Society and a recipient of a grant from the Sweobius Fund. J. C. Unkeless has a Faculty Research Award from the American Cancer Society.

Received for publication 23 August 1982, and in revised form 11 November 1982.

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