PHAGOCYTOSIS OF IMMUNE COMPLEXES BY MACROPHAGES

Different Roles of the Macrophage Receptor Sites for Complement (C3) and for Immunoglobulin (IgG)

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Immunological phagocytic recognition by monocytes and macrophages can be mediated by cell surface receptors for IgM, IgG, and a modified component of complement (C3) (1, 2). Two stages are involved: the attachment of a sensitized particle to the plasma membrane and its subsequent interiorization (3). The relative roles of the above immune factors in particle attachment and ingestion have not been adequately examined. In this paper we describe a quantitative technique which permits a separate evaluation of these two successive stages of phagocytosis. This technique was used to study the encounter of mouse peritoneal macrophages with sheep erythrocytes (E)[1] coated with either IgG (EA) or with IgG antibody and complement (EAC), and it enabled us to estimate the relative importance of complement and antibody in phagocytosis. We have obtained evidence that the C3 and Ig membrane receptor sites may play different roles in the attachment and ingestion phases of particle uptake.

Materials and Methods

Media.—Phosphate-buffered saline (PBS) was prepared as in reference 4. Dulbecco's modified Eagle's medium was obtained from Grand Island Biological Co., Grand Island, N. Y.

Animals.—Random-bred Swiss Webster female mice, 20–25 g body weight, were used as a source of serum and macrophages. New Zealand rabbits were used as donors of normal or of immune serum.

Erythrocytes.—Sheep red cells (E) were obtained from the Animal Blood Center, Syracuse, N. Y., and stored in Alsever's medium.

Antisera.—Rabbit antiserum to E was obtained after multiple intravenous injections of red cells washed in PBS. Agglutination tests were performed with 0.2% red cells and the titer of the serum was 1:256,000. A 7S fraction of a mouse antiserum to E was prepared as described

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[1] *Abbreviations used in this paper:* C, complement; E, sheep erythrocytes; EA, sheep erythrocytes sensitized with IgG antibody; EAC, sheep erythrocytes sensitized with IgG antibody and complement; PBS, phosphate-buffered saline.

in reference 5. Antiserum to mouse C3 was prepared in rabbits by the procedure described in reference 6. It precipitated mouse C3, but not mouse Ig, as shown by the immunoelectrophoretic analysis of mouse serum. The agglutination titer of the antiserum to mouse C3 was 1:50,000 when tested with E sensitized with antibody and mouse complement (C) (see method below). No agglutination with antiserum diluted 1:20 was observed with E sensitized with antibody alone. Purified mouse immunoglobulins (Ig) were given to us by Dr. I. Schenkein, and they contained γ2a, γ2b, and γ1 immunoglobulins. Rabbit antiserum to mouse Ig was prepared by injecting rabbits with purified mouse Ig. A total of 0.5 mg of protein was emulsified in 1 ml of complete Freund's adjuvant and injected into the four footpads of rabbits, which were bled from 6 to 8 wk later. The antiserum precipitated mouse Ig, but not mouse C3, as determined by immunoelectrophoretic analysis of mouse serum.

Rabbit IgG was obtained from normal or immune rabbit serum by three cycles of precipitation with $\frac{1}{3}$ saturated ammonium sulfate. The redissolved final precipitate was dialyzed against 0.02 M phosphate buffer, pH 7.5, and filtered through a column of diethylaminoethyl (DEAE)-cellulose previously equilibrated with the same buffer. Nonimmune IgG was absorbed three times with $\frac{1}{10}$ its volume of E at 4°C, reprecipitated with ammonium sulfate, and rechromatographed on DEAE-cellulose.

Papain fragments of rabbit IgG were prepared (7) by digestion for 6 hr with a $\frac{1}{100}$ enzyme (papain [twice crystallized], Mann Research Labs, New York) to substrate ratio. After digestion, the crystals of the Fc fragment were removed by centrifugation and the supernatant was filtered through a column of Sephadex G-200 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) equilibrated with saline. The protein eluted under the 7S peak, containing incompletely digested protein, was discarded. The peak tubes containing the 3.5S fraction were pooled, concentrated in an ultrafiltration cell (Amicon Corp., Lexington, Mass.), and frozen at −20°C. No contamination of this fraction with 7S antibody was detected. The 3.5S fraction of the IgG (0.6 mg/ml) isolated from the antiserum to C3 did not agglutinate EAC cells while the agglutinating titer of native IgG preparation was $\frac{1}{5,000}$ when tested at the same protein concentration.

Labeling of IgG with ^{125}I.—The procedure used was that of McFarlane (8) as described in reference 9. Iodine monochloride was added to a concentration necessary to obtain 1 iodine atom/IgG molecule. After iodination the protein solution was extensively dialyzed against saline and chromatographed on a column of Sephadex G-200 equilibrated with PBS. No aggregation of the protein was observed and the specific activities of the fractions were almost identical (8.100 cpm/mg of protein).

^{51}Cr Labeling of Sheep Erythrocytes.—The procedure used was similar to that described in reference 10. 200–500 μCi of $Na_2\ ^{51}Cr\ O_4$ were added per milliliter of 10% sheep red cell suspension in Alsever's solution and the mixture was incubated for 1 hr at 37°C with occasional agitation. The cells were washed four times with PBS at 4°C and the red cell concentration determined after hemolysis by absorbancy at 415 nm.

Treatment of E with Antibody or with Antibody and Complement.—The red cells in PBS ($8 \times 10^4/\mu$l) were incubated with IgG solutions at the final concentrations given in the Results section. The mixtures were incubated at 30°C for 1 hr and the E were washed in PBS four times by centrifugation at 4°C. The final red cell pellet (EA) was suspended in Dulbecco's medium. To one aliquot of the EA suspension, fresh mouse serum (which is very poorly lytic) was added as the source of complement. The mouse serum was previously absorbed with 1/10 vol of packed E for 10 min at 0°C and added to the EA to a final dilution of 1/15. After 30 min at 37°C, the red cells (EAC) were washed twice with Dulbecco's medium by centrifugation at 4°C and resuspended to a concentration of $7 \times 10^4/\mu$l.

Procedure for the Determination of Attached or Ingested E-^{51}Cr.—Macrophages were obtained from normal mice after sacrifice with chloroform. 2.5 ml of PBS were injected into the peritoneal cavity and the washings were removed with a Pasteur pipette. 0.1 ml of the washings, containing about 7×10^4 macrophages, were layered on 9 × 35 mm cover slips which had

been previously treated with a 1% solution of bovine serum albumin in distilled water for 30 min, washed with water, and dried. Coating of the cover slips with albumin was found to reduce the nonspecific binding of erythrocytes to glass.

Macrophages were allowed to attach to the cover slips for 10 min at room temperature in a moist atmosphere and were gently rinsed in PBS. The cover slips were drained and overlaid with 0.1 ml of the red cell suspensions containing 7×10^6 red cells. Macrophages and red cells were incubated for 1 hr at 37°C in a 10% CO_2-air atmosphere saturated with water vapor.

Preliminary experiments showed that higher red cell concentrations did not appreciably increase the binding of ^{51}Cr-labeled E. Attachment and ingestion were separately measured by the following procedure. The macrophage monolayers were thoroughly washed in PBS and incubated for 45 sec in 1 ml of PBS diluted $\frac{1}{5}$ with water to effect lysis of bound but uningested E; after another rinse in PBS, the cover slips were transferred to tubes containing 1 ml of 3% acetic acid. Radioactivity in the hypotonic lysates was a measure of red cells bound to the surface of macrophages; radioactivity that remained associated with the cover slips was a measure of the red cells ingested by the phagocytes. Radioactivity measurements were made in a gamma counter.

Because no direct determination was made of the number of macrophages that attached to the cover slips, the radioactivity determinations provide relative values of binding and ingestion of red cells and permit comparison only within a particular experiment. For this reason results are expressed as counts per minute and not as average numbers of erythrocytes attached to or ingested by macrophages. It should also be emphasized that the technique used does not measure rates of binding and ingestion but only the number of red cells taken-up after 1 hr. Most determinations were performed in triplicate. The reproducibility of the method was estimated by inspection of replicate determinations. Their values were usually within ±15% of the mean. In several experiments the cover slips were monitored under the phase-contrast microscope. These evaluations showed that (*a*) the binding of E to glass was negligible; (*b*) the hypotonic treatment led to complete lysis of the E attached to the surfaces of macrophages while ingested E could be visualized under the microscope; and (*c*) the binding and ingestion of E as visually assessed were consistent within the same experiment with the ^{51}Cr counts obtained.

RESULTS

Binding and Ingestion of EA as a Function of the Average Number IgG Molecules per Erythrocyte.—The following experiments were performed in order to study the interaction of E with macrophages as a function of the degree of sensitization of the red cells with IgG antibodies.

(*a*) The average number of IgG anti-E antibody bound to erythrocytes was estimated as follows: IgG purified from the serum of rabbits immunized with E was labeled with ^{125}I. E were treated with several different concentrations of the labeled IgG, washed by centrifugation, and the number of counts per minute determined. As the specific activity of the iodinated IgG was known, it was possible to determine the number of IgG molecules bound as a function of the concentration of IgG, assuming that the specific activities of anti-E IgG and of nonspecific IgG were identical. When the concentration of labeled IgG in the incubation mixture was plotted against the number of molecules of IgG bound per erythrocyte, a linear relationship was obtained with no evidence of saturation of the sites for antibody on the membrane of the erythrocyte (Fig. 1). In order to estimate the amount of nonspecific IgG which could have bound to the

erythrocytes, IgG was isolated from normal rabbit serum, absorbed with $\frac{1}{10}$ of its volume of packed washed E, and iodinated with ^{125}I. E were treated with different concentrations (0.75, 1.5, and 2.5 $\mu g/\mu l$) of labeled IgG, washed, and counted. Counts were less than 1% of those obtained when specific IgG was used. In addition, we found that up to a 100-fold of normal, unlabeled IgG could be added to the labeled specific IgG without affecting the uptake of radioactivity by the erythrocytes. Thus, it appears that most of the counts remaining on the erythrocytes after incubation with ^{125}IgG from an antiserum to E were due to the specific binding of antibodies to the red cells.

(*b*) In most of the experiments to be reported below, we used only unlabeled antibodies to sensitize E. This was done in order to avoid changes in the Fc por-

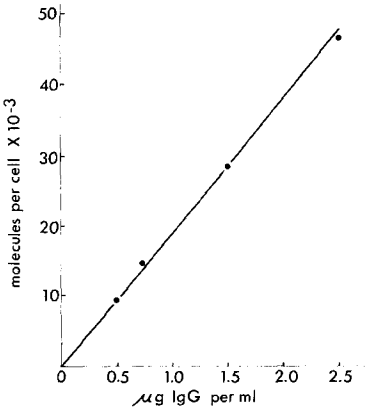

FIG. 1. Average number of ^{125}I-labeled IgG molecules bound to E as a function of the concentration of IgG. IgG was purified from the serum of a rabbit immunized with E. Sensitized cells were subsequently washed three times by centrifugation and counted. Each point in the graph represents the means of triplicates.

tion of the IgG which might be induced by the labeling procedure, and which could alter the interaction with the receptor for Fc on the macrophages. In this case, the number of unlabeled IgG molecules attached to the red cells was assumed to be identical to that obtained with equimolar concentrations of labeled IgG. The validity of this assumption was supported by the observation that labeled and unlabeled IgG had the same hemagglutination titers for E. In addition, the expected binding of labeled IgG antibody to E in the region of large antibody excess was obtained in the presence of different and increasing concentrations of unlabeled specific IgG, as shown in the experiments summarized in Fig. 2. Separate aliquots of E were incubated with equimolar concentrations of antibodies which contained labeled and unlabeled IgG in varying ratios, and after extensive washing of the erythrocytes, the number of counts bound was measured. A constant proportion of labeled molecules was bound to the E in the presence of varying concentrations of unlabeled antibody molecules (Fig. 2),

which indicates that the combining sites of the immunoglobulin had not been altered by the labeling procedure.[2]

(*c*) In order to study phagocytosis of erythrocytes as a function of the number of specific IgG molecules bound, separate suspensions of E were sensitized with increasing concentrations of unlabeled IgG antibodies. For these experiments we used E which had been previously labeled with ^{51}Cr. The ^{51}Cr-labeled erythrocytes were incubated with monolayers of mouse peritoneal macrophages attached to cover slips, and then were treated as described in the Materials

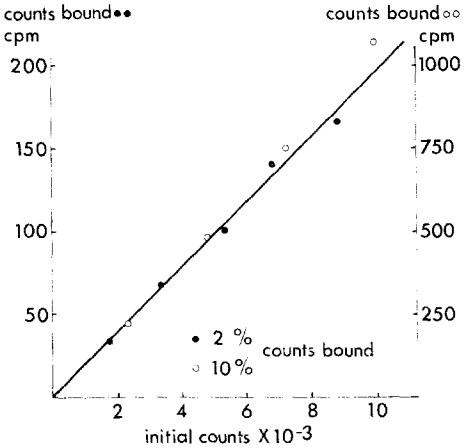

FIG. 2. ^{125}I-labeled IgG molecules bound to E after incubation with equimolar concentrations of specific IgG containing ^{125}I-labeled and unlabeled molecules in varying ratios. IgG was purified from the serum of a rabbit immunized with E and an aliquot was labeled with ^{125}I. Mixtures of labeled and unlabeled IgG were prepared and incubated with E. Sensitized cells were subsequently washed by centrifugation and counted. Only the amounts of ^{125}I-labeled IgG in the mixtures are ploted in the abscissa, but in all points the total concentration of IgG (labeled and unlabeled) was the same. Two experiments were performed using different concentrations of E. In one, 2% of the counts were bound and in the other 10% were bound. In each experiment, the ratio of labeled/unlabeled molecules varied widely but a constant proportion of ^{125}I molecules bound to E.

and Methods section for the evaluation of the attachment and ingestion of red cells. Fig. 3 summarizes the results of one such experiment and shows that interaction between the mononuclear phagocytes and E requires that 10^3–10^4 IgG molecules are bound to E. As the number of IgG molecules bound per red cell increases, ingestion also increases, but no plateau was reached with up to 40×10^3 IgG molecules per red cell. These results were confirmed in two other separate experiments.

[2] As these experiments were performed in a region of large antibody excess, the maximum number of 7S antibody molecules which could bind to each red cell could be determined. The figure obtained was 6×10^5, which coincides with that reported by Humphrey (11).

(*d*) During the incubation with macrophages only a fraction of the EA were taken-up, and therefore it could be argued that those EA which did interact had been sensitized with above average numbers of antibody molecules. In order to exclude this possibility, the number of IgG molecules coating macrophage-bound erythrocytes was determined and compared with the number of IgG molecules bound to the bulk of the red cell suspension. In these experiments

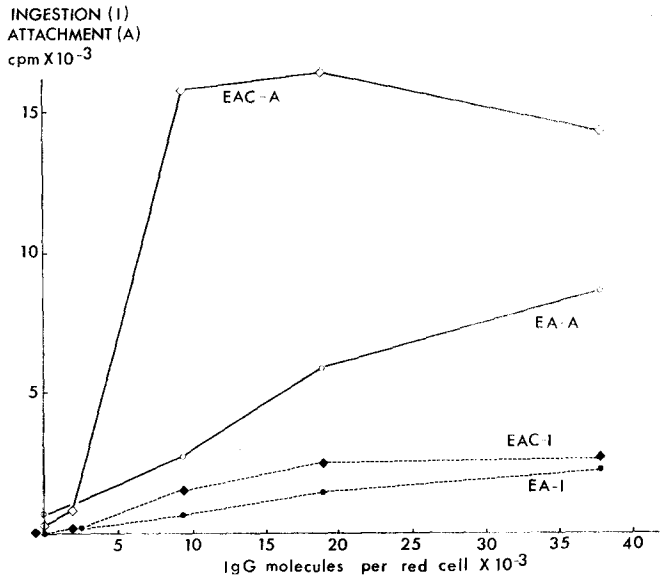

FIG. 3. Attachment and ingestion of erythrocytes as a function of the number of specific IgG molecules bound. Separate suspensions of ^{51}Cr-labeled E were incubated with increasing concentrations of unlabeled/labeled IgG antibodies, washed three times by centrifugation, and overlayed on monolayers of mouse peritoneal macrophages. Separate aliquots of the sensitized erythrocytes were incubated with fresh mouse serum (diluted 1/15) as a source of complement, washed three times, and incubated with the macrophages. Attachment and ingestion of red cells to macrophages were measured as described in the Materials and Methods section. Each point in the graph represents the means of duplicates.

A, attachment; I, ingestion.

^{51}Cr-labeled E were sensitized with ^{125}I-labeled IgG antibodies (1 μg/ml) and then were incubated with monolayers of macrophages. In eight such determinations, the average number of IgG molecules per red cell in the initial preparation of EA and on the macrophage-bound EA were not significantly different. These results discount the possibility that the macrophages selectively bound erythrocytes coated with the highest numbers of IgG molecules.

The Effect of Complement.—In Fig. 3, the interactions of macrophages with EA and EAC are compared. At low concentrations of IgG per cell, i.e. about 10^4 molecules per erythrocyte, complement markedly enhanced both attach-

ment and ingestion, with a greater effect on the attachment step. However, when the amount of IgG antibodies to E is increased a plateau is rapidly reached. This plateau could be due to the saturation of the macrophage surface by EAC. Indeed, microscope observations showed that virtually 100% of the phagocytic cells were covered with erythrocytes. Alternatively, the plateau could be due to a limiting number of effective C sites being generated on the red cell membrane under the conditions of sensitization. The actual number of effective C sites on the membrane in the various EAC preparations was

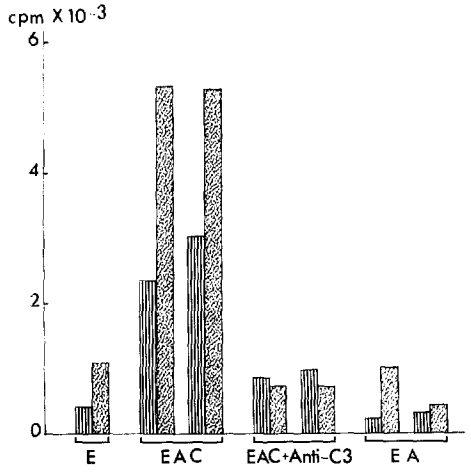

FIG. 4. Inhibitory effect of papain fragments of rabbit IgG anti-mouse C3 on the binding and ingestion of EAC by mouse peritoneal macrophages. The results of two separate experiments are plotted. Aliquots of E, EA, and EAC, prepared with ^{51}Cr-labeled E, were incubated with monolayers of mouse peritoneal macrophages in the presence or absence of papain fragments of IgG from a rabbit antiserum to mouse C3. Attachment (stippled bars) and ingestion (striped bars) of red cells were measured as described in the Materials and Methods section. The concentration of papain fragments was 200 μg/ml. The same concentration of papain fragments of normal rabbit IgG had no effect on ingestion or attachment of EAC. Also, papain fragments of the IgG anti-C3 did not inhibit the binding or ingestion of EA. Bars represent means of duplicate determinations.

not determined. However, the importance of membrane-bound C3 in the attachment of EAC to the macrophages was demonstrated in the experiments illustrated in Fig. 4. Papain fragments of rabbit IgG antibodies to mouse C3 were incubated with EAC for 1 hr at room temperature, and the mixture was applied to macrophage monolayers. Papain fragments and not intact IgG were used in order to avoid both agglutination of EAC and the opsonizing contribution of the Fc portion of IgG anti-C3. It can be seen that anti-C3 antibodies markedly inhibited both attachment and ingestion of EAC by the macrophages. However, the same preparation of the antibodies to mouse C3 had no significant effect on either attachment or ingestion of EA. Control experiments

showed that the same concentration of papain fragments obtained from non-immune IgG had no effect on the binding and ingestion of EAC. In addition, anti-C3 antibodies did not inhibit the binding of EA to macrophages.

Different Roles of C3 and IgG in the Interaction of EAC with Macrophages.—The following experiments were performed with mouse antibodies to E (7S fraction). As shown in Fig. 5, very low concentrations of papain fragments of IgG from a rabbit antiserum to mouse Ig inhibit the binding and ingestion of EA to the macrophages. For example, when 2 μg/ml of fragments were present in

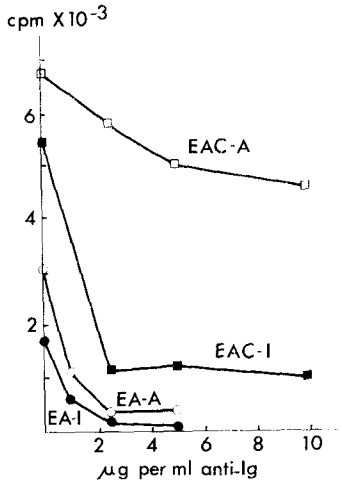

FIG. 5. Inhibitory effect of papain fragments of rabbit IgG anti-mouse Ig on the binding and ingestion of EA and EAC by mouse peritoneal macrophages. Separate suspensions of ^{51}Cr-labeled E were incubated with unlabeled mouse IgG anti-E (4 μg/ml). The red cells were washed three times by centrifugation and incubated with monolayers of mouse peritoneal macrophages in the presence of increasing concentrations of papain fragments of rabbit IgG anti-mouse Ig. Separate aliquots of sensitized erythrocytes were incubated with fresh mouse serum (diluted 1/15) as a source of complement, washed three times, and incubated in the same way with other cover slips containing attached macrophages. The ingestion and attachment of E to macrophages were measured as described in the Materials and Methods section. Each point in the graph represents the means of duplicates.

the incubation medium, the binding of EA (prepared with 4.0 μg of antibody) declined to background levels. This suggests that under these conditions no contact, or ineffective contacts, are made between the macrophage receptor and the Fc portion of the mouse antibodies to E. This observation provided the basis for the experiments described below, and summarized in Fig. 5, in which we tried to discriminate between the roles of macrophage receptors for C3 and for antibody in the successive steps of phagocytosis. Samples of ^{51}Cr-labeled EAC (prepared with 4 μg/ml of mouse antibodies to E and a 1/15 dilution of fresh mouse serum as a source of complement) were incubated with increasing concentrations of papain fragments of rabbit antibodies to mouse IgG for 1 hr

at room temperature and the mixtures were then applied to a series of macrophage monolayers. These were then treated in the standard way in order to measure binding and ingestion of erythrocytes. With small doses of antibody to Ig there was a clear dissociation between the effects on attachment *versus* ingestion; the attachment was slightly affected, while ingestion was markedly reduced. This finding, confirmed in two other separate experiments, contrasts with many previous observations which have consistently showed that a large proportion of the EAC cells which attach to macrophages are subsequently ingested. Control experiments showed that papain fragments of normal rabbit IgG had no effect on the binding and ingestion of EAC.

DISCUSSION

In the present paper, we studied the roles of the C3 and IgG receptors of the membrane of macrophages during the uptake of sensitized particles. Monolayers of peritoneal macrophages were overlaid with E, EA, or EAC prepared with mouse (or rabbit) antibody against E and fresh mouse serum as a source of complement. A method was developed which allowed the separate measurement of attachment and ingestion of the erythrocytes. The new significant observations which were made can be summarized as follows.

(*a*) The binding and ingestion of EA by macrophages depends on the number of IgG molecules present on the membrane of the erythrocyte. The minimum number for detectable interaction is 10^3–10^4 molecules of antibody bound per red cell (Fig. 3). The erythrocytes could maximally accomodate about 6×10^5 molecules of rabbit IgG antibody molecules as determined from the experiments shown in Fig. 2.[2] Thus, only a small percentage of the sites available on the red cell have to be occupied in order to establish effective contact with a macrophage. The high sensitivity of opsonization is probably a reflection of the large number of receptor sites on the macrophage membrane, which was recently estimated as 2×10^6 per cell (12). In addition, in our experiments, the likelihood of an encounter between the macrophage receptor site and the IgG bound to the red cell membrane was probably increased by the prolonged contact between the cells and by the mobility of the macrophage plasma membrane infoldings (13). The only previous estimate of the number of IgG molecules needed for opsonization is that of Huber et al. (14) and the figures obtained were of the same order of magnitude as ours, despite the fact that their system was different (human monocytes and Rh-positive erythrocytes sensitized with anti-Rh antibodies).

b) The presence of membrane-bound C3 on Ig-sensitized erythrocytes enhances their interaction with macrophages. Interestingly, at low doses of IgG, the C3-mediated increase in interaction was mainly the result of an enhanced attachment of the erythrocytes to the macrophages. As shown in Fig. 3, at 10^4 molecules of IgG per red cell, binding of EAC was sixfold higher but ingestion only 2.4 times higher than that of EA. The important role of C3 for the attachment of EAC to macrophages is suggested by the marked inhibitory effect

exerted by antibodies to C3 (Fig. 4). This finding is in agreement with previous observations that EAC1423, but not EAC 142, binds to macrophages (15) and that preincubation of EAC with a serum enzyme which inactivates membrane-bound C3b destroys the ability of the red cells to interact with leukocytes (16, 17).

The present data do not bear on the binding affinities of the macrophage receptors for IgG and C3 and the respective target molecules since the increased "stickiness" of EAC when compared to EA may be a reflection of the very large number of membrane-bound C3 sites which can be generated around antigen-IgG sites (18, 19). It must be pointed out that these experiments were performed in tissue culture media in the absence of serum proteins. If normal IgG were present in the medium, as would be expected under physiological conditions, and as shown by many investigators (1, 2, 20), the normal IgG molecules would effectively compete with the specific antibody for the Fc receptor on the macrophages. Under these circumstances the macrophage receptor for C3 would be expected to play a decisive role in the binding of immune complexes, since this receptor binds configurations of C3 which are revealed only after it is enzymatically split and membrane bound (16, 17, 20).

(*c*) Evidence was obtained suggesting that the receptors for IgG and C3 on the macrophage membrane may not have the same function during phagocytosis, and that the C3 sites on the immune complexes are primarily involved in their attachment to the macrophage while the ingestion phase may depend mainly on the cytophilic receptor. This hypothesis is based on the results of two sets of experiments. (*1*) The addition of complement to EA enhanced markedly the attachment of erythrocytes to macrophages, particularly when small doses of specific IgG were used (Fig. 3). Ingestion was also enhanced but to a smaller degree and this may simply mean that more EAC than EA were available on the macrophage surface when the process of interiorization took place. (*2*) If antibodies directed against IgG were present in the incubation medium before EAC entered in contact with macrophages, ingestion, but not attachment of the red cells, was markedly affected (Fig. 5). In this situation, and with a relatively low concentration of antibodies to IgG, it was possible to decrease fivefold the ingestion of EAC as compared to controls and only affect to a small degree the binding of the red cells.

The hypothesis that the macrophage membrane receptors for IgG and C3 have different roles during phagocytosis is supported by some previous observations. Thus, Rabinovitch (21) found that IgG, but not IgM, stimulated the ingestion of glutaraldehyde-treated erythrocytes which were bound to the membrane of mouse peritoneal macrophages. Lay and Nussenzweig (5) have reported that erythrocytes sensitized with 19S antibody were less readily ingested by macrophages than red cells sensitized with IgG antibody. Also, erythrocytes sensitized with cold agglutinins (IgM) and complement were observed to attach to human monocytes but little interiorization was observed. Similar results were obtained with erythrocytes coated with complement by nonimmunological

means (2).[3] In addition it may be pertinent that receptors for IgG and C3 were also found among neutrophils which are very efficient phagocytes, while only receptors for C3, and not for IgG, have been detected on certain lymphocytes (20, 22) which can bind but not ingest immune complexes.

We think that the presence on leukocytes of receptors specialized for attachment or for ingestion of immune complexes may be relevant to the mechanism of phagocytosis and may provide an explanation for some contradictory findings about the role of macrophages in the immune response. Thus, our results support the idea that complement components may play a more important role than IgG antibodies in opsonization in vivo since in most circumstances this must take place in the presence of normal Ig, which competes with antibody for the receptors for Fc on macrophages (1, 2). Accordingly, the first contact between a phagocyte and the immune complex is perhaps made through the C3 receptor and the IgG receptor would be of primary importance only for interiorization. As shown by Muller-Eberhard et al. (18), through the action of one C(42)a site several hundred molecules of C3 can bind to E and one C(42)a site may be created by only two closely placed IgG antibody molecules (19). This amplification mechanism should increase the effectiveness of recognition by leukocytes of an antibody-sensitized site on a particle or cell, and perhaps play a primary role in resistance to infection. Because complement activity is characterized in many of its steps by proteolysis and exposure of new configurations on the complement components, it can generate specific signals to induce leukocyte activity even in the presence of native complement molecules in the serum or other fluids. Therefore, it appears that the C3 receptor is particularly well fitted for the recognition of antigen-antibody interactions, and this may be the reason why it is so widely distributed among leukocytes of many mammalian species.

Finally, our results suggest that the ratio of immune complexes bound/immune complexes ingested by macrophages may depend on the class of antibody in the complexes. If, for example, IgM is the predominant species, immune complexes would tend to remain exteriorized on the macrophage membrane, and thus be in a position adequate for the stimulation of immunocompetent cells (23). This would provide a rational basis for the observation that 19S antibodies very effectively enhance the immune response (24, 25). On the other hand, when 7S antibodies predominate, as occurs during the secondary immune response, macrophages might function mainly as scavenger cells, with effective ingestion and breakdown of the immune complexes inside phagocytic vacuoles (26).

SUMMARY

Sheep red cells (E) sensitized with IgG antibody (EA) or with antibody and complement (EAC) interact in vitro with mouse peritoneal macrophage monolayers. The role of IgG and of C3 in the attachment and ingestion of the erythrocytes was examined by means of quantitative technique utilizing ^{51}Cr-labeled

[3] Lay, W. H., and V. Nussenzweig. Unpublished observation.

E. Controlled osmotic lysis permitted the separate measurement of the radioactivity associated with bound or with ingested E. IgG-^{125}I was used to estimate the number of IgG molecules bound per E as a function of the IgG concentration. Control experiments showed that iodination did not influence the extent of binding of IgG to E and that the binding of IgG prepared from immune serum could be essentially ascribed to its anti-E antibody content. Only between 10^3 and 10^4 rabbit anti-E IgG molecules per erythrocyte were needed for detectable attachment and ingestion of EA (a maximum number of 6×10^5 IgG antibody molecules could be accomodated on one erythrocyte).

Evidence was obtained that C3 is primarily involved in particle attachment, whereas only IgG is able to markedly promote the ingestion of particles attached to macrophages: (*a*) Addition of complement to the EA substantially increased the binding to the macrophages, whereas ingestion was increased to a smaller extent. Both binding and ingestion of EAC were markedly inhibited by papain fragments of IgG obtained from a rabbit antiserum to mouse C3. (*b*) Low doses (2 μg/ml) of papain fragments of IgG from a rabbit antiserum to mouse IgG markedly reduced the ingestion of EAC, whereas attachment of EAC to macrophages was inhibited to a much smaller degree.

The possible relevance of these findings for the in vivo fate of particulate immune complexes as they interact with macrophages is discussed. It is suggested that in the primary immune response, when the complexes are predominantly in the form of EA (IgM) or EA (IgM) C3, they would tend to remain on the surface of the macrophages and thus be in a position to stimulate immunocompetent cells. In the secondary response, when EA (IgG) or EA (IgG) C3 predominate, the complexes would tend to be more rapidly interiorized and degraded by the mononuclear phagocytes.

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