A NEW Fc RECEPTOR ON MOUSE MACROPHAGES
BINDING IgG₃*

BY BETTY DIAMOND AND DALE E. YELTON‡

From the Departments of Microbiology and Immunology and Medicine, and of Cell Biology, Albert Einstein College of Medicine, Bronx, New York 10461

Among the effector functions exhibited by immunoglobulins is the ability to bind to Fc receptors on macrophages, lymphocytes, and granulocytes (1-10). It has recently become evident that there are different types of Fc receptors on different types of cells (11) and, moreover, that the same cell may express more than one type of Fc receptor (1, 2, 5). For example, mouse macrophage Fc receptors do not bind IgM, whereas some lymphocyte Fc receptors do (7). Mouse macrophages are known to express at least two different Fc receptors, one specifically binding mouse IgG₁ and IgG₂b, the other binding IgG₂a (12, 13). The Fc receptors are distinguishable by certain other properties as well. The IgG₂a receptor is sensitive to trypsin and shows decreased binding at 4°C and in the presence of cytochalasin B; the IgG₁-IgG₂b receptor is trypsin resistant and unaltered at 4°C by cytochalasin B (1, 13).

The IgG₂ class of mouse immunoglobulins has not been studied extensively with respect to its binding to Fc receptors. IgG₂ has an Fc fragment that is serologically and structurally different from IgG₁ and IgG₂ (14). Functionally, it has been shown to have an increased ability to cross the placenta (14). Earlier experiments have shown that monomeric IgG₂ does not inhibit the binding of monomeric IgG₂a to its Fc receptor (14). These and other early results have led to the assumption that IgG₂ does not bind to Fc receptors. In light of the evidence for multiple Fc receptors, we felt it necessary to examine again the binding of IgG₃ to Fc receptors.

We generated a monoclonal IgG₂ anti-sheep erythrocyte (SRBC) antibody from a mouse spleen-mouse myeloma fusion in order to study directly the binding of IgG₂ to macrophage Fc receptors. Monoclonal antibodies are especially useful in such studies because they are homogeneous, they are the only mouse immunoglobulin in culture medium from the hybridoma cultures, and when reacted with their antigen, they form antigen-antibody complexes more natural for binding studies than artificial aggregates. Using this monoclonal IgG₂ anti-SRBC antibody, we found that IgG₂ does bind to macrophages through a third, independent IgG Fc receptor.

Materials and Methods

Cells. J774 is a reticulum cell sarcoma from a BALB/c mouse with macrophagelike properties that has been adapted to culture (15). J774.2 is a clone from the tissue culture line.

* Supported by grants AI16116, AI10702, AI13811, AI5231, and CA24300 from the National Institutes of Health, grant PCM77-25635 from the National Science Foundation, and grants IM-216 from the American Cancer Society.

‡ Medical scientist trainee supported by grant ST32GMF288 from the National Institutes of Health.

Abbreviations used in this paper: BDB, bis-diazotized benzidine; PBS, phosphate-buffered saline; SRBC, sheep erythrocytes.

514 J. Exp. Med. ©The Rockefeller University Press • 0022-1007/81/03/0514/06 $1.00
Volume 153 March 1981 514-519
A series of subclones of J774.2 were picked and screened for their ability to bind IgG. J774.2.1 was found to lack IgG receptors. It has grown to mass culture and recloned to produce J774.2.1.4. P388D1 is a macrophagelike cell line with Fc receptors and phagocytic ability. FC1.4 and FC1.6 are subclones of the FC1 cell line which arose during a fusion of MPC 11 myeloma cells to spleen cells from a BALB/c mouse immunized with SRBC. FC1.4 and FC1.6 were selected for their inability to phagocytize IgG~a-SRBC.

Primary macrophages were obtained from resident peritoneal cells and from peritoneal cells 4 d after an intraperitoneal injection of thioglycolate broth (Difco Laboratories, Detroit, Mich.). The method for isolating adherent cells has been described previously (1).

Myeloma Proteins. Myeloma proteins were obtained by injecting 10^7 MOPC21 (IgG1), MPC11 (IgG3), MOPC173 (IgG2a), or J606 (IgG3) cells into the peritoneal cavity of pristine-primed BALB/c mice (16). The ascites fluid was precipitated with 50% saturated ammonium sulfate. A purified γ-globulin fraction was obtained by DEAE chromatography (1). It was determined by agarose gel electrophoresis and by Ouchterlony analysis that no fraction had any contaminating protein of another subclass. Flopc 21 was purchased from Bionetics, Kensington, Md. Monomeric protein was prepared by centrifuging the protein for 30 min at 150,000 g.

Preparation of Ig-coated SRBC. The methods of Bianco et al. (17) were used. SRBC were incubated with antibody for 30 min at 37°C, washed, and resuspended to 0.5%. The antibodies were obtained from cloned hybridoma lines making anti-SRBC antibody. 5 μl of ascites fluid was incubated with 1 ml of a 5% solution of SRBC.

The IgG2-producing line was derived from a fusion of drug-marked P3 cells with spleen cells from a BALB/c mouse immunized with SRBC. Segregants no longer making the myeloma IgG1 heavy chain were selected. The IgG2 antibody was identified by Ouchterlony analysis with commercial anti-IgG3 (Meloy Laboratories, Inc., Springfield, Va.) and with antiserum raised in the laboratory of Dr. John Cebra, University of Pennsylvania, against J606 protein and absorbed with IgG1, IgG3a, and IgG2b.

Fc Rosettes and Fc-mediated Phagocytosis. This was done as previously described (1). Cells adhered to glass coverslips were incubated for 30 min at 37°C, washed, and assayed for rosettes. Attachment of three or more SRBC signified a rosette. For phagocytosis, the cells were incubated for 1 h at 37°C, free SRBC were lysed in hypotonic solution, and intracellular SRBC were assayed. A phagocytic cell was any cell with three or more ingested SRBC. Controls were run with SRBC incubated in normal mouse serum, and <5% of cells rosetted or phagocytized. To study the effect of trypsin and cytochalasin, macrophages were incubated in 1 mg/ml of crystallized trypsin in phosphate-buffered saline (PBS), or 10 μg/ml of cytochalasin B (Aldrich Chemical Co., Inc., Milwaukee, Wis.) in PBS for 30 min at 37°C, washed, and assayed for rosetting ability.

Inhibition of Binding by Myeloma Proteins. MPC11 (IgG2a), MOPC173 (IgG2a), MOPC21 (IgG1), J606 (IgG3), or Flopc 21 (IgG3) protein was aggregated with bis-diazotized benzidine (BDB) (18). Macrophages were pretreated for 5 min with the myeloma protein in PBS and then Ig-SRBC were added. Rosettes were assayed at 20 min.

Inhibition of Binding by Protein A. 20 μl of a 5% solution of SRBC were incubated with 50 μl of protein A at 5 mg/ml and 200 μl of either IgG1, IgG2a, IgG2b, or IgG3 anti-SRBC antibody. The titer of the anti-SRBC antibody in each case was 1:500 using indirect hemagglutination with an anti-κ antibody. The SRBC were washed and assayed for rosette formation.

Results

IgG3-coated SRBC formed rosettes with primary resident and thioglycolate-induced peritoneal macrophages, and with J774.2 and P388D1 cell lines (Table I).

In competitive inhibition experiments (Tables I and II), aggregates of IgG1, IgG2a, and IgG2b all failed to inhibit rosetting of IgG3-coated SRBC, suggesting a separate receptor for IgG3. In contrast, two IgG3 myeloma proteins, Flopc 21 and J606, did inhibit rosetting when aggregated chemically with BDB. These results imply that the
IgG₃ antibody is binding to the macrophage through its Fc terminal. Monomeric IgG₃ did not inhibit rosetting.

The rosetting of IgG₃-SRBC was unaffected at 4°C, by cytochalasin B, or by trypsinization of the macrophages. In this respect, the IgG₃ receptor behaves like the receptor for IgG₁ and IgG₂b and unlike the receptor for IgG₂a, which is altered at 4°C, and by both cytochalasin and trypsin.

Although inhibition of rosetting by aggregated IgG₃ suggested that the binding of antibody-coated SRBC was Fc mediated, we wanted to show by a second method that IgG₃ was bound to receptors on macrophages through its Fc portion. We therefore examined whether protein A could inhibit the binding of IgG₃. Protein A will bind to the Fc portion of several immunoglobulins, including IgG₃ and IgG₂. It binds poorly to IgG₁ (19). It has been further shown that protein A will compete with Fc receptors for Fc binding (20). When protein A was incubated with IgG₃-coated SRBC, it inhibited rosette formation. As expected, it also inhibited rosetting of IgG₂-coated SRBC and not of IgG₁-coated SRBC (Table III).

J774.2.1.4 is a randomly picked subclone of J774.2. It did not rosette IgG₃-SRBC.
at all. It did, however, rosette IgG1-, IgG2a-, and IgG2b-coated SRBC in a manner indistinguishable from the parent cell line (Tables II and IV). These data again suggest that the receptor for IgG3 is independent of the other IgG receptors.

We have previously reported variants from the FC1 macrophage cell line that have altered phagocytosis through the IgG2a receptor (1). These variant lines with a selective defect in phagocytosis were both able to phagocytize IgG2a-coated SRBC, suggesting again that the IgG3 receptor is independent of the IgG2a receptor (Table V).

Discussion

It has previously been thought that IgG3 does not bind to Fc receptors on mouse macrophages. This was based on data showing the inability of IgG3 to compete successfully with IgG2a for Fc binding (14). It has since become clear that macrophages possess more than one Fc receptor and that each receptor has a restricted specificity (1, 5, 12). Two receptors have been clearly defined: one for IgG1 and IgG2b, and the other for IgG2a (4, 13).

The studies reported here indicate that there is also a separate Fc receptor on mouse macrophages for IgG3. This Fc receptor is present on resident peritoneal macrophages as well as on thioglycolate-induced macrophages. In addition, the J774.2 cell line and the P388D1 cell line exhibit this receptor. This receptor is independent from the two previously identified receptors because (a) the other IgG subclasses do not compete with IgG3 for binding; (b) J774.2.1.4, a subclone of J774.2, is unable to bind or phagocytize IgG3 complexes but is unaffected in its IgG2a and IgG1-IgG2b receptors; and, (c) variants of the FC1 line that are altered in their ability to phagocytize through the IgG2a receptor have an intact ability to phagocytize IgG3-SRBC.

The IgG3 Fc receptor is similar to the receptor for IgG1 and IgG2b in that it is not affected at 4°C or by cytochalasin B or trypsin. It resembles the other IgG receptors in its ability to mediate phagocytosis. In addition, we have reported that IgG3 can also mediate antibody-dependent cell-mediated cytolysis of SRBC.
That IgG3 should bind through a separate receptor is not surprising, because structurally and serologically it is the most dissimilar of the IgG subclasses (14). The identification of this third receptor, however, raises again the question of why several independent Fc receptors are present on macrophages. On macrophages, each receptor is capable of mediating both phagocytosis and antibody-dependent cell-mediated cytolysis (21). Other cell types that bear Fc receptors may possess only one or two of these receptors in order to restrict their response to antibody or immune complexes (8, 22, 23). If Fc receptors on other cell types have the same specificity as on macrophages, perhaps the multiplicity of receptors allows for antibody-specific responsiveness in other cell types. The multiplicity may be a consequence of the amount of information that must be carried in the constant region of the immunoglobulin molecule; the constraints of subclass specificity may make separate Fc receptors necessary. Further studies on both the biologically active sites of the IgG molecules and the nature of Fc receptors on other cell types are necessary to resolve these questions.

Summary

Monoclonal antibodies to sheep erythrocytes (SRBC) have proved useful in identifying two Fc receptors on mouse macrophages, one for IgG2a, and one for IgG1 and IgG2b. We have used monoclonal IgG3 anti-SRBC to identify a third Fc receptor on mouse macrophages which binds IgG3 uniquely. This receptor is present on primary resident and thioglycolate-induced peritoneal macrophages and on some macrophage cell lines. The binding of IgG3-coated SRBC is inhibited by aggregated but not monomeric IgG3, and not by IgG1, IgG2a, and IgG2b aggregates. It is unaffected by treating the macrophages with trypsin or cytochalasin B and occurs at both 4°C and 37°C. IgG3, like all other IgG subclasses, mediates phagocytosis. We have also generated a variant macrophage line which bears the receptors for IgG1 and IgG2b and for IgG2a, but not for IgG3.

We would like to acknowledge the help of Dr. Peter Ralph in initiating these studies and in giving advice throughout.

Received for publication 29 September 1980.

References

1. Diamond, B., B. R. Bloom, and M. D. Scharff. 1978. The Fc receptors of primary and cultured phagocytic cells studied with homogeneous antibodies. J. Immunol. 121:1329.
2. Heusser, C. H., C. L. Anderson, and H. M. Grey. 1977. Receptors for IgG: subclass specificity of receptors on different mouse cell types and the definition of two distinct receptors on a macrophage cell line. J. Exp. Med. 145:1316.
3. Segal, D. M., and J. A. Titus. 1978. The subclass of specificity for the binding of murine myeloma proteins to macrophage and lymphocyte cell lines and to normal spleen cell. J. Immunol. 120:1395.
4. Unkeless, J. C. 1979. Characterization of a monoclonal antibody directed against mouse macrophage and lymphocyte Fc receptors. J. Exp. Med. 150:580.
5. Walker, W. S. 1976. Separate Fc receptors for immunoglobulins IgG2a and IgG2b on an established cell line of mouse macrophages. J. Immunol. 116:911.
6. Paraskevas, F., and S. T. Lee. 1976. The helper cell function of primed T-cells. I. Marked
amplification of antibody formation by antigen-educated T-cells carrying surface Ig 6 h after priming. *Eur. J. Immunol.* **6**:856.

7. Andersson, B., A.-C. Skoglund, and A. Rosen. 1979. Functional characterization of mouse T lymphocytes with IgM-Fc receptors. *J. Immunol.* **123**:1936.

8. Strober, W., N. E. Hague, L. G. Lum, and P. A. Henkart. 1978. IgA-Fc receptors on mouse lymphoid cells. *J. Immunol.* **121**:2440.

9. Scribner, D. J., and D. Farhney. 1976. Neutrophil receptors of IgG and complement: their roles in the attachment and ingestion phases of phagocytosis. *J. Immunol.* **116**:892.

10. Mantovani, B. 1975. Different roles of IgG and complement receptors in phagocytosis by polymorphonuclear leukocytes. *J. Immunol.*, **115**:15.

11. McNabb, T., T. Y. Koh, K. Y. Dorrington, and R. H. Painter. 1976. Structure and function of immunoglobulin domains. V. Binding of immunoglobulin G and fragments to placental membrane preparations. *J. Immunol.* **117**:882.

12. Unkeless, J. C. 1977. The presence of two Fc receptors on mouse macrophages: evidence from a variant cell line and differential trypsin sensitivity. *J. Exp. Med.* **142**:931.

13. Diamond, B., and M. D. Scharff. 1980. IgG1 and IgG2b share the Fc receptor on mouse macrophages. *J. Immunol.* **125**:631.

14. Grey, H. M., J. W. Hirst, and M. Cohn. 1971. A new mouse immunoglobulin IgG3. *J. Exp. Med.* **133**:289.

15. Ralph, P., J. Prichard, and M. Cohn. 1975. Reticulum cell sarcoma: an effector cell in antibody-dependent cell-mediated immunity. *J. Immunol.* **114**:898.

16. Potter, M., J. G. Pumphrey, and J. L. Walters. 1972. Growth of primary plasmacytomas in the mineral oil conditioned peritoneal environment. *J. Natl. Cancer Inst.* **49**:305.

17. Bianco, C., F. M. Griffin, and S. C. Silverstein. 1975. Studies of the macrophage complement receptor. Alteration of receptor function upon macrophage activation. *J. Exp. Med.* **141**:1278.

18. Pressman, D., D. H. Campbell, and L. Pauling. 1942. The agglutination of intact azoerythrocytes by antisera homologous to the attached groups. *J. Immunol.* **44**:101.

19. Kronvall, G., H. M. Grey, and R. C. Williams. 1970. Protein A reactivity with mouse immunoglobulins. *J. Immunol.* **105**:1116.

20. Dossett, J. W., G. Kronvall, R. C. Williams, Jr., and P. G. Quie. 1969. Antiphagocytic effects of staphylococcal protein A. *J. Immunol.* **103**:1405.

21. Ralph, P., I. Nakoinz, B. Diamond, and D. Yelton. 1980. All classes of murine IgG antibody mediate macrophage phagocytosis and lysis of erythrocytes. *J. Immunol.* **125**:1885.

22. Gordon, J., and R. A. Murgita. 1975. Suppression and augmentation of the primary in vitro response by different classes of antibody. *Cell. Immunol.* **15**:392.

23. Paraskevas, F., and S. T. Lee. 1976. Helper cell function of primed T cells. II. T-T' cell synergism between Ig' and Ig" subpopulations of primed thymocytes: a mechanism for amplification of helper cell function. *Eur. J. Immunol.* **6**:862.