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SPECIFICITY OF Fc RECEPTORS FOR IgG2a, IgG1/IgG2b, AND IgE ON RAT MACROPHAGES*

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The presence of receptors specific for the Fc fragment of IgG (FcγR)[1] on monocytes and macrophages (Mϕ) is well established (1–4). Recently, mouse Mϕ or macrophage-like cell lines were shown to have two FcγR, one being trypsin sensitive and binding monomeric or aggregated mouse IgG2a. The other is trypsin resistant and binds aggregated or immune-complexed mouse IgG1 and IgG2b (5, 6). Unkeless (7, 8) provided additional evidence for the presence of two types of FcγR on mouse Mϕ by demonstrating a mutant mouse Mϕ line that binds IgG2a but not aggregated IgG1 and IgG2. Anderson and Grey (9) noted that the detergent-solubilized FcγR for IgG2a is phospholipase C sensitive, whereas the solubilized FcγR for aggregated IgG is not. Furthermore, Lane et al. (10) obtained structural evidence for the presence of two FcγR on mouse peritoneal macrophages (PMϕ). They showed that a trypsin-sensitive peptide of 67,000 mol wt binds IgG2a, whereas a trypsin-resistant 52,000 mol wt peptide binds IgG1 and IgG2b. Guinea pig PMϕ also seems to have two FcγR: one for IgG1 and one for IgG2 (11).

Rat Mϕ were shown to form rosettes with rabbit IgG- (12) and rat IgE-coated (13) erythrocytes and to bind aggregated radiolabeled IgE (14), demonstrating that they bear FcR for IgG and IgE (FcεR). However, the specificity of FcγR for different IgG subclasses on rat Mϕ is unknown, and little is known of FcR for Ig classes other than IgE and IgE. Therefore, we investigated the ability of rat alveolar macrophages (AMϕ) and PMϕ to form rosettes with fixed ox erythrocytes (Eo′) coated with rat myeloma proteins of all five rat Ig classes and of the four IgG subclasses. Evidence will be presented demonstrating that rat Mϕ have an FcR for rat IgG2a, an FcR for IgG1 and IgG2b, and an FcR for IgE.

Materials and Methods

Animals. Lewis and Brown Norway rats weighing 200–300 g were obtained from the breeding colony of the Scripps Clinic and Research Foundation, La Jolla, Calif.

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[1] *Abbreviations used in this paper:* AMϕ, alveolar macrophage; DNP, dinitrophenyl; Eb, burro erythrocyte; Eo, fresh ox erythrocyte; Eo′, fixed ox erythrocyte; EoAγ, EoAμ, EoAγ1, EoAγ2, EoAα, fresh ox erythrocytes sensitized with rabbit IgG, rabbit IgM, rat IgG1 and rat IgG2 anti-Eo antibodies, and mouse IgA anti-DNP MOPC315 myeloma protein, respectively; FcR, FcγR, FcεR, Fc receptor, FcR for IgG and IgE, respectively; Mϕ, macrophage; MEM, minimum essential medium; PMϕ, peritoneal macrophage; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SRS-A, slow-reacting substances of anaphylaxis.

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Macrophages. Rat AMϕ were obtained by bronchial lavage with Hanks' balanced salt solution containing 0.25% EDTA and 2.5% fetal calf serum (FCS; 15). PMϕ were harvested 4 d after proteose-peptone stimulation by rinsing the peritoneal cavities, as previously described (15). The Mϕ were then purified by adherence to plastic petri dishes (13). Over 95% of the recovered adherent cells consisted of Mϕ, as judged by morphologic criteria after Wright staining of cytocentrifuge preparations or nonspecific esterase staining (16), and by the ability to phagocytose latex particles (15). Human U937 (17) and murine P388D1 (18) macrophagelike cell lines were cultured in RPMI-1640 medium supplemented with 10% FCS, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Flow Laboratories, Inc., Rockville, Md.). The viability of the cultured cells was 92–98%, as determined by trypan blue dye exclusion.

Myeloma Proteins. Rat myeloma proteins were isolated from the sera or ascitic fluids of Lou/M/Wsl rats bearing the corresponding immunocytomas, as previously described (19, 20). They included IgG1 (IR10, IR27, and IR595), IgG2a (IR33, IR418, and IR530), IgG2b (IR863), IgG2c (IR304), IgA (IR22), IgD (IR731), IgE (IR2, IR162), and IgM (IR473). The purity of these isolated myeloma proteins was evaluated by immunoelectrophoresis and Ouchterlony analysis with class and subclass specific antisera and by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). To remove aggregates, isolated immunoglobulins were ultracentrifuged for 90 min at 100,000 *g* in a Beckman SW50.1 rotor (Beckman Instruments, Inc., Fullerton, Calif.) and separated into three fractions. The upper third of the supernatant fluid was used immediately for coating Eo′ and for inhibition studies. IgG was heat aggregated at 63°C for 30 min at a concentration of 10–20 mg/ml in phosphate-buffered saline (PBS; 21). IgE was heat denatured by incubation in a 56°C water bath for 3 h. Human myeloma proteins of different Ig classes and subclasses were isolated as previously described (22). Purified mouse IgG and IgA meyloma proteins were purchased from Bionetics Laboratory Products, Litton Bionetics, Kensington, Md.

Immunoglobulins. Rat anti-ox erythrocyte (Eo) antibodies were raised by subcutaneous biweekly injections of 0.5 ml of 10% Eo in complete Freund's adjuvant. Rat IgG anti-Eo antibodies were isolated by a combination of ammonium sulfate fractionation at 40% saturation and stepwise DEAE-cellulose chromatography using 0.005 M phosphate buffer, pH 8.0, for elution of IgG2, and 0.05 M of the same buffer for elution of IgG1. Normal rat IgG and rabbit IgG were isolated from normal sera with 0.005 M buffer. Rabbit IgG and IgM anti-Eo antibodies were purchased from N. L. Cappel Laboratories Inc., Cochranville, Pa. Antisera to rat IgG subclasses were purchased from Miles Laboratories, Inc., Elkhart, Ind.

Rosette Assays. Two types of indicator cells were prepared and used in a rosette assay to detect FcR on Mϕ. For the first, Eo was trypsinized, washed, and treated with pyruvic aldehyde and formaldehyde (23). These Eo′ were then coated with myeloma proteins or normal IgG. Ig in amounts ranging from 0.01 to 1.0 mg/ml Eo′ were tested for the most effective coating of the Eo′; usually a final concentration of 0.25 mg Ig/ml Eo′ provided optimal Eo′ coating. Routinely, 50 μl of 10% Eo′ was washed twice with 0.1 M acetate buffer, pH 5.0, and suspended in 450 μl of the same buffer. 50 μl of a 2.5 mg/ml Ig was then added, and the cells were incubated on a rotator for 2 h at room temperature, washed three times in PBS, and resuspended in 0.5 ml PBS. To prepare the second type of indicator cells, washed, fresh Eo were sensitized with the highest subagglutinating concentration of rabbit IgG (EoAγ) or IgM (EoAμ) anti-Eo antibodies, and rat IgG (EoAγ1) or IgG2 (EoAγ2) anti-Eo antibodies. Trinitrophenyl-conjugated burro erythrocytes (Eb) were optimally sensitized with mouse IgA antidinitrophenyl myeloma protein MOPC315 (EbAα; 24), as described by Strober et al. (25). The rosette assay and rosette inhibition procedures have been previously described (13). To prevent phagocytosis of Eo′ and to reduce the percentage of spontaneously formed rosettes, all rosette assays were carried out at 4°C.

Trypsin and Neuraminidase Treatment. Mϕ at a concentration of 4×10^6 cells/ml PBS were incubated for 20 min at 37°C with different concentrations of trypsin [L-(tosylamide-2-phenyl)-ethyl-chloromethyl-ketone treated, 272 U/mg, 76% protein, Worthington Biochemical Corp., Freehold, N. J.]. An equivalent amount of soybean trypsin inhibitor (Worthington Biochemical Corp.) was then added and after brief incubation the cells were washed three times with cold minimum essential medium (MEM) containing 2.5% FCS (MEM-2.5% FCS). For rosette assays they were resuspended at 4×10^6 cells/ml MEM-2.5% FCS.

Mϕ suspended in MEM were incubated with 50 U/ml neuraminidase (Vibrio cholera, Calbiochem-Behring Corp., American Hoechst, San Diego, Calif.), for 1 h at 37°C. The cells were then washed and suspended in MEM-2.5% FCS and used immediately for the rosette assay.

Results

Rat Mϕ Forming Rosettes with Eo′ Coated with Rat Myeloma Proteins and Heterologous Ig. The percentage of rosettes formed by rat AMϕ with Eo′ coated with rat myeloma proteins of all classes and subclasses is shown in Table I. AMϕ formed rosettes with Eo′ coated with rat myeloma proteins of classes IgG1, IgG2a, IgG2b, and IgE. In contrast, the percentage of rosettes formed with Eo′ coated with rat IgG2c, IgA, IgM, and IgD was similar to the percentage of rosettes formed spontaneously with uncoated Eo′. Eo′ that were coated optimally with individual myeloma proteins within an Ig class varied in their ability to form rosettes with AMϕ.

Of the heterologous Ig tested (Table I), human IgG1, IgG3, and IgG4, murine

TABLE I

Rat AMϕ Forming Rosettes with Eo′ Coated with Ig of Different Classes and Subclasses

Rat myeloma protein	Percent rosettes*	Heterologous Ig	Percent rosettes*
None	6.4 ± 3.8	Human IgG1 (Ben)	89.9 ± 5.3
IgG1 (IR595)	86.2 ± 8.8	Human IgG2 (Kel)	6.6 ± 3.9
IgG1 (IR10)	51.4 ± 7.1	Human IgG3 (Fis)	90.4 ± 4.8
IgG1 (IR27)	58.3 ± 9.5	Human IgG4 (Hof)	44.6 ± 17.9
IgG2a (IR33)	45.1 ± 12.3	Human IgE (Sha)	6.3 ± 3.1
IgG2a (IR418)	38.7 ± 6.5	Human IgA1 (Pat)	7.9 ± 2.7
IgG2a (IR530)	54.7 ± 9.4	Human IgA2 (Kra)	5.8 ± 1.3
IgG2b (IR863)	89.2 ± 5.1	Human IgM (Huf)	6.9 ± 2.3
IgG2c (IR304)	9.4 ± 5.3	Human IgD (Dil)	8.1 ± 2.5
IgE (IR162)	84.5 ± 5.1	Mouse IgG1 (MOPC21)	52.7 ± 7.7
IgE (IR2)	65.2 ± 13.9	Mouse IgG2a (UPC10)	57.4 ± 7.9
IgM (IR473)	7.9 ± 4.2	Mouse IgG2b (MOPC141)	26.9 ± 8.1
IgA (IR22)	9.2 ± 3.8	Mouse IgG3 (J606)	6.8 ± 2.8
IgD (Ir731)	7.1 ± 3.3	Rabbit IgG (normal)	78.7 ± 8.0

* Mean ± SD of five to eight experiments.

TABLE II

AMϕ and PMϕ Forming Rosettes with Eo or Eb Sensitized with IgG, IgM, and IgA Antibodies

Indicator erythrocytes	Percent rosettes*	
	AMϕ	PMϕ
Eo	01. ± 0.3	0.2 ± 0.4
EoAγ1 (rat IgG1 anti-Eo)	85.4 ± 6.1	87.5 ± 5.4
EoAγ2 (rat IgG2 anti-Eo)	88.5 ± 4.2	88.6 ± 4.7
EoAγ (rabbit IgG anti-Eo)	86.1 ± 5.9	87.7 ± 4.5
EoAμ (rabbit IgM anti-Eo)	0.3 ± 0.4	0.1 ± 0.3
EbAα (mouse IgA anti-DNP)‡	0.2 ± 0.5	0.3 ± 0.6

* Mean ± SD of three experiments.

‡ Trinitrophenyl-conjugated Eb sensitized with the IgA mouse anti-DNP myeloma protein MOPC315.

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IgG1, IgG2a, IgG2b, and rabbit IgG induced significant rosette formation, whereas human IgG2, IgE, IgA, IgM, and IgD, and murine IgG3 did not.

Mϕ Forming Rosettes with Antibody-sensitized Erythrocytes. To compare the results obtained with Eo′ coated with myeloma proteins with antibody-sensitized Eo, we used rat IgG1 and IgG2, and rabbit IgG and IgM anti-Eo antibodies. In addition, trinitrophenyl-conjugated Eb sensitized with the mouse IgA anti-dinitrophenyl (DNP) myeloma protein MOPC315 were used to detect Fc receptors for IgA. As shown in Table II, >80% of both AMϕ and PMϕ formed rosettes with Eo sensitized with rat IgG1 and IgG2 and with rabbit IgG antibodies. In contrast, AMϕ and PMϕ did not form rosettes with rabbit IgM and mouse IgA-sensitized erythrocytes. Rat AMϕ and PMϕ were cultured for up to 72 h in media free of rat serum or were treated with neuraminidase to determine whether such treated Mϕ form EoAμ or EbAα rosettes. However, neither of these preparations formed rosettes with IgM or IgA antibody-coated erythrocytes above background (data not shown).

Ig Class and Subclass Specificity of Rosette Formation. The specificity of the rat Mϕ rosette formation was tested by inhibition with various rat myeloma proteins. As shown in Fig. 1, IgG1 and IgG2b rosettes were inhibited equally by IgG1, IgG2b, and IgE. In contrast, IgG2a and IgE rosettes were inhibited only by IgG2a or IgE, respectively. Rat IgG2c, IgA, IgM, and IgD did not inhibit IgG1, IgG2b, or IgE rosettes even at the highest concentration (3 mg/ml) tested. Identical results were obtained with Eo sensitized with rat IgG1 or IgG2 antibodies; again, IgG1 rosettes were inhibited by IgG1, IgG2b, and IgE, whereas IgG2 rosettes were inhibited only with IgG2a myeloma proteins (data not shown).

Although IgE myeloma proteins inhibited IgG1/IgG2b rosettes on a milligram per milliliter basis as much as did IgG1 and IgG2b, the inhibition could have resulted from trace contamination by normal IgG, particularly aggregated IgG. To eliminate this possibility, we ultracentrifuged the IgE preparation and tested the top, middle, and bottom fractions, the latter being expected to be enriched in IgG aggregates. In

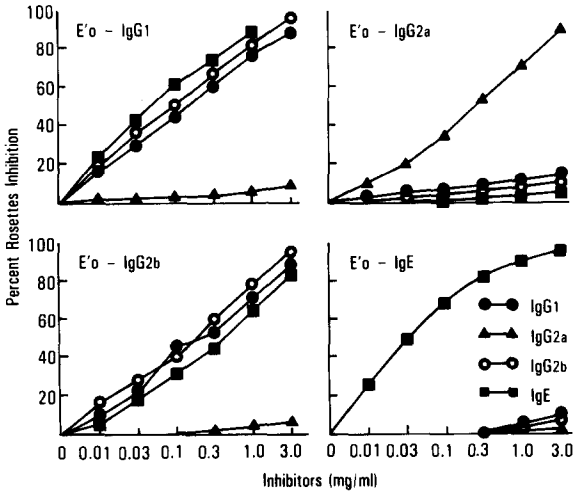

FIG. 1. Inhibition of Eo′-IgG1 (IR595), IgG2a (IR530), IgG2b (IR863), and IgE (IR162) rosettes on rat AMϕ by rat myeloma protein IgG1 (IR595), IgG2a (IR530), IgG2b (IR863), and IgE (IR162).

addition, we heated the IgE preparation at 56°C for 3 h, which destroys the ability of IgE to bind to FcεR (26) but does not affect IgG binding to FcγR (13). As shown in Table III, the ultracentrifuged IgE preparations, whether taken from the top or bottom of the tube, inhibited equally the IgG1, IgG2b, and IgE rosettes, making it very unlikely that IgG aggregates in the IgE preparations were responsible for the inhibition. Furthermore, when the IgE was heated at 56°C, it lost its ability to inhibit IgG1/IgG2b and IgE rosettes, again indicating that IgE, and not traces of contaminating IgG, was responsible for IgG1/IgG2b rosette inhibition.

To determine whether inhibition of IgG rosettes by IgE is a general phenomenon,

TABLE III

Inhibition of IgG and IgE Rosette Formation on Rat AMϕ by Ultracentrifuged or Heated Rat IgE

Inhibitor rat IgE, 1.0 mg/ml	Percent rosette inhibition*						
	Eo′-IgG1 IR595	EoAγ1	Eo′-IgG2a IR33	EoAγ2	Eo′-IgG2b IR863	EoAγ	Eo′-IgE IR162
IR162	85.2	75.7	8.6	10.2	66.9	81.6	86.6
IR162 top ⅓‡	83.1	74.1	7.9	10.6	58.7	79.9	83.4
IR162 middle ⅓	83.9	75.6	8.1	9.7	63.0	82.6	82.8
IR162 bottom ⅓	86.7	78.1	9.5	12.6	65.8	83.9	87.1
IR162 Δ56§	18.5	7.5	0.6	11.5	0.2	7.2	10.1
IR2	78.2	72.1	6.8	14.1	61.7	63.1	82.4

* Mean of three experiments.

‡ Ultracentrifuged at 100,000 *g* for 90 min and fractionated into three portions.

§ Heated for 3 h at 56°C and used immediately for inhibition at a final concentration of 3.0 mg/ml.

TABLE IV

Inhibition of IgG and IgE Rosette Formation by Rat and Human IgE on Mouse and Human Macrophagelike Cell Lines

Macrophage cell line	Indicator erythrocytes	Percent rosettes*		
		Without inhibitor	With inhibitor‡	Percent inhibition
			Rat IgE	
Mouse P388D1	Eo′-rat IgE (IR162)	53.8 ± 12.4	1.3 ± 6.3	97.6
	Eo′-rat IgG1 (IR595)	78.9 ± 9.9	81.5 ± 6.0	(+3.3)§
	Eo′-rat IgG2a (IR530)	43.1 ± 4.2	45.3 ± 3.9	(+5.1)
	Eo′-rat IgG2b (IR863)	86.6 ± 5.2	88.7 ± 8.4	(+2.4)
	Eo′-mouse IgG1 (MOPC21)	46.3 ± 8.7	47.7 ± 7.6	(+3.0)
	Eo′-mouse IgG2a (UPC10)	78.8 ± 5.7	81.5 ± 6.6	(+3.4)
	Eo′-mouse IgG2b (MOPC141)	32.8 ± 5.8	35.5 ± 5.3	(+8.2)
	EoAγ (rabbit IgG)	90.6 ± 3.1	87.8 ± 6.3	3.1
			Human IgE	
Human U937	Eo′-human IgE (Sha)	78.5 ± 5.7	7.3 ± 2.1	90.7
	Eo′-human IgG1 (Ben)	80.5 ± 7.1	78.3 ± 8.9	2.7
	Eo′-human IgG2 (Kel)	5.3 ± 2.1	5.5 ± 3.3	(+3.8)
	Eo′-human IgG3 (Fis)	81.8 ± 9.6	84.6 ± 11.2	(+3.4)
	Eo′-human IgG4 (Hof)	26.7 ± 5.4	25.9 ± 6.1	3.0
	EoAγ (rabbit IgG)	90.3 ± 5.5	86.6 ± 8.5	4.1

* Mean ± SD of three experiments.

‡ Rat IgE (IR162) or human IgE (Sha) myeloma proteins at a final concentration of 1.0 mg/ml.

§ (+) = percentage increase in rosette formation.

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TABLE V

Species Specificity of IgG Rosette Formation on Rat AMϕ

Inhibitor, 1.0 mg/ml	Percent rosette inhibition*								
	Rat IgG			Human IgG			Mouse IgG		Rabbit
	Eo′-IgG1 IR595	Eo′-IgG2a IR530	Eo′-IgG2b IR863	Eo′-IgG1 Ben	Eo′-IgG3 Fis	Eo′-IgG4 Hof	Eo′-IgG1 MOPC21	Eo′-IgG2a UPC10	EoAγ
Rat IgG1 (IR595)	77.6	1.7	78.5	73.0	71.9	85.6	93.4	93.7	81.8
Rat IgG2a (IR530)	8.9	66.4	7.7	4.1	3.4	0.4	10.9	16.2	2.8
Rat IgG2b (IR863)	81.2	17.0	83.2	89.2	84.4	83.8	NT‡	NT	87.1
Rat IgE (IR162)	84.8	8.6	71.4	72.1	77.3	85.4	85.4	81.2	83.3
Human IgG1 (Ben)	79.0	19.6	65.4	79.1	77.7	88.0	NT	NT	82.9
Human IgG3 (Fis)	84.1	18.5	67.2	81.2	81.7	84.1	NT	NT	87.6
Mouse IgG1 (MOPC21)	68.2	3.1	NT	NT	48.8	NT	85.3	88.9	NT
Mouse IgG2a (UPC10)	64.3	3.7	NT	NT	53.9	NT	75.1	88.4	NT
Rabbit IgG (normal)	84.0	18.5	68.4	80.2	89.2	88.4	NT	NT	73.9

* The data represent the percentage of rosette inhibition (mean of three experiments).

‡ Not tested.

TABLE VI

Trypsin Sensitivity of Fc Receptors for IgG and IgE on Rat AMϕ

Trypsin	Percent inhibition*						
	Eo′-IgG1 IR595	EoAγ1	Eo′-IgG2a IR530	EoAγ2	Eo′-IgG2b IR863	EoAγ	Eo′-IgE IR162
mg/ml			*mg/ml*				
0.05	(+5.5)	(+5.6)	(+5.0)	(+7.4)	(+1.5)	(+6.5)	(+6.2)
0.1	10.0	9.3	(+13.4)	(+5.3)	(+4.7)	8.7	(+5.6)
0.5	51.1	28.4	(+5.5)	(+2.8)	23.9	21.4	9.0
1.0	83.2	50.6	16.3	5.2	48.7	40.5	43.5
5.0	96.9	84.3	26.1	18.7	77.6	71.8	82.1

* Mean of three to four experiments. The data represent percentage decrease or increase (+) in the capacity of rat AMϕ to form rosettes after treatment with trypsin for 20 min at 37°C.

we inhibited IgG rosettes with IgE and IgG on mouse P388D1 and human U937 macrophagelike cell lines. As shown in Table IV, ~50% of the mouse Mϕ formed rosettes with Eo′ coated with rat IgE, and 80% of the human Mϕ formed rosettes with Eo′ coated with human IgE; both were inhibitable by >90% with the homologous IgE. In contrast, neither rat nor human IgE inhibited IgG rosettes formed by different rat, mouse, and human IgG subclasses, or those formed by rabbit IgG antibodies on both mouse and human macrophagelike cell lines.

Species Specificity of IgG Rosettes on Rat Mϕ. To test the species specificity of the two putative FcγR on rat Mϕ, Eo′ were coated with rat, human, and mouse IgG of different subclasses or Eo were sensitized with rabbit IgG anti-Eo antibodies and used in the rosette assay. As shown in Table V, rat IgG1 and IgG2b rosettes were inhibited by all IgG preparations tested, except rat IgG2a. Conversely, rat IgG2a rosettes were inhibited only by rat IgG2a. IgG rosettes formed by Eo′ coated with human and mouse IgG of different subclasses or by rabbit IgG antibodies were all inhibitable with rat IgG1 and IgG2b, heterologous IgG, and rat IgE but not with rat IgG2a.

Rosettes Formed by Trypsin-treated Mϕ. To determine the trypsin sensitivity of the putative Fc receptors on rat Mϕ, the cells were incubated with various concentrations of trypsin at 37°C for 20 min, washed, and analyzed for rosette formation. As shown

in Table VI, trypsin treatment reduced IgG1, IgG2b, and IgE rosette formation by up to 97%, whereas IgG2a rosette formation decreased only slightly, even at very high trypsin concentrations. The decreased percentage of trypsin-treated AMϕ forming rosettes with Eo sensitized with rabbit IgG anti-Eo antibodies offers further support for the binding of heterologous IgG to the FcγR reacting with rat IgG1 and IgG2b.

Discussion

Rat Mϕ were shown to form rosettes with Eo′ coated with rat IgG1, IgG2a, IgG2b, and IgE but not with rat IgM, IgA, IgD, and IgG2c myeloma proteins. The IgG2a and IgE rosettes were inhibited only by IgG2a and IgE, respectively, whereas the IgG1 and IgG2b rosettes were equally inhibited by both IgG1 and IgG2b myeloma proteins. These findings indicate that rat Mϕ bear three types of Fc receptors: one specific for IgG2a, one specific for IgE, and one reacting with both IgG1 and IgG2b. The IgG2a FcR was trypsin resistant, whereas the IgG1/IgG2b and the FcεR were trypsin sensitive. As shown by inhibition studies, heterologous IgG reacted with the IgG1/IgG2b FcR but not with the IgG2a FcR. The rat Mϕ resemble mouse Mϕ that also possess two FcγR, one for IgG2a, and one for IgG1 and IgG2b (5–8). However, the two murine FcγR differ from the rat FcγR in their trypsin sensitivity in that the mouse IgG2a FcR is trypsin sensitive and the IgG1/IgG2b FcR is resistant. In addition to the FcγR, FcεR seem to be a characteristic of Mϕ and the related blood monocytes. Mouse macrophagelike cells P388D1 formed specific rosettes with Eo′ coated with rat IgE, and recent experiments performed in our laboratory showed that the majority of mouse AMϕ form rosettes with mouse IgE-coated indicator erythrocyte, and bind radiolabeled mouse IgE (unpublished observations). Similarly, human U937 macrophagelike cells form IgE rosettes and bind radiolabeled human IgE (27). We have previously shown that human peripheral blood monocytes form IgE rosettes and lyse specifically IgE-coated target cells (28). It appears from these experiments that FcγR and FcεR are the major FcR on Mϕ and monocytes.

By using a rosette assay with Eo′ coated with rat myeloma proteins, we could not demonstrate FcR for IgM, IgA, IgD, or IgG2c on rat Mϕ. Although other investigators similarly failed to demonstrate FcR for IgM and IgA on Mϕ (5, 8, 29–31), Haegert (32) reported that neuraminidase-treated human and rabbit monocytes form IgM rosettes. Therefore, we treated the rat Mϕ with neuraminidase and, in addition, cultured them in media free of rat serum to allow elution of in vivo FcR bound IgM, which appears to inhibit IgM rosette formation on T lymphocytes (33). However, after such treatment, rat Mϕ did not form IgM rosettes. Fanger et al. (34) showed that human monocytes have IgA FcR by formation of rosettes with Eo sensitized with rabbit secretory IgA anti-Eo antibodies. However, by using either rabbit IgM anti-Eo or mouse MOPC315 IgA anti-DNP-sensitized erythrocytes, we still could not demonstrate IgM or IgA FcR on the rat Mϕ. Recently, Diamond and Yelton (35) characterized a new FcR specific for IgG3 on mouse Mϕ using sheep erythrocytes sensitized with a mouse IgG3 hybridoma anti-Eo antibody. In contrast to IgG2a and IgG1/IgG2b rosettes, the IgG3 rosettes were inhibited only with aggregated but not monomeric IgG3. Rat Mϕ may have FcR for IgG2c, IgM, and IgA, but FcR were not detected by our method because they may be few in number and/or of low affinity. Binding studies of radiolabeled monomeric and aggregated Ig of these classes will be necessary to address this possibility.

Rat IgE inhibited IgG1/IgG2b rosettes as much as did IgG1 and IgG2b, but it did not inhibit IgG2a rosettes. This inhibition could not be explained by IgG1 and IgG2b contamination of the IgE preparations. First, IgG was not detected by gel diffusion methods and SDS-PAGE. Second, no evidence for IgG aggregates in the IgE preparation was obtained because both top and bottom portions of ultracentrifuged IgE preparations inhibited IgG1/IgG2b rosettes equally. Finally, heating the IgE at 56°C, which induces irreversible conformational changes in the Cε3 and Cε4 regions of the IgE Fc fragments (26), destroyed the ability of IgE to inhibit not only IgE but also IgG1/IgG2b rosettes. In contrast, heating rat IgG at 56°C did not destroy the ability of IgG to react with FcγR (13). A cross-reaction of rat IgE with FcγR was also observed by Yodoi and Ishizaka (36) on rat lymphocytes and by Segal et al. (37) on rat basophilic leukemia cells. These investigators used rabbit IgG to detect FcγR, which, based on the present experiments, presumably reacted only with IgG1/IgG2b FcR. The cross-inhibition by rat IgE of IgG1/IgG2b rosettes seems to be unique to the rat cells. Mouse Mϕ formed rosettes with rat IgG1 and IgG2b, but rat IgE failed to inhibit these or any other IgG rosettes. Similarly, human IgE did not inhibit IgG rosettes on human Mϕ. It could not be determined from our studies whether IgE reacted with the IgG1/IgG2b FcR or whether binding of IgE to the FcεR inhibited IgG1/IgG2b rosettes by steric hindrance. However, rat IgE inhibits IgG rosettes on FcεR negative lymphocytes (36), suggesting that rat IgE may bind to the rat IgG1/IgG2 FcR. The biological significance of IgE interaction with IgG1/IgG2b receptors is unknown. Yodoi and Ishizaka (36, 38) suggested that IgE reacting with FcγR may provide a signal to the lymphocytes to switch from expressing FcγR to FcεR. However, because rat IgE failed to cross-react with FcγR on mouse Mϕ, and human IgE failed to cross-react with FcγR on human Mϕ and lymphocytes (23), further evidence is necessary to determine whether the interaction of IgE with FcγR induces FcεR.

Like mouse Mϕ, rat Mϕ presumably possess different membrane proteins or protein complexes that form the FcγR. The differences in trypsin sensitivity of the rat and mouse FcγR, the occurrence of mutant mouse Mϕ cell lines having only FcγR for IgG2a (7, 8), and the demonstration of two different peptides binding either murine IgG2a or IgG1/IgG2b (10), suggest structurally different Fcγ receptors, at least on the intact cell. However, recent experiments reported by Mellmann and Unkeless (39) and by Schneider et al. (40) have demonstrated a lack of IgG subclass specificity of the solubilized FcγR. The FcγR may be composed of different peptides that rearrange upon solubilization of the cell membrane, thus altering the specificity of the FcR. Isolation and physicochemical characterization of the peptides binding Fc fragments will be necessary to determine structural differences among various FcR on macrophages.

Established functions of FcR on Mϕ are attachment, ingestion, and lysis of IgG-coated particles. Whether IgG1/IgG2b and IgG2a FcR each mediate special functions is unresolved. Walker (41) reported that IgG2b FcR on murine Mϕ mediate killing of antibody-coated target cells, whereas IgG2a FcR do not. In contrast, Ralph et al. (42) showed that antibodies of all murine IgG subclasses mediate phagocytosis and antibody-dependent cellular cytotoxicity. Moreover, FcεR on rat Mϕ mediate the killing of *Schistosoma mansoni* schistosomules (43, 44) and secretion of β-glucuronidase (45). Mouse Mϕ release slow reacting substances of anaphylaxis (SRS-A) after incubation with ionophore A23187 (46, 47) or in response to phagocytic stimuli (48,

49). If the interaction of IgE immune complexes with IgE FcR on Mϕ and on monocytes can induce SRS-A release, it may establish a previously unrecognized pathway for the induction of anaphylactic reactions.

Summary

To characterize the Fc receptors on rat alveolar and peritoneal macrophages (Mϕ), we analyzed their ability to form rosettes with fixed ox erythrocytes (Eo′) coated with myeloma proteins of all rat Ig classes and with fresh erythrocytes (Eo) sensitized with rat IgG1 and IgG2, rabbit IgG and IgM, and mouse IgA antibodies. The Mϕ formed rosettes with Eo′ coated with rat myeloma proteins of classes IgG1, IgG2a, IgG2b, and IgE but not IgG2c, IgA, IgM, and IgD. Rat Mϕ also formed rosettes with Eo′ coated with human IgG1, IgG3, IgG4, mouse IgG1, IgG2a, IgG2b, and rabbit IgG. Furthermore, rat Mϕ formed rosettes with Eo sensitized with rat IgG1, IgG2, or rabbit IgG antibodies but not with Eo sensitized with rabbit IgM or mouse IgA antibodies. Trypsin treatment of rat Mϕ abolished IgG1/IgG2b and IgE but not IgG2a rosettes.

The IgG2a and IgE rosettes were Ig class specific because they were inhibited only by rat IgG2a and rat IgE, respectively. In contrast, IgG1 and IgG2b rosettes were inhibited equally by IgG1 and IgG2b. Heterologous IgG inhibited IgG1/IgG2b but not IgG2a rosettes. Rat IgE inhibited rat IgG1, IgG2b, and heterologous IgG rosette formation on rat Mϕ. Although Eo′ coated with rat IgE formed rosettes with mouse P388D1 macrophagelike cells, rat IgE did not inhibit IgG rosettes on these cells. Similarly, Eo′ coated with human IgE formed rosettes with human U937 macrophage-like cells, but human IgE did not inhibit IgG rosettes on these cells.

The results indicate that rat Mϕ have at least three distinct Fc receptors: one is specific for rat IgG2a and is trypsin resistant; a second is specific for rat IgE and is trypsin sensitive; and a third reacts with rat IgG1 rat IgG2b, and heterologous IgG and is trypsin sensitive. Rat IgE inhibited IgG1/IgG2b rosettes unidirectionally and uniquely on rat Mϕ.

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References

1. Berken, A., and B. Benacerraf. 1966. Properties of antibodies cytophilic for macrophages. *J. Exp. Med.* **123:**119.
2. Lobuglio, A. F., R. S. Cotran, and J. H. Jandl. 1967. Red cells coated with immunoglobulin G: binding and sphering by mononuclear cells in man. *Science (Wash. D. C.).* **158:**1582.
3. Huber, H., and H. H. Fudenberg. 1968. Receptor sites of human monocytes for IgG. *Int. Arch. Allergy Appl. Immunol.* **34:**18.
4. Unkeless, J. C., and H. N. Eisen. 1975. Binding of monomeric immunoglobulins to Fc receptors of mouse macrophages. *J. Exp. Med.* **142:**1520.
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. 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.
7. 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.* **145:**931.

8. Unkeless, J. C., G. Kaplan, H. Plutner, and Z. A. Cohn. 1979. Fc-receptor variants of a mouse macrophage cell line. *Proc. Natl. Acad. Sci. U. S. A.* **76:**1400.
9. Anderson, C. L., and H. M. Grey. 1978. Physicochemical separation of two distinct Fc receptors on murine macrophage-like cell lines. *J. Immunol.* **121:**648.
10. Lane, B. C., J. Kan-Mitchell, M. S. Mitchell, and S. M. Cooper. 1980. Structural evidence for distinct IgG subclass-specific Fc receptors on mouse peritoneal macrophages. *J. Exp. Med.* **152:**1147.
11. Alexander, M. D., J. A. Andrews, R. G. Q. Leslie, and N. J. Wood. 1978. The binding of human and guinea-pig IgG subclasses to homologous macrophage and monocyte Fc receptors. *Immunology.* **35:**115.
12. Boltz-Nitulescu, G., and O. Foerster. 1980. Effect of proteinases on EA (IgG)-binding receptors of rat macrophages. *In* Macrophages and Lymphocytes. Nature, Functions, and Interaction. Part A. M. R. Escobar and H. Friedman, editors. Plenum Publishing Corp., New York. 157.
13. Boltz-Nitulescu, G., and H. L. Spiegelberg. 1981. Receptors specific for IgE on rat alveolar and peritoneal macrophages. *Cell. Immunol.* **59:**106.
14. Dessaint, J. P., G. Torpier, M. Capron, H. Bazin, and A. Capron. 1979. Cytophilic binding of IgE to the macrophage. I. Binding characteristics of IgE on the surface of macrophages in the rat. *Cell. Immunol.* **46:**12.
15. Boltz-Nitulescu, G., and O. Foerster. 1979. Antigenic differences between alveolar and peritoneal macrophages of the rat. Lack of population-specific determinants. *Immunology.* **38:**621.
16. Tucker, S. B., R. V. Pierre, and R. E. Jordan. 1977. Rapid identification of monocytes in a fixed mononuclear cell preparation. *J. Immunol. Methods.* **14:**267.
17. Sundstrom, C., and K. Nilsson. 1976. Establishment and characterization of a human histiocytic lymphoma cell line (U937). *Int. J. Cancer.* **17:**565.
18. Koren, H. S., B. S. Handwerger, and J. R. Wunderlich. 1975. Identification of macrophage-like characteristics in a cultured murine tumor line. *J. Immunol.* **114:**894.
19. Bazin, H., A. Beckers, and P. Querinjean. 1974. Three classes and four (sub)classes of rat immunoglobulins: IgM, IgA, IgE, and IgG_1, IgG_{2a}, IgG_{2b}, IgG_{2c}. *Eur. J. Immunol.* **4:**44.
20. Bazin, H., A. Beckers, G. Urbain-Vansanten, R. Pauwels, C. Bruynus, A. F. Tilkin, B. Platteau, and J. Urbain. 1978. Transplantable IgD immunoglobulin-secreting tumors in rat. *J. Immunol.* **121:**2077.
21. Augener, W., and H. M. Grey. 1970. Studies on the mechanism of heat aggregation of human γG. *J. Immunol.* **105:**1024.
22. Lawrence, D. A., W. O. Weigle, and H. L. Spiegelberg. 1975. Immunoglobulins cytophilic for humal lymphocytes, monocytes, and neutrophils. *J. Clin. Invest.* **55:**368.
23. Gonzalez-Molina, A., and H. L. Spiegelberg. 1977. A subpopulation of normal human peripheral B lymphocytes that bind IgE. *J. Clin. Invest.* **59:**616.
24. Eisen, H. N., E. S. Simms, and M. Potter. 1968. Mouse myeloma proteins with antihapten antibody activity. The protein produced by plasma cell tumor MOPC-315. *Biochemistry.* **7:** 4126.
25. Strober, W., N. E. Hague, L. G. Lum, and P. A. Henkart. 1978. IgA-Fc receptors on mouse lymphoid cells. *J. Immunol.* **121:**2440.
26. Dorrington, K. J., and H. H. Bennich. 1978. Structure-function relationships in human immunoglobulin E. *Immunol. Rev.* **41:**3.
27. Anderson, C. L., and H. L. Spiegelberg. 1981. Macrophage receptors for IgE: binding of IgE to specific IgE Fc receptors on human macrophage cell line, U937. *J. Immunol.* In press.
28. Melewicz, F. M., and H. L. Spiegelberg. 1980. Fc receptors for IgE on a subpopulation of human peripheral blood monocytes. *J. Immunol.* **125:**1026.
29. Bianco, C., F. M. Griffin, Jr., and S. C. Silverstein. 1975. Studies of the macrophage

complement receptor. Alteration of receptor function upon macrophage activation. *J. Exp. Med.* **141:**1278.

30. Ralph, P., J. Prichard, and M. Cohn. 1975. Reticulum cell sarcoma: an effector cell in antibody-dependent cell-mediated immunity. *J. Immunol.* **114:**898.
31. Reynolds, H. Y., J. P. Atkinson, H. H. Newball, and M. M. Frank. 1975. Receptors for immunoglobulin and complement on human alveolar macrophages. *J. Immunol.* **114:**1813.
32. Haegert, D. G. 1979. Phagocytic peripheral blood monocytes from rabbits and humans express membrane receptors specific for IgM molecules: evidence that incubation with neuraminidase exposes crytpic IgM (Fc) receptors. *Clin. Exp. Immunol.* **35:**484.
33. Moretta, L., M. Ferrarini, M. L. Durante, and M. C. Mingari. 1975. Expression of a receptor for IgM by human T cells *in vitro*. *Eur. J. Immunol.* **5:**565.
34. Fanger, M. W., L. Shen, J. Pugh, and G. M. Bernier. 1980. Subpopulations of human peripheral granulocytes and monocytes express receptors for IgA. *Proc. Natl. Acad. Sci. U. S. A.* **77:**3640.
35. Diamond, B., and D. E. Yelton. 1981. A new Fc receptor on mouse macrophages binding IgG3. *J. Exp. Med.* **153:**514.
36. Yodoi, J., and K. Ishizaka. 1979. Lymphocytes bearing receptors for IgE. III. Transition of FcγR(+) cells to FcεR (+) cells by IgE. *J. Immunol.* **123:**2004.
37. Segal, D. M., S. O. Sharrow, J. F. Jones, and R. P. Siraganian. 1981. Fc (IgG) receptors on rat basophilic leukemia cells. *J. Immunol.* **126:**138.
38. Yodoi, J., and K. Ishizaka. 1980. Induction of Fcε-receptor bearing cells *in vitro* in human peripheral lymphocytes. *J. Immunol.* **124:**934.
39. Mellman, I. S., and J. C. Unkeless. 1980. Purification of a functional mouse Fc receptor through the use of a monoclonal antibody. *J. Exp. Med.* **152:**1048.
40. Schneider, R. J., J. P. Atkinson, V. Krause, and A. Kulczycki, Jr. 1981. Characterization of ligand-binding activity of isolated murine Fcγ receptor. *J. Immunol.* **126:**735.
41. Walker, W. S. 1977. Mediation of macrophage cytolytic and phagocytic activities by antibodies of different classes and class-specific Fc-receptors. *J. Immunol.* **119:**367.
42. 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.
43. Capron, A., J.-P. Dessaint, M. Capron, and H. Bazin. 1975. Specific IgE antibodies in immune adherence of normal macrophages to *Schistosoma mansoni* schistosomules. *Nature (Lond.).* **253:**474.
44. Capron, A., J.-P. Dessaint, M. Joseph, R. Rousseaux, M. Capron, and H. Bazin. 1977. Interaction between IgE complexes and macrophages in the rat: a new mechanism of macrophage activation. *Eur. J. Immunol.* **7:**315.
45. Dessaint, J.-P., A. Capron, M. Joseph, and H. Bazin. 1979. Cytophilic binding of IgE to the macrophage. II. Immunologic release of lysosomal enzyme from macrophages by IgE and anti-IgE in the rat: a new mechanism of macrophage activation. *Cell. Immunol.* **46:**24.
46. Bach, M. K., and J. R. Brashler. 1978. Ionophore A 23187-induced production of slow reacting substance of anaphylaxis (SRS-A) by rat peritoneal cells *in vitro*: evidence for production by mononuclear cells. *J. Immunol.* **120:**998.
47. Orange, R. P., E. G. Moore, and E. W. Gelfand. 1980. The formation and release of slow reacting substance of anaphylaxis (SRS-A) by rat and mouse peritoneal mononuclear cells induced by ionophore A23187. *J. Immunol.* **124:**2264.
48. Rouzer, C. A., W. A. Scott, Z. A. Cohn, P. Blackburn, and J. M. Manning. 1980. Mouse peritoneal macrophages release leukotriene C in response to a phagocytic stimulus. *Proc. Natl. Acad. Sci. U. S. A.* **77:**4928.
49. Rouzer, C. A., W. A. Scott, A. L. Hamill, and Z. A. Cohn. 1980. Dynamics of leukotriene C production by macrophages. *J. Exp. Med.* **152:**1236.