Fc RECEPTOR MODULATION IN MONONUCLEAR PHAGOCYTES MAINTAINED ON IMMOBILIZED IMMUNE COMPLEXES OCCURS BY DIFFUSION OF THE RECEPTOR MOLECULE*

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The inhibition of Fc receptor (FcR)[1] function that occurs when macrophages are plated on surfaces coated with antigen-antibody complexes is due to the physical removal of these receptors from the nonadherent surface of the cell's plasma membrane (1). In the studies reported here we analyze the mechanism of removal of the trypsin-resistant FcRII of mouse macrophages from this segment of the cell's plasma membrane. Our results show that this receptor diffuses rapidly in the plasma membrane and that this leads to the entrapment of FcRII molecules in the portion of the plasma membrane in contact with substrate-adherent immune complexes.

Materials and Methods

Resident and thioglycollate broth-elicited macrophages, immunoglobulins, dinitrophenyl (DNP)-poly-L-lysine (PLL)-coated coverslips, binding and ingestion of IgG-coated erythrocytes, and binding of ^{125}I-monoclonal antibodies were obtained and used as described (1). Rabbit IgG against bovine serum albumin (RαBSA IgG), sheep erythrocytes (E) coated with rabbit IgG (Cordis Laboratories, Inc., Miami, FL) or with rabbit IgM (Cordis Laboratories) and mouse complement [E(IgM)C] were prepared as described (2).

Formation of Immune Complexes on Substrates Containing Adherent Macrophages ("Sequential Method"). PLL-DNP-coated coverslips were placed into 16-mm wells of Costar plates (Costar, Data Packaging, Cambridge, MA) and were overlaid with 0.4 ml Eagle's Minimum Essential Medium (MEM) with Earle's salt solution (Gibco Laboratories, Grand Island, NY). 4×10^5 resident peritoneal cells or 1.25×10^5 thioglycollate-elicited peritoneal cells in 0.1 ml MEM were added per well and the cultures were incubated for 30–60 min at 37°C in a 95% air/5% CO_2 mixture to allow macrophages to spread on the DNP-coated surfaces. The coverslips were

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; C, complement; 2-dG, 2-deoxy-D-glucose; DMSO, dimethylsulfoxide; DNP, dinitrophenyl; E, sheep erythrocytes; E(IgG), E coated with anti-E IgG; E(IgM)C, E coated with anti-E IgM and mouse complement components; FcR, receptor for the Fc portion of IgG; HBSS, Hanks' balanced salt solution without sodium bicarbonate and phenol red; hFBS, heat-inactivated fetal bovine serum; HRP, horseradish peroxidase; MEM, Eagle's Minimum Essential Medium with Earle's salt solution; PBS, phosphate-buffered saline with CA^{++} and Mg^{++} ions; PD, PBS without Ca^{++} and Mg^{++}; PLL, poly-L-lysine; PMA, phorbol myristate acetate; RαBSA IgG, rabbit anti-BSA IgG; RαDNP IgG, rabbit anti-DNP IgG.

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removed, dipped five times vigorously into ~200 ml phosphate-buffered saline (PBS) without Ca^{++} and Mg^{++} (PD) at 2–3°C to dislodge nonadherent cells, and transferred to fresh Costar plates kept on ice in the cold room. Each well of these Costar plates was filled with 0.5–1 ml of Hanks' balanced salt solution (HBSS) (without sodium bicarbonate and phenol red) containing 1 mg/ml BSA (Sigma Chemical Co., St. Louis, MO) and 0.02 M Hepes, pH 7.2 (Hepes-HBSS-BSA) (Sigma Chemical Co.). After 15 min at 2–3°C, some of the coverslips were removed from the wells, drained of surplus buffer on tissue paper, and placed with their dry and cell-free surface down onto a precooled Costar plate cover, in an ice bath in the cold room. These coverslip cultures were overlaid with 60 μl of ice-cold PD containing 9–13 μg of affinity-purified RαDNP IgG, and protected by another Costar plate cover. The little chamber containing these coverslips was buried in ice for 60 min to allow DNP-RαDNP IgG complexes to form. The excess soluble RαDNP IgG was removed by dipping the coverslips 10 times into 200 ml ice-cold PD. The coverslips were placed into fresh Costar wells containing 0.5 ml MEM at 2–3°C and further processed as described in Results.

Trypsinization of Peritoneal Cells. Trypsinization of macrophage cultures that were prepared according to the sequential procedure was done directly on the coverslips after the initial 30–60 min period of macrophage adherence. The macrophage-laden PLL-DNP-coated coverslips were placed into Costar wells filled with 0.5 ml warm PBS containing 0.75 mg/ml TPCK-trypsin (Worthington Biochemical Corp., Freehold, NJ). After 30 min at 37°C the Costar plates were transferred onto ice, the trypsin-containing PBS removed, and 1 ml of ice-cold PD supplemented with 1 mg/ml BSA was added to each well followed by two washings with 1 ml of the same buffer. After additional dipping of the coverslips into ~200 ml of ice-cold PD, they were transferred to Costar wells containing fresh medium. >92% of the cells were viable as measured by trypan blue (Gibco Laboratories) exclusion (3). Trypsin treatment of preadhered macrophages was not accompanied by an obvious loss of macrophages. As expected, trypsinization of the macrophages on the DNP-coated coverslips did not affect the binding of RαDNP IgG and the formation of the immobilized DNP-RαDNP IgG complexes, nor did it affect the capacity of the macrophages to modulate their FcR under permissive experimental conditions.

Pinocytosis Assay. 35-mm diam tissue culture dishes (3001; Falcon Labware, Oxnard, CA) were coated with PLL-DNP as described above for coverslips but the initial acid treatment was omitted. 1×10^6 thioglycollate-elicited peritoneal cells in 1 ml MEM were seeded into each dish and incubated for 30 min at 37°C. After washing the cultures three to five times with 2 ml warm PD to remove nonadherent cells, the cultures were incubated with control or drug-containing medium, and then with RαDNP IgG as described in Results. At the appropriate point in the experiment the cultures were incubated in 1 ml MEM supplemented with 5% heat-inactivated fetal bovine serum (hFBS) (Flow Laboratories, Inc., Rockville, MD), 1 mg/ml horseradish peroxidase (HRP) (Sigma Chemical Co.) as a marker for pinocytosis, and drugs where indicated, and the incubation was continued at 37°C. At the time points indicated duplicate cultures were removed, the cells were washed six times with 2 ml ice-cold MEM and once with 2 ml ice-cold PD, dissolved in 1 ml 0.05% Triton X-100 in H_2O, and processed for HRP uptake as described (4). The results of these experiments are expressed in micrograms HRP ingested per microgram cell protein over a period of up to 1 h. They are corrected for nonspecific adsorption of HRP to the cell-free culture dish (0.008 μg). Protein was quantitated according to the method of Lowry et al. (5).

Measurements of the Effect of Temperature on FcR Movement. Macrophages on PLL-DNP-coated coverslips were incubated with RαDNP-IgG at 2–3°C as described above for the sequential procedure. After washing the coverslips in ice-cold Hepes-HBSS-BSA to remove unbound RαDNP IgG, some coverslips were kept at 2–3°C to control for receptor modulation at this temperature; others were placed onto a specially designed perfusable "cooling" block (Fig. 1 *A*) that was brought to the desired temperature by connecting it to a Lauda type K/R circulating water bath (Brinckmann Instruments, Westbury, NY). To achieve rapid temperature equilibration the block was made out of copper. It was placed in a humidified chamber to avoid desiccation of the macrophages, which were each covered with 60 μl of Hepes-HBSS-BSA. To achieve precise temperature control of the coverslips on which the macrophages were plated, a disc-shaped surface temperature probe (421; Yellow Springs Instrument Co., Yellow Springs, OH) connected to a telethermometer (Yellow Springs Instrument Co.) was in contact with the

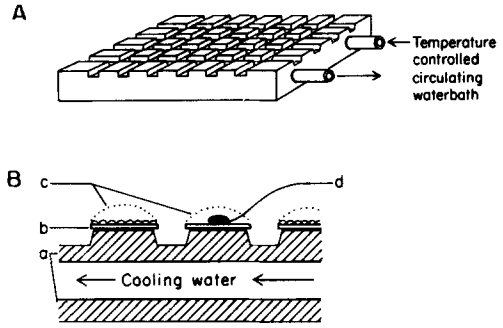

FIG. 1. (*A*) Diagram of copper cooling block used to regulate the temperature of macrophages on glass coverslips. The individual pedestals of the cooling block are 8.5 × 8.5 mm and the entire block measures 15 × 7 × 1.4 cm. (*B*) Diagram of a coverslip on a single pedestal of the cooling block shown above. (*a*) Copper cooling block. (*b*) Macrophages on 12-mm glass coverslip. (*c*) "Bubble" of culture medium overlying macrophages. (*d*) Thermistor probe.

surface of a control coverslip on the cooling block throughout the experiment (Fig. 1 *B*). At the end of the incubation period the supernatant fluid was removed from the coverslips while still on the cooling block, and the coverslips were rapidly transferred into 2–3°C Hepes-HBSS-BSA to stop further receptor movement. The macrophages on these coverslips were then used to measure the extent of FcR modulation by rosetting with E(IgG) and/or by binding of ^{125}I-2.4G2 IgG as described (1).

Treatment of Macrophages with Inhibitors of Metabolism, Protein Synthesis, or Cytoskeletal Function. Macrophages were plated on PLL-DNP-coated coverslips. Where indicated the cells were trypsinized as described above to remove FcRI (2) and washed in medium containing hFBS to inhibit further trypsin activity. The cells were incubated in MEM containing the indicated concentrations of NaF, NaCN, 2-deoxy-D-glucose (2-dG) (Sigma Chemical Co.), cycloheximide, cytochalasins B or D (Aldrich Chemical Co., Milwaukee, WI), colchicine (Sigma Chemical Co.), podophyllotoxin or taxol (gifts of Dr. John Loike, The Rockefeller University), phorbol myristate acetate (PMA) (Consolidated Midland Corp., Brewster, NY) at 37°C for 30–120 min and then cooled to 2–3°C. RαDNP IgG in PBS-BSA with the appropriate drug(s) was added to some cultures at 2–3°C for 1 h to form substrate-adherent DNP-RαDNP IgG complexes according to the sequential method. The macrophages were washed and incubated at 37°C in drug-containing medium to allow FcR to respond to the immobilized ligands. To measure FcR modulation the macrophages were incubated at the indicated temperature with E(IgG) or ^{125}I-2.4G2 IgG in the presence of the respective drug(s). To assure that the macrophages were exposed to the drug(s) continuously, the washing media used throughout these experiments contained the drug(s) at the appropriate concentrations. Colchicine was prepared at a concentration of 100 mM in water, cytochalasin B and D at 20 mM in dimethyl sulfoxide (DMSO), and PMA at 10 mg/ml in DMSO. All stock solutions were stored at −70°C.

Results

Previous work documented the modulation of FcR when macrophages were plated on preformed antigen-antibody complexes (1, 2). Under those conditions FcR modulation occurred during the relatively asynchronous processes of cell settling, adherence, and spreading. To study the rate and mechanism of receptor modulation and to examine the effects of drugs that inhibit cell attachment and spreading we developed a method for forming immune complexes underneath macrophages already adherent to antigen (DNP)-coated surfaces. Macrophages plated on PLL-DNP-coated coverslips were washed and incubated with RαDNP IgG at 2–3°C for 45 min. After this period RαDNP IgG that had not bound to the substrate was removed by washing at 2–3°C. Some of the coverslips were maintained at 2–3°C; others were incubated at 37°C for

1 h and then tested for their capacity to bind E(IgG). Binding of E(IgG) was markedly inhibited in macrophages incubated at 37°C, but not at 2–3°C (Table I). Moreover, the activities of complement and mannose receptors, as measured by binding of complement-coated E (Table I) and phagocytosis of zymosan (6) (data not shown), were fully expressed. These results indicate that the sequential method of immune complex formation caused selective modulation of FcR.

As indicated in Table I, some macrophages were trypsinized before assay for E(IgG) binding. Previous work (2) established that Ig2a receptors (FcRI) are incompletely modulated on DNP-RαDNP IgG complexes and that trypsinization blocks residual E(IgG) binding by these trypsin-sensitive receptors. As expected, trypsin treatment abolished virtually all residual E(IgG) binding by macrophages on both preformed and sequentially prepared immune complex-coated coverslips, but had little effect on E(IgG) binding by macrophages on control coverslips (compare lines 6 and 9, Table I, with line 4).

To define quantitatively the specificity of receptor modulation by substrate-adherent immune complexes we measured binding of ^{125}I-labeled monoclonal antibodies 2.6 and 2.4G2 to macrophages to these substrates. Monoclonal antibody 2.6 (7) binds to a 20,000-D membrane protein. Monoclonal antibody 2.4G2 binds to FcRII (8). The conditions used for quantitating the number of ^{125}I-labeled monoclonal antibodies bound to each coverslip were identical to those used and validated previously (1).

Macrophages were plated at 37°C on PLL-DNP-coated coverslips. The coverslips were then cooled to 2–3°C, and some were overlaid with RαDNP IgG to permit the formation of DNP-RαDNP IgG complexes. Control coverslips were incubated in buffer alone. After 45 min at 2–3°C, the cultures were washed to remove unreacted

TABLE I

Comparison of FcR Modulation in Macrophages on Preformed vs. Sequentially Assembled Substrate-adherent DNP-RαDNP IgG Complexes

Sequence of treatment of PLL-coated coverslips	Resident macrophages		Thioglycollate macrophages			
	Percent macrophages attaching E(IgG)	Attachment index E(IgG)	Percent macrophages attaching E(IgG)	Attachment index E(IgG)	Percent macrophages attaching E(IgM)C	Attachment index E(IgM)C
None	ND*	ND	84	1,730	99	2,000
RαDNP IgG (22°C)	ND	ND	92	1,122	98	2,000
DNP → Macrophages plated (37°C)	94	1,225	92	1,806		
DNP → Macrophages plated (37°C) → trypsin (37°C)	83	879	86	1,536		
DNP → RαDNP IgG (22°C) → macrophages plated (37°C)	43	221	38	388		
DNP → RαDNP IgG (22°C) → macrophages plated (37°C) → trypsin (37°C)	5	19	8	54		
DNP → Macrophages plated (37°C) → RαDNP IgG (2.5°C)	89	904	90	1,384		
DNP → Macrophages plated (37°C) → RαDNP IgG (2.5°C) → (37°C)	48	206	29	144		
DNP → Macrophages plated (37°C) → trypsin (37°C) → RαDNP IgG (2.5°C) → (37°C)	13	37	19	48		

Assay conditions were as described in Materials and Methods and Results. Coverslips containing preformed immune complexes were prepared as described (1).

* Not done.

IgG. Some cultures were exposed immediately at 2°C to saturating amounts of ^{125}I-labeled monoclonal antibody; other cultures were warmed to 37°C for 2 h and then reacted with the ^{125}I-labeled monoclonal antibodies. When the macrophages were maintained continuously at 2–3°C on DNP-RαDNP IgG-coated coverslips, there was no decrease in binding of ^{125}I-2.4G2 IgG or ^{125}I-2.6 IgG to their respective antigens (Table II). Upon warming to 37°C, binding of ^{125}I-2.4G2 IgG as well as of 2.6 to macrophages on control coverslips remained virtually unchanged (Table II, line 3). However, when the cells were allowed to react at 37°C with the immobilized DNP-RαDNP IgG complexes, binding of ^{125}I-2.4G2 IgG to the macrophages was decreased by 72%, while the binding of ^{125}I-2.6 was reduced by only 24% (Table II, line 4). These results provide insight into the mechanism by which ligand-induced receptor modulation inhibits the binding of ^{125}I-2.4G2 IgG to FcR. We address this issue in the Discussion. For the moment, it is sufficient to point out that these data provide quantitative proof that modulation induced by DNP-RαDNP IgG-coated surfaces specifically affects FcR, and does not cause uniform redistribution of other macrophage membrane proteins.

Inhibitors of Cytoskeletal Function Do Not Affect FcR Modulation. The movement of many cell surface proteins requires the participation of the cytoskeleton (9, 10). FcR-mediated phagocytosis is inhibited by drugs that interfere with actin filament elongation (11). To determine whether intact cytoskeletal functions are required for FcR modulation to occur, macrophages were plated on PLL-DNP-coated coverslips, treated with cytochalasin B or colchicine at 37°C, incubated with RαDNP IgG at 2–3°C, and further incubated at 37°C in medium containing the same inhibitor. Control cultures were treated similarly except that the cytoskeletal inhibitors and/or RαDNP IgG were omitted. FcR modulation was assayed by binding of E(IgG). To reduce background binding due to incomplete modulation of FcRI (2), the macrophages were trypsinized before drug treatment as described above.

As shown in Table III, neither cytochalasin B nor colchicine, at concentrations that completely inhibit phagocytosis (12) and microtubule assembly (13), respectively, had any effect on the extent of FcR modulation in macrophages on immune complex-coated coverslips. Quantitatively similar results were obtained when podophyllotoxin (14) (10^{-4} - 10^{-6} M) or taxol (15) (10^{-5} M) were substituted for colchicine in this experiment (data not shown). Similarly, phorbol myristate acetate (10 ng/ml) had no

TABLE II

Comparison of the Effect of Immobilized Immune Complexes on the Expression of FcRII and an Unrelated 20,000-D Plasma Membrane Protein

Treatment of PLL-DNP coverslips	Conditions of incubation	Binding of ^{125}I-antimacrophage antibodies	
		2.4G2	2.6
None	2°C, 45 min*	9.69	4.58
RαDNP IgG	2°C, 45 min*	9.23	4.46
None	2°C, 45 min → 37°C, 2 h	9.30	4.02
RαDNP IgG	2°C, 45 min → 37°C, 2 h	2.79	3.48

* At the end of this incubation period the macrophage-coverslip cultures were washed in ice-cold PD and immediately covered with 60 μl ice-cold PD containing 1 mg/ml BSA and the radio-iodinated monoclonal antibody, and further incubated at 2°C for 60 min. The coverslips were then washed and assayed for radiolabeled antibody as described in Materials and Methods.

TABLE III

Effect of Colchicine and Cytochalasin B on FcR Modulation *

Treatment of PLL-coated coverslips	Treatment of macrophages		Resident macrophages		Thioglycollate-elicited macrophages	
	Inhibitor	Concentration	Macrophages attaching E(IgG)	Attachment index	Macrophages attaching E(IgG)	Attachment index
		M	%		%	
DNP‡			76	1,398	80	1,240
DNP + RαBSA IgG§			82	1,025	ND	ND
DNP + RαDNP IgG			2	14	4	31
DNP	Colchicine‖	100	88	739	ND	ND
DNP	Colchicine	1	93	846	ND	ND
DNP + RαDNP IgG	Colchicine	100	0	0	ND	ND
DNP + RαDNP IgG	Colchicine	1	2	16	ND	ND
DNP	Cytochalasin B¶	100	ND	ND	82	1,148
DNP	Cytochalasin B	10	80	800	91	1,474
DNP	Cytochalasin B	1	96	874	89	1,709
DNP + RαDNP IgG	DMSO**		5	21	ND	ND
DNP + RαDNP IgG	Cytochalasin B	100	ND	ND	14	63
DNP + RαDNP IgG	Cytochalasin B	10	3	11	9	72
DNP + RαDNP IgG	Cytochalasin B	1	3	21	30	291

* Cells were cultured in 24-well Linbro dishes. Each well contained a PLL-DNP-coated 12 mm diam glass coverslip and 0.4 ml MEM. 0.1 ml of MEM containing resident peritoneal cells (4×10^6 cells/ml) or thioglycollate-elicited peritoneal cells (2×10^6 cells/ml) was added to each well; the cells were incubated at 37°C for 0.5 h, washed to remove nonadherent cells, and trypsinized as described in Materials and Methods. The coverslip cultures were transferred to fresh Linbro wells containing 0.4 ml MEM and the drug indicated, and incubated at 37°C for an additional 0.5 h in MEM or MEM and cytochalasin B, and for 1.5 h in MEM and colchicine. The cultures were then placed at 2°C; each coverslip was treated with 10 μg RαDNP IgG in 0.05 ml PD as described in Materials and Methods, washed, and further incubated in fresh medium containing the indicated drug for 0.5 h at 37°C. 0.1 ml of a 1% suspension of E(IgG) was added for 0.5 h at 37°C. The indicated drug was present in all media and washing buffers with the exception of those used in control cultures. ND, not done.

‡ Overlaid with 0.05 ml PD-BSA.

§ RαBSA IgG was used at 17 μg in 0.05 ml PD.

‖ Colchicine was freshly prepared on the day of an experiment from a stock solution.

¶ Cytochalasin B was freshly prepared on the day of an experiment from a stock solution.

** DMSO was present in MEM at 0.5% vol/vol, i.e., equivalent to the amount present in MEM containing 10 μM cytochalasin B.

inhibitory effect.

Treatment of macrophages on control coverslips with colchicine or cytochalasin B caused a small (35%) reduction in the number of bound E(IgG) (Table III). This could be due to the decrease in cell surface area caused by these drugs (13). However, the almost complete abolition of E(IgG) binding observed in cytochalasin B-treated macrophages on DNP-RαDNP IgG-coated coverslips cannot be due to plasma membrane retraction since these macrophages remained well spread in the presence of cytochalasin B (see Fig. 2).

To examine the possibility that inhibitors of cytoskeletal function slowed FcR modulation we used ^{125}I-2.4G2 IgG to measure quantitatively the rate of FcRII modulation. Macrophages plated on PLL-DNP-coated coverslips were pretreated with colchicine or with cytochalasin B or D at 37°C, incubated with RαDNP IgG at 2–3°

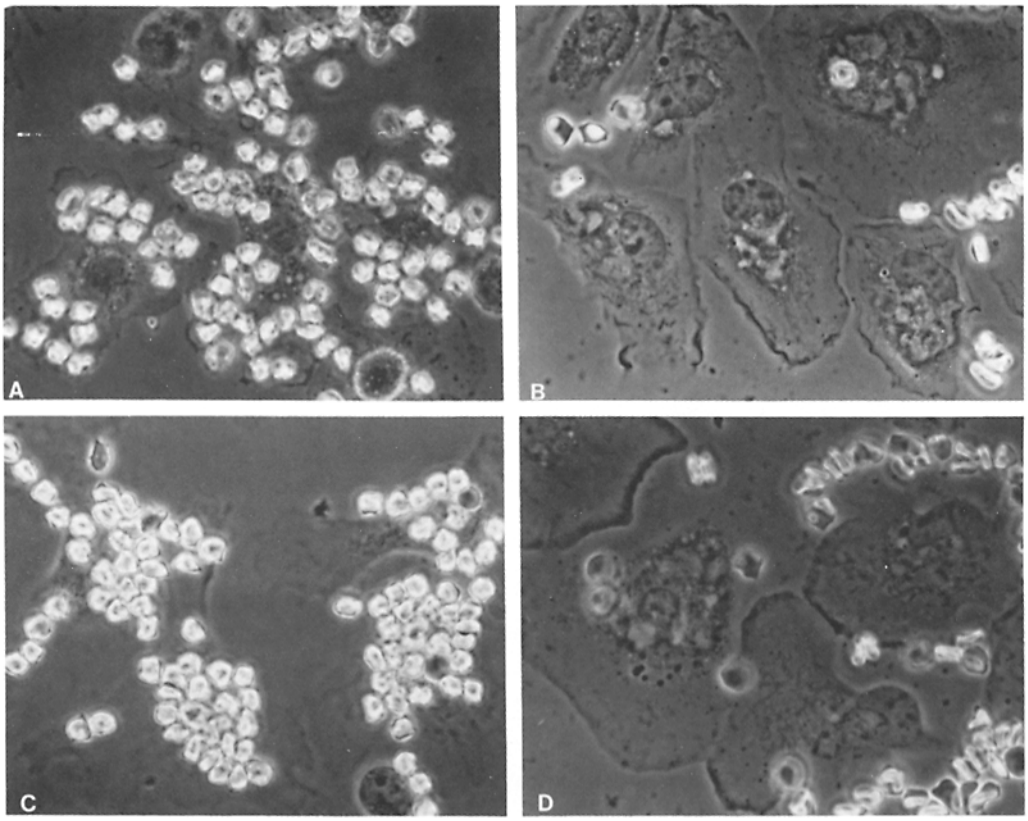

FIG. 2. Phase-contrast micrographs of colchicine- or cytochalasin B-treated thioglycollate-elicited macrophages on control and immune complex-coated coverslips. Experimental conditions are exactly as described in Table III. Macrophages were pretreated with colchicine (10 μM) (*A* and *B*), or cytochalasin B (10 μM) (*C* and *D*), and maintained in the presence of these drugs on PLL-DNP-coated (*A* and *C*) or PLL-DNP RαDNP IgG-coated (*B* and *D*) coverslips. Cells were assayed for E(IgG) binding at the end of the experiment. Note the well-spread appearance and reduced E(IgG) binding of drug-treated cells on immune complex-coated coverslips (*B* and *D*). × 800.

to allow immune complex formation, washed, and then warmed to 37°C for varying time periods in the presence of the corresponding drugs. At the times indicated (Fig. 3) the cells were again cooled to 2–3°C and assayed for their capacity to bind ^{125}I-2.4G2 IgG. Neither colchicine nor cytochalasin B had any measurable effect on the rate or extent of FcRII modulation (Fig. 3*A* and *B*). The rate of FcR modulation may have been slightly enhanced in cells treated with cytochalasin D, but we have not studied this effect further. These experiments establish that intact microtubule and microfilament systems are not required for FcRII modulation to occur.

Metabolic Inhibitors Do Not Affect FcR Modulation. To determine whether receptor modulation requires metabolic energy, macrophages on PLL-DNP-coated coverslips were incubated at 37°C in medium containing 2-dG and NaCN. (Macrophages incubated in medium containing 2-dG for 2 h exhibit a 75% decrease in ATP content [16] and a 90% decrease in creatine phosphate content [J. D. Loike and S. C. Silverstein, unpublished observations]). The coverslip cultures were then treated with RαDNP IgG at 2–3°C in medium containing 2-dG and NaCN, washed, and further

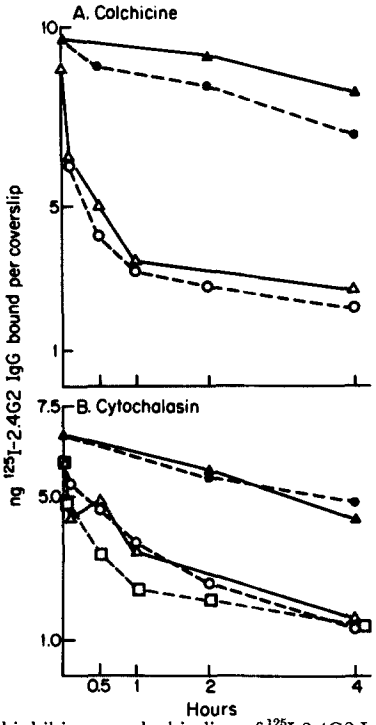

FIG. 3. Effect of cytoskeletal inhibitors on the binding of ^{125}I-2.4G2 IgG by macrophages on DNP-RαDNP IgG complexes. 2.5×10^5 thioglycollate-elicited macrophages were plated on PLL-DNP coated coverslips pretreated with colchicine (10^{-5} M) for 1.5 h at 37°C (*A*), or with cytochalasin B or D (10^{-5} M) for 0.5 h at 37°C (*B*), and processed exactly as described in the Table III legend and in the text. (*A*) Colchicine-treated macrophages on PLL-DNP-coated (●) or on PLL-DNP/RαDNP IgG-coated (○) coverslips. Control macrophages on PLL-DNP-coated (▲) or on PLL-DNP/RαDNP IgG-coated (△) coverslips. (*B*) Cytochalasin B-treated macrophages on PLL-DNP-coated (●) or on PLL-DNP/RαDNP IgG-coated (○) coverslips. Cytochalasin D-treated macrophages on PLL-DNP/RαDNP IgG-coated (□) coverslips. Control macrophages on PLL-DNP-coated (▲) or on PLL-DNP/RαDNP IgG-coated (△) coverslips.

incubated in 2-dG and NaCN at 37°C. The rate and extent of FcR modulation was measured by the binding of E(IgG) and of ^{125}I-2.4G2 IgG. Treatment of the macrophages with these metabolic inhibitors did not decrease the rate or extent of FcRII modulation (Fig. 4, *B* and *C*). Similar results were obtained when resident macrophages were incubated for 0.5–2 h in medium containing 2-dG (5×10^{-2} M) or NaF (10^{-2} M), and when thioglycollate-elicited macrophages were incubated for similar intervals with these inhibitors (data not shown). Preincubation of macrophages for 45 min in medium supplemented with cycloheximide (10 μg/ml) also had no effect on FcR modulation.

Inhibition of Pinocytosis Does Not Affect FcR Modulation. Macrophages exhibit high rates of pinocytosis and membrane recycling (17–19). The experiments described above do not resolve whether FcR modulation occurs by internalization of these receptors and their reinsertion into the substrate-adherent membrane, or by lateral movement of receptors in the plane of the macrophage membrane. To distinguish between these two possibilities we measured the rate of pinocytosis in macrophages treated with 2-dG and NaCN (Fig. 4*A*). As expected, in the presence of these

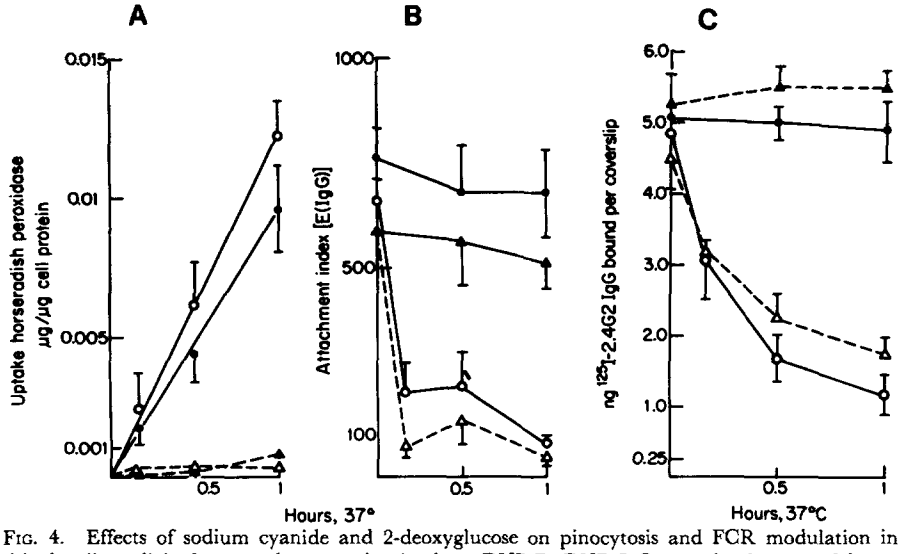

FIG. 4. Effects of sodium cyanide and 2-deoxyglucose on pinocytosis and FCR modulation in thioglycollate-elicited macrophages maintained on DNP-RαDNP IgG-coated substrates. Macrophages in PLL-DNP-coated 35-mm petri dishes or on PLL-DNP-coated glass coverslips were incubated for 0.5 h at 37°C in medium containing NaCN (1 mM) and 2-dG (50 mM). The cells were then overlayed with fresh cold (2°C) medium containing the same combination of inhibitors and RαDNP IgG (200 μg in 1 ml PD for cells in petri dishes; 10 μg in 0.05 ml PD for cells on coverslips), maintained at this temperature for 45 min to allow immune complexes to form, washed, and further incubated at 37°C in NaCN and 2-dG-containing medium. Control cultures were processed similarly, except that the treatments with NaCN and 2-dG and/or RαDNP IgG were omitted. At the indicated time intervals the cultures were assayed as follows: (*A*) HRP (1 mg/ml) was added to the medium of cells in petri dishes for the final 0.5–1 h incubation at 37°C. The cells were then washed and assayed for their content of HRP as described in Materials and Methods. (*B*) 1.5×10^5 thioglycollate-elicited peritoneal cells were plated on glass coverslips for 0.5 h at 37°C, washed, and trypsinized as described in Materials and Methods. E(IgG) binding was assayed on duplicate sets of coverslip cultures at 2–3°C as described (2). (*C*) Binding of ^{125}I-2.4G2 IgG to macrophages was assayed at 2–3°C as described (1). Symbols in all panels as follows: macrophages in medium without inhibitors on PLL-DNP-coated (●) or on PLL DNP-RαDNP IgG-coated (○) surfaces; macrophages in NaCN- and 2-dG-containing medium on PLL-DNP-coated (▲) or PLL DNP-RαDNP IgG-coated (△) surfaces.

inhibitors pinocytosis was reduced by >95% in macrophages cultured on control or immune complex-coated coverslips. Thus endocytosis and reinsertion of receptors cannot account for the receptor modulation observed in metabolically poisoned cells. Modulation must occur via movement of receptors within the plane of the membrane. The insensitivity of FcR movement to metabolic and cytoskeletal inhibitors suggests that it is diffusion limited. If so, FcR movement should be affected by low temperatures.

FcR Modulation Is Slowed at Low Temperatures. To examine the effect of temperature on FcRII modulation, macrophages on PLL-DNP-coated coverslips were incubated at 2–3°C with RαDNP IgG, washed at 2°C and processed as follows: Some coverslips were further incubated at this temperature whereas other coverslips were transferred to a specially designed cooling block (see Materials and Methods) and kept at the desired temperature. At the time points indicated the coverslip cultures were removed, cooled in <0.5 min to 2–3°C, and kept at this temperature for 15 min at which point the macrophages' capacity to form rosettes with E(IgG) and to bind ^{125}I-2.4G2 IgG

was examined. Control cultures on DNP-coated coverslips were processed in the same way but in the absence of RαDNP IgG. In experiments in which E(IgG) binding was measured, the macrophages were trypsinized to reduce binding due to incomplete modulation of FcRI.

Within 2 min of transfer of macrophages on DNP-RαDNP IgG-coated coverslips to 37°C, FcRII-mediated binding of E(IgG) decreased by 82%, and reached its maximal extent (88% reduction) by 10 min. FcRII modulation occurred at about equal speed at 21 and 15°C (Fig. 5*A*). It was significantly slowed at 4°C, and at 2°C there was only a small (12%) reduction in E(IgG) binding after 30 min. Complete FcR modulation occurred at 2°C, but only after the macrophages were incubated on immune complex-coated coverslips for >18 h at this temperature (data not shown).

The effect of reduced temperature on FcRII modulation also was quantitated using ^{125}I-2.4G2 IgG. No decrease in ^{125}I-2.4G2 IgG binding was observed in macrophages maintained on immune complexes at 2–3°C (Fig. 5*B*). Increasing the temperature to 15, 21, or 37°C led to stepwise increases in the rate and extent of FcRII modulation. ^{125}I-2.4G2 IgG binding fell to 60% of control value within 2 min of warming macrophages on immune complexes to 37°C; ^{125}I-2.4G2 IgG binding further decreased to 25% of control after 60 min at 37°C. Macrophages maintained at 2–3°C on DNP-coated coverslips, or exposed to control IgG and then warmed to 37°C for 30 min, showed no decrease in ^{125}I-2.4G2 IgG binding capacity.

From a comparison of Fig. 5*A* and *B* it is evident that there are differences in the rate and extent of FcRII modulation as measured by binding of E(IgG) and ^{125}I-2.4G2 IgG. Our interpretation of these differences is described in the Discussion. For the moment, it is sufficient to point out that these data show that FcRII modulation is a

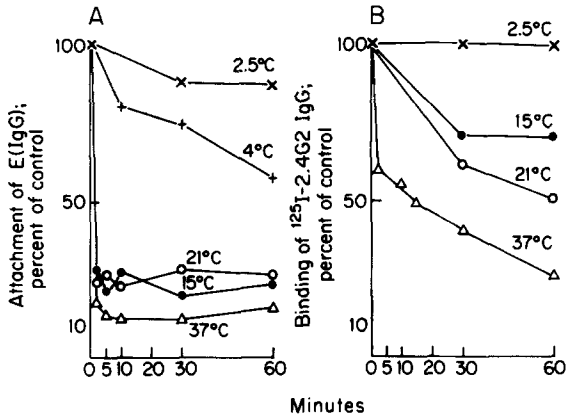

FIG. 5. Effect of reduced temperature on FcR modulation. Thioglycollate-elicited peritoneal cells were plated on PLL-DNP-coated coverslips at a concentration of 1.5 × 10^5 per coverslip for the experiment described in (*A*) and 2.5 × 10^5 per coverslip for the experiment described in (*B*). Duplicate coverslips were used for each time point in both experiments. The cultures were incubated with RαDNP IgG for 45 min at 2–3°C and then washed at 2–3°C (as described in Materials and Methods). Some coverslip cultures were used immediately thereafter to measure E(IgG) binding (*A*) or ^{125}I-2.4G2 IgG binding (*B*) to the macrophages at 2°C for 1 h. The values obtained are indicated as 100%. Additional coverslip cultures were overlaid with MEM and incubated at the temperatures indicated, using the cooling block described in Materials and Methods. At appropriate time intervals coverslip cultures were cooled to 2–3°C and assayed for E(IgG) or ^{125}I-2.4G2 binding at this temperature. The results are expressed as percent of the initial value. (*A*) E(IgG) attachment. (*B*) ^{125}I-2.4G2 IgG binding.

temperature-dependent process. In conjunction with previous data (Figs. 3 and 4 and Table III), they provide strong support for the concept that FcRII modulation occurs by lateral diffusion in the plane of the membrane.

FcR Modulation in Formaldehyde-treated Macrophages. Poo and Cone (20) showed that diffusion of rhodopsin in outer rod segment membranes is not affected by fixation of the tissue with formaldehyde, but is abolished by fixation with glutaraldehyde. To determine the feasibility of similar studies in this system, macrophages were treated with varying concentrations of these fixatives and then tested for their capacity to bind E(IgG) and ^{125}I-2.4G2 IgG. Binding of E(IgG) and of ^{125}I-2.4G2 IgG was abolished by all concentrations of glutaraldehyde tested (0.5–2%). However, cells treated with 0.1–0.5% formaldehyde exhibited no decrease in binding of E(IgG) or of ^{125}I-2.4G2 IgG (Fig. 6, *A* and *B*) and continued to exclude trypan blue (data not shown). As expected, 0.1% formaldehyde completely abolished E(IgG) ingestion (Fig. 6*A*) and HRP pinocytosis (data not shown). Formaldehyde concentrations >0.5% caused significant inactivation of FcRII function (as measured by E(IgG) binding), and antigenicity (as measured by ^{125}I-2.4G2 IgG binding) (Fig. 6, *A* and *B*). For this reason, all subsequent experiments were performed using 0.1–0.5% formaldehyde-treated phagocytes.

To determine if formaldehyde-fixed macrophages could modulate FcR, macrophages on PLL-DNP coverslips were trypsinized to remove FcRI, and further incubated in the presence or absence of 0.1–0.5% formaldehyde. The cells were then incubated at 2–3°C with RαDNP IgG according to the sequential method, warmed to 37°C for 1 h, and assayed for E(IgG) binding. Formaldehyde-treated macrophages on immune complex-coated coverslips showed an 83–92% decrease in E(IgG) binding capacity (Table IV). Macrophages on PLL-DNP coverslips treated in parallel with

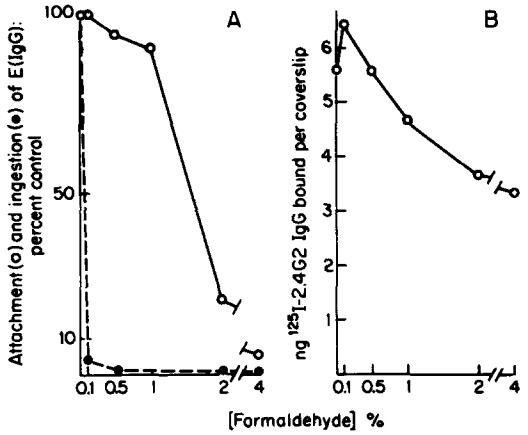

FIG. 6. Effect of various concentrations of formaldehyde on the capacity of macrophages to bind E(IgG) and ^{125}I-2.4G2 IgG. Thioglycollate-elicited peritoneal cells (1.5×10^5 [*A*] and 2.5×10^5 [*B*]) were plated on PLL-DNP-coated coverslips at 37°C for 30 min. The coverslip cultures were then washed, treated with the indicated concentrations of formaldehyde in 0.1 M sodium phosphate buffer (pH 7.2) for 15 min at room temperature, washed, and incubated for an additional 15 min in Hepes-HBSS-BSA. The cells were then treated as follows. (*A*) Macrophages incubated for 1 h at 37° with 0.05 ml of a 1% suspension of E(IgG) in Hepes-HBSS-BSA and assayed for E(IgG) binding (○) and phagocytosis (●). (*B*) Macrophages incubated at 2–3°C with ^{125}I-2.4G2 IgG in Hepes-HBSS-BSA and assayed for the amount of ^{125}I-2.4G2 IgG bound as described in Materials and Methods.

TABLE IV

Effect of Formaldehyde on FcR Modulation

Treatment of macrophages*	Treatment of PLL-DNP-coated coverslips	Percent macrophages attaching E(IgG)‡	Attachment index
Phosphate buffer 0.1 M	None	87.7 ± 8.7	701 ± 102
Phosphate buffer 0.1 M	RαDNP IgG§	15 ± 7	45 ± 17
Formaldehyde 0.5%	None	90 ± 2	728 ± 38
Formaldehyde 0.5%	RαDNP IgG	43 ± 5.2	125 ± 28
Formaldehyde 0.5%	RαBSA IgG‖	99 ± 0	635 ± 106
Formaldehyde 1%	None	93.2 ± 1.5	548 ± 24
Formaldehyde 1%	RαDNP IgG	12.9 ± 11.1	46 ± 25

* 1.5×10^5 thioglycollate-elicited peritoneal cells were plated for 30 min at 37°C on PLL-DNP-coated coverslips, washed, trypsinized, and incubated for 15 min at 21°C with 0.1 M phosphate buffer (pH 7.2) with or without formaldehyde. After washing, the cultures were transferred to Linbro covers on ice, and substrate-adherent immune complexes were formed according to the "sequential" method. The cultures were then washed and transferred to Linbro wells containing 0.5 ml MEM at 37°C. 1 h later 0.1 ml of 1% E(IgG) was added per well, and the incubation was continued in PBS for 1 h at 2°C for cultures treated with phosphate buffer (controls) and at 37°C for formaldehyde-treated cultures.

‡ Mean ± SD of two to four experiments using duplicate coverslips.

§ RαDNP IgG was used at 0.26 mg/ml in PD.

‖ RαBSA IgG was used at 0.34 mg/ml in PD.

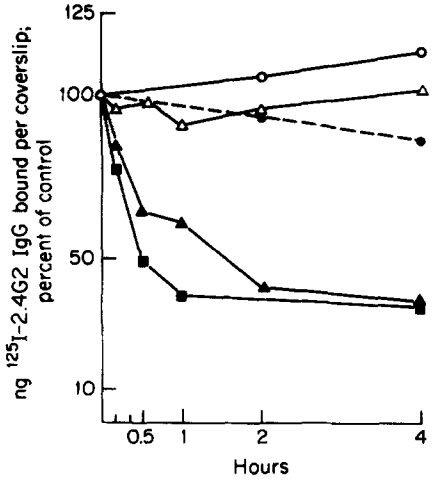

FIG. 7. Effect of formaldehyde on the binding of ^{125}I-2.4G2 IgG to macrophages maintained on immune complexes. Thioglycollate-elicited macrophages on PLL-DNP-coated coverslips were prepared as described for Fig. 6*B*. Some of the cultures were fixed with 0.5% formaldehyde (vol/vol) as described for Fig. 6. Control and formaldehyde-fixed cultures were then cooled to 2–3°C, incubated at 2–3°C with RαDNP IgG (described as the sequential method in Materials and Methods), washed, incubated at 2–3°C or 37°C for the indicated time periods, and assayed for ^{125}I-2.4G2 IgG binding at 2°C as described in Materials and Methods. Formaldehyde-fixed macrophages incubated at 2–3°C (○) or 37° (△) in 0.1 M phosphate buffer. Unfixed macrophages incubated at 2–3°C (●) or 37°C (▲) in 0.1 M phosphate buffer. Unfixed macrophages incubated at 37°C in Hepes-HBSS (■).

control IgG (RαBSA IgG) showed no FcRII modulation (Table IV). These results show that receptor modulation does not require viable macrophages, and are consistent with our interpretation that FcR modulation on immune complex-coated surfaces occurs via diffusion in the plane of the membrane. Similar experiments were per-

formed using ^{125}I-2.4G2 IgG to measure FcR modulation. No modulation was detected in formaldehyde-treated cells with this assay (Fig. 7). These findings, like those in Fig. 5 *B*, are in apparent conflict with the results obtained using E(IgG) to measure FcRII modulation. However, examination of the mechanism by which immune complex-coated surfaces block binding of ^{125}I-2.4G2 IgG indicates that these results are not inconsistent with one another and that only E(IgG) binding provides an accurate assessment of the rate of FcR removal from the macrophages' upper surface. This is explained in the Discussion.

Discussion

Using the sequential method of immune complex formation described in this paper, we have been able to initiate FcR modulation synchronously in a population of macrophages already adherent to and spread upon coverslips. This has allowed us to analyze the mechanism of FcR modulation. Our studies show that receptor modulation induced by substrate-adherent antigen-antibody complexes occurs in macrophages preincubated with cytoskeletal (Fig. 3) and metabolic (Fig. 4) inhibitors, and in macrophages treated with formaldehyde (Table IV). These results rule out endocytosis or shedding of receptors as causes of modulation, since neither process would occur in metabolically poisoned or formaldehyde-fixed cells. Only incubation of macrophages at low temperature inhibited FcR modulation. These findings lead us to conclude that unligated FcR diffuse in the plane of the macrophage membrane, and that the effect of substrate-adherent immune complexes is to trap FcR that diffuse into the segment of plasma membrane in contact with the substrate. Our findings using E(IgG) binding as a probe for FcR modulation are entirely consistent with this hypothesis.

The results obtained using ^{125}I-2.4G2 IgG binding to measure FcR modulation agree with those obtained using E(IgG) binding except in the experiments (Figs. 5 and 7) in which low temperature and formaldehyde were used. These exceptions are easily reconciled once the mechanism by which FcR become inaccessible to ^{125}I-2.4G2 IgG is understood. Macrophages maintained on immune complex-coated substrates at 37°C adhere so tightly to the substrate as to prevent access of macromolecules, such as fluorescein-labeled IgG, into the space between the substrate and the macrophage's substrate-adherent plasma membrane (21). Closure of this space to large protein molecules does not occur when macrophages are maintained at 37°C on control coverslips, or on immune complex-coated coverslips at 2°C, or after fixation with formaldehyde (Michl, unpublished observations), and under these conditions ^{125}I-2.4G2 IgG has access to the cell's undersurface.

Moreover, 2.4G2 IgG has a high affinity (Ka, 10^{-9} M) for FcRII (22) and under appropriate conditions can be shown to compete efficiently for this receptor with substrate-adherent immune complexes containing rabbit IgG (Ka, 10^{-7} M) (23, 24). For instance, formaldehyde-fixed macrophages that had been rosetted with E(IgG) were incubated with 2.4G2 IgG (4 μg/ml) at 2–3°C, or 37°C for 1 h. This caused a 70 or 84% decrease, respectively, in the number of E(IgG) that remained bound to these macrophages compared with formalin-fixed macrophages incubated under similar conditions in the absence of 2.4G2 IgG. We think this explains why there is no decrease in binding of ^{125}I-2.4G2 IgG to formaldehyde-fixed macrophages incubated at 37°C (Fig. 7), or to viable macrophages incubated at 2–3°C on immune complex-

coated coverslips (Table I, Figs. 4, 5, and 7).

The failure of metabolic (Fig. 4) and cytoskeletal (Fig. 3) inhibitors to slow or decrease ^{125}I-2.4G2 IgG binding to macrophages on immune complex-coated coverslips indicates that the macrophages are able to adhere tightly to the substrate in the presence of these compounds. (In the case of colchicine- and cytochalasin-treated cells this is not surprising, since these drugs do not cause significant membrane retraction in macrophages on immune complex-coated coverslips [Fig. 2]) Similarly, the decreased rate of disappearance of FcR measured by ^{125}I-2.4G2 IgG binding in macrophages maintained at 15 or 21°C on immune complexes (Fig. 5) probably reflects a slower rate of sealing and/or incomplete closure of the space between macrophage and coverslip at these temperatures.

Like FcRII, the macrophage surface antigen recognized by monoclonal antibody 2.6 remains completely accessible to this antibody in macrophages maintained on control coverslips, or at 2–3°C on DNP-RαDNP IgG-coated coverslips (Table II). However, 2.6 IgG shows a 24% decrease in binding to macrophages maintained at 37°C on DNP-RαDNP IgG-coated coverslips (Table II). From electron micrographs of macrophages plated on DNP-RαDNP IgG-coated surfaces we estimate that ~35% of the cell's surface is contiguous to the substrate. Assuming that the antigen recognized by 2.6 IgG is randomly distributed on the macrophage plasma membrane, the 24% reduction in binding of this antibody to macrophages maintained at 37°C on DNP-RαDNP IgG-coated coverslips could result from the inaccessibility of the corresponding antigens on the macrophage's substrate-adherent membrane to this antibody.

These findings indicate that the rate of disappearance of ^{125}I-2.4G2 IgG-binding sites reflects several processes operating simultaneously. Because of this complexity the apparent rate of loss of these binding sites is an unsuitable parameter for estimating the rate of FcRII removal from the macrophage's apical plasma membrane. However, ^{125}I-2.4G2 IgG binding provides a minimal estimate of the extent of FcRII removal once receptor modulation is complete.

The 2.6 IgG binding data show that 24% of the binding sites for this antibody are masked in macrophages plated on immune complex-coated substrates (Table II). Therefore, 24% of the FcRII on the plasma membranes of these macrophages also will be masked by this interaction, for the reasons cited above. Masking of these IgG-binding sites does not require removal of FcRII from the upper surface of the cell. After complete FcRII modulation 25% of the cell's FcRII remain accessible to ^{125}I-2.4G2 IgG (Tables I and II, Figs. 3–5, and reference 1). Thus, the 76% of the plasma membrane that is accessible to 2.6 IgG and that presumably constitutes the cell's upper surface retains ~33% (25%/76%) of its original FcRII concentration. This is surely an overestimate of FcRII remaining on the cell's upper surface since ^{125}I-2.4G2 IgG has access to FcRII at the edge of the macrophage's undersurface where these receptors are inaccessible to large particles such as E(IgG). These data indicate that, at a minimum, there is a 67% reduction in FcRII concentration in the upper surface of macrophages maintained at 37°C on immune complex-coated coverslips, and that this reduction is sufficient to drastically inhibit E(IgG) binding (Table I and Fig. 5*A*).

The modulation of FcRII following phagocytosis of IgG-coated erythrocyte ghosts has been studied by Mellman et al. (25). They observed a maximal decrease of 60% in the binding of ^{125}I-2.4G2 Fab after ingestion of the ghosts. This result, and our

findings of incomplete modulation of FcRI (2) and of FcRII (1) in macrophages plated on immune complex-coated surfaces, suggest that these cells bear a subpopulation of immobile receptors. A subpopulation (20–30%) of immobile membrane proteins (26–28), including the receptor for IgE (28), has been observed on a variety of cells using fluorescence photobleaching and recovery methods. It is possible that the fraction of macrophage FcR that is not modulated after ingestion of IgG-coated ghosts (25) or cultivation on immune complex-coated surfaces (1, 2) corresponds to the immobile receptors detected by the fluorescence photobleaching and recovery method.

The data in Fig. 5*A* show that E(IgG) binding is inhibited by ~82% 2 min after warming to 37°C. We estimate that the half-time for FcRII disappearance is about 100 s. Assuming thioglycollate-elicited macrophages are spread on the immune complex-coated coverslips like flattened discs with diam 40 μm (i.e., that they cover a surface area of ~1,200 μm^2), and using the formula described in the appendix to this paper, we calculate that FcRII has a diffusion coefficient of 2.5×10^{-9} cm^2/s at 37°C. This is approximately the same as the diffusion coefficient of $2–3 \times 10^{-9}$ cm^2/s estimated by Poo and Cone (20) for rhodopsin in the outer segments of frog rod cells, and of 1×10^{-9} cm^2/s by Woda et al. (28) for the mobile fraction of H-2 antigens on the surfaces of rat macrophages. It is 10-fold faster than the diffusion coefficients of 2×10^{-10} cm^2/s determined by Schlessinger et al. (29) for IgG FcR on rat mast cells, and of $2–3 \times 10^{-10}$ cm^2/s determined by Hafeman et al. (30) for C3b receptors on human neutrophils.

The relative insensitivity of FcR modulation to decreased temperature (Fig. 5) is consistent with the results of Vaz et al. (31) and Criado et al. (32) who used photobleaching methods to measure the diffusion of acetyl choline receptors and sarcoplasmic reticulum ATPase in cholesterol-containing liposomes. Their data show that these integral membrane proteins have diffusion coefficients of $1–2 \times 10^{-8}$ cm^2/s at 37°C and that decreasing the temperature to 14°C caused, at most, a twofold decrease in their rates of diffusion.

The finding that FcRII diffuses in the macrophage membrane provides two important insights into the way this receptor functions during phagocytosis and cell movement. First, FcR diffusion explains the perfect fit observed between the macrophage membrane and irregularly shaped IgG-coated particles during phagocytosis. Mobile receptors can accommodate spatially to the distribution of ligands on the particle's surface. Second, it indicates that physical contact between ligated FcR and the cytoskeleton may not be necessary for these receptors to regulate membrane movement. According to this view, FcR provide transmembrane signals that regulate both spatially and temporally the assembly of the cytoskeleton, but are not themselves linked to the cytoskeleton. In principle, this is the relationship between acetyl choline receptors and contractile elements in skeletal muscles. The finding that FcRII is an ion channel (33) suggests that a similar relationship between receptors and cytoskeleton may prevail in macrophages and other motile cells.

Summary

We describe a method for synchronously assembling antigen-antibody complexes underneath macrophages adherent to an antigen-coated surface. We have used this method to study the mechanism of Fc receptor (FcR) disappearance that occurs when

resident and thioglycollate-elicited mouse macrophages are cultured on immune complex-coated surfaces. Erythrocytes opsonized with IgG (E(IgG)) and a monoclonal antibody (2.4G2 IgG) directed against the trypsin-resistant FcR (FcRII) were used as indicators of the presence and distribution of FcRII molecules on the macrophage plasma membrane. Inhibitors of aerobic (NaCN) and anerobic (2-deoxyglucose, NaF) glycolysis and pinocytosis, of protein biosynthesis (cycloheximide), and of cytoskeletal function (cytochalasin B and D, colchicine, podophyllotoxin, taxol) did not reduce the rate or extent of FcRII modulation. Moreover, treatment of the macrophages with 0.1–0.5% formaldehyde did not reduce the extent of FcRII modulation as measured by the disappearance of E(IgG) binding sites. FcRII modulation was markedly slowed when the temperature was decreased to 2–4°C. These results prove that FcRII modulation is governed by diffusion of the receptor in the plasma membrane. From the speed of FcRII disappearance from the macrophage's upper surface we calculate that the receptor has a diffusion coefficient at 37°C of 2.5×10^{-9} cm^2/s. This finding indicates that FcRII, in its unligated form, is not linked to the macrophage's cytoskeleton, and that the receptor is capable of accommodating spatially to any distribution of ligands on a particle's surface.

Appendix

Estimation of Receptor Diffusion Constant from Rate of Receptor Modulation. Consider a macrophage spread on a ligand-bearing surface. As receptors move about on the membrane of the cell we suppose they are likely to become bound to ligand molecules on the substrate whenever they are on portions of the membrane adjacent to the substrate. We may estimate the receptor diffusion constant (D) from the rate of receptor disappearance from the apical surface of the cell, by using the following simple model.

Let us approximate a spread macrophage by a circular disc of radius R. Initialy, at time (t) = 0 when the cells are first warmed, let the number of receptors per unit area on the upper surface be a constant, n_0, independent of position. Thereafter assume that the receptors diffuse about on the upper surface, and that any receptor that reaches the edge of the disc sticks there, bound by ligand on the substrate. The number of receptors per unit area, n(r, t) then satisfies the diffusion equation

$$\frac{\partial n}{\partial t} = D\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial n}{\partial r}\right) = D\left[\frac{\partial^2 n}{\partial r^2} + \frac{1}{r}\frac{\partial n}{\partial r}\right] \tag{1}$$

with initial condition $r(r, 0) = n_0$ ($r \leq R$) and boundary condition $n(R, t) = 0$ ($t > 0$).

This problem is mathematically equivalent to the radial flow of heat in an infinitely long rod initially at a uniform temperature and with the surface thereafter held at zero temperature. The solution to this problem is given in reference 34 and as follows:

$$n(r, t) = \frac{2n_0}{R}\sum_{n=1}^{\infty}\exp(-D\alpha_n^2 t)\frac{J_0(r\alpha_n)}{\alpha_n J_1(R\alpha_n)} \tag{2}$$

where J_0 and J_1 are Bessel functions, and α_n are the roots of the equation

$$J_0(\alpha_n R) = 0. \tag{3}$$

Thus for example $\alpha_1 R = 2.4048$ and $\alpha_2 R = 5.5201$. Moreover, the fraction f of the receptors remaining on the upper surface after time t (cf. equation 6, p. 199 in reference 34) is

$$f = \frac{4}{R^2}\sum_{n=1}^{\infty}\frac{1}{\alpha_n^2}\exp(-D\alpha_n^2 t). \tag{4}$$

For $t > (D\alpha_1^2)^{-1}$ the first term alone is a good approximation (within 1% of the full series):

$$f = \frac{4}{(2.405)^2} e^{-t/\tau_D} \tag{5}$$

where

$$\tau_D = \frac{R^2}{(2.405)^2 D}. \tag{6}$$

The full solution is plotted in Fig. 8, where f is shown as a function of t/τ_D.

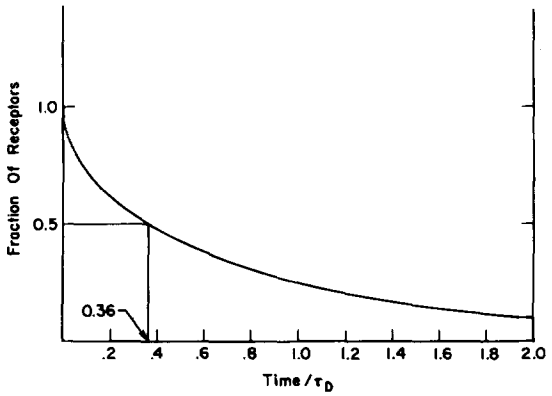

FIG. 8. Fraction (f) of receptors on the top surface of macrophage as a function of time. Time (t) is in units of $\tau_D = R^2/(2.405)^2 D$ where R denotes radius and D, diffusion constant. Note that half the receptors are gone ($f = \frac{1}{2}$) when $t/\tau_D = 0.36$.

The asymptotic formula, equation 5, would suggest that $f = \frac{1}{2}$ when $t/\tau_D = \ln[2 \times 4/(2.405)^2] = 0.32$ but the full solution predicts $f = \frac{1}{2}$ when $t = 0.36\ \tau_D$. If $t_{1/2}$ is an experimental value of the time for disappearance of half the receptors, then we may estimate D from the equation

$$D = \frac{R^2\, 0.36}{(2.405)^2 t_{1/2}} = 0.062 \frac{R^2}{t_{1/2}}. \tag{7}$$

Thus, for the values in the text ($R \cong 20\ \mu m$; $t_{1/2} \cong 100$ s), $D \cong 2.5 \times 10^{-9}\ cm^2/s$.

It may be noted that equation 5 predicts that the receptors will decrease by a factor of 2 whenever t/τ_D increases by a factor of 0.693 (= ln2). However, at early times, the other terms in equation 4 cannot be neglected, causing a more rapid drop. This is because at early times, the distribution of receptors is flat; as receptors near the edge are absorbed first, they will cause a more rapid drop of the free receptor population than can be sustained.

It appears that the estimate of the diffusion constant obtained by this model will be a lower limit to the true value, because as a receptor diffuses onto the lower (adjacent to the substrate) membrane of the cell, it will have to diffuse for a while until it encounters a free ligand to bind, during which migration it may return to the upper membrane. The model neglects this possibility, by assuming that every receptor that moves to the lower membrane never returns. Hence the model overestimates the rate of receptor modulation for a given diffusion constant or underestimates the diffusion constant that would produce a given rate of modulation.

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