LOSS OF Fc RECEPTOR ACTIVITY AFTER CULTURE OF HUMAN MONOCYTES ON SURFACE-BOUND IMMUNE COMPLEXES

Mediation by Cyclic Nucleotides*

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The properties of mononuclear phagocytes are influenced by contact with surface-bound stimuli. Spreading is greatly enhanced when the surface to which monocytes or macrophages adhere incorporates immune complexes (1, 2), and monocytes cultured on surface-bound IgG aggregates undergo a respiratory burst that generates superoxide anion and other oxygen products (3). Monocyte neutral protease activity also is greatly modified; granule-associated protease activity is elicited, and secretion of plasminogen activator is inhibited by culture on surface-bound immune complexes (4). Immune complex-stimulated monocyte spreading can be inhibited by cytoskeletal disruption with colchicine or cytochalasin B and by membrane-active local anesthetics (5). Superoxide anion generation and lysosomal enzyme release also can be inhibited by local anesthetics (6, 7).

Surface-bound immune complexes also affect the Fc receptor activity of mononuclear phagocytes. Resident mouse peritoneal macrophages plated onto surface-bound immune complexes lose the capacity to phagocytose IgG-sensitized sheep erythrocytes (SRBC)[1] (EA) but not latex particles or zymosan (8). Rosetting of such macrophages with EA is not affected, which suggests the maintenance of some Fc receptor function with a selective inhibition of receptor-triggered interiorization. In contrast, human monocytes lose the ability to form EA rosettes when incubated upon surface-bound immune complexes (2). The mechanism of reduction in Fc receptor function or number has not been elucidated; it is not known whether Fc receptors are interiorized, capped, or whether their activity is altered by some other means.

In this paper, we confirm that human peripheral blood monocytes lose EA rosetting ability when in contact with surface-bound immune complexes. Our results indicate

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[1] *Abbreviations used in this paper:* 2dG, 2-deoxy-D-glucose; 8 Br-cAMP, 8-bromoadenosine 3′:5′-cyclic monophosphoric acid; 8 Br-cGMP, 8-bromoguanosine 3′:5′-cyclic monophosphoric acid; DE-52, diethylaminoethyl cellulose; DFP, di-isopropylfluorophosphate; DME, Dulbecco's Modified Eagle's Medium; DME-LH, DME with 0.05% lactalbumin hydrolysate; EA, IgG-sensitized sheep erythrocytes; EAC, sheep erythrocytes sensitized with IgM and C3; FCS, fetal calf serum; GVB, veronal buffer containing 1% gelatin; HBSS, Hanks' balanced salt solution; HSA, human serum albumin; MIBX, methyl isobutyl xanthine; MNL, mononuclear leukocyte(s); PBS, phosphate-buffered saline; SOD, superoxide dismutase; SRBC, sheep erythrocyte(s).

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that this loss is not a result of Fc receptor migration or interiorization and is not mediated by cytoskeletal elements. Also, the loss does not result from damage of Fc receptors by lysosomal enzymes or oxygen radicals. We present data that suggest that after monocytes adhere to surface-bound immune complexes, Fc receptor activity is modulated by cyclic nucleotide-dependent mechanisms.

Materials and Methods

Materials. Dulbecco's Modified Eagle's Medium (DME), Hanks' balanced salt solution (HBSS), and fetal calf serum (FCS) were obtained from Grand Island Biological Co., Grand Island, N. Y. Lactalbumin hydrolysate (LH) was purchased from ICN Nutritional Biochemicals, Cleveland, Ohio. Bovine fibrinogen, superoxide dismutase (SOD), and human serum albumin (HSA) were obtained from Miles Laboratories, Inc., Elkhart, Ind. SRBC hemolysin was purchased from N. L. Cappel Laboratories Inc., Cochranville, Pa. Catalase was purchased from Boehringer Mannheim Biochemicals, Indianapolis, Ind. Cytochalasin B, acetaldehyde, and methyl isobutyl xanthine (MIBX) were obtained from Aldrich Chemical Co., Inc., Milwaukee, Wis. Cycloheximide, colchicine, D-L-propranolol, 8-bromoadenosine 3′:5′-cyclic monophosphoric acid (8 Br-cAMP), 8-bromoguanosine 3′:5′-cyclic monophosphoric acid (8 Br-cGMP), 2-deoxy-D-glucose (2dG), and ATP were purchased from Sigma Chemical Co., St. Louis, Mo. Lidocaine was obtained from Astra Pharmaceutical Products, Inc., Worcester, Mass., and 1-octanol from Mallinckrodt Inc., St. Louis, Mo. Ficoll-Hypaque and Sephadex G-200 were obtained from Pharmacia Fine Chemicals, Inc., Piscataway, N. J., and diethylaminoethyl cellulose (DE-52) from Whatman, Inc., Clifton, N. J. C5-deficient mice were kindly supplied by Dr. George Martin of the University of Washington, Seattle, Wash.

Preparation of Monolayers. Mononuclear leukocytes (MNL) were obtained by Ficoll-Hypaque centrifugation of EDTA-anticoagulated human blood. The cells were washed once in 0.005 M phosphate-buffered saline (PBS) that contained 1 mM EDTA, and twice in HBSS, and then were suspended in DME with 0.05% lactalbumin hydrolysate (DME-LH).

For immediate application to substrates, MNL were diluted to 0.8×10^6 cells/ml in DME-LH with or without drugs, and 2.5-ml aliquots were pipetted into 35-mm culture plates coated with fibrin or fibrin that contained adherent immune complexes. The plates were incubated at 37°C in a 5% CO_2 atmosphere for 1 h and then vigorously washed four times with warm HBSS. The medium was replaced before further incubation with reagents for determination of Fc or C3 receptor activity. In some experiments monolayers were cultured in DME with 10% FCS for 21 h.

If prolonged preincubation with drugs was required, MNL at 1.6×10^6 cells/ml were incubated with the drug in a 37°C shaking water bath in 50-ml gassed, sealed culture tubes for 30–180 min. At the end of the incubation period the cells were spun, resuspended in their original volume of DME-LH that contained the drug, and applied to substrates, as described above. Preincubation of MNL with some drugs reduced total adherence, but no drug reduced the percentage of adherent cells that were peroxidase positive.

For some experiments, cell monolayers were formed and then incubated with enzymes or drugs before determination of Fc receptor activity. Superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) were generated in the presence of adherent cells using xanthine oxidase with acetaldehyde as substrate (9). In other experiments, cell suspensions were cooled to 2–4°C on ice before application to substrates. For studies with macrophages, alveolar cells were obtained from rabbits after repeated intravenous administration of complete Freund's adjuvant (10). 85–90% of the alveolar cells appeared to be macrophages by Wright's stain examination and <5% were heterophiles.

Preparation of Substrates. For preparation of culture plates, bovine fibrinogen was dissolved in warm PBS, then diluted to 333 μg/ml with warm distilled water and filtered. 0.3-ml aliquots of the fibrinogen solution were applied to 35-mm culture plates and spread with a sterile glass rod. Plates were then dried at 45°C for 2 d. (11). For preparation of plates that contained surface-bound immune complexes, HSA as antigen was added to the bovine fibrinogen solution to give a final HSA concentration of 8–660 μg/ml, to make plates with 2.5, 10, or 200 μg of HSA incorporated into the substrate. Before use, plates were incubated with HBSS that

contained 10% FCS for 2 h at 37°C to convert the fibrinogen to fibrin, then were washed twice with HBSS. Immune complexes were prepared by incubating plates that contained fibrin-HSA with 1-ml vol of HBSS that contained 10, 25, 100, or 1,000 μg of monomeric rabbit IgG anti-HSA. Plates were incubated for 15 min at room temperature and then washed twice with HBSS. When fibrin plates that contained 2.5 μg HSA were incubated with 10, 25, and 100 μg of anti-HSA, 0.3, 0.5, and 1 μg, respectively, of antibody adhered to the plates, as determined by control experiments using ^{125}I-anti-HSA. Plates that contained 10 μg of HSA incubated with 100 μg of anti-HSA had 2 μg of adherent antibody, and plates that contained 200 μg of antigen incubated with 1,000 μg of antibody contained 20 μg of adherent antibody. Unless otherwise indicated, plates prepared with immune complexes contained 2 μg of adherent antibody.

Some plates containing standard amounts of antibody were coated with C3 by incubation with 1 ml of a 1:5 dilution of C5-deficient mouse serum in veronal buffer containing 1% gelatin (GVB) for 30 min at 37°C, then washed twice.

Rabbit IgG anti-HSA was isolated from the serum of hyperimmunized rabbits by affinity chromatography and gel filtration (10).

Coating of SRBC with IgG (EA) or with IgM and C3 (EAC). Rabbit IgG SRBC hemolysin was obtained from antiserum by ion-exchange chromatography on DE-52 and gel filtration chromatography on G-200 and stored in PBS at 2.8 mg/ml. The IgM fraction was obtained by similar purification on DE-52 and Sephadex G-200 and stored in PBS at 1.45 mg/ml.

SRBC were obtained from a single animal for all experiments, stored in Alsever's solution, and used within 2 wk. The SRBC were washed three times in GVB with aspiration of the buffy coat after each wash, then suspended in GVB at 10^9 cells/ml. Antibody preparations were diluted 1:100 (IgG) and 1:300 (IgM) in GVB to give maximal subagglutinating sensitization. Volumes of antibody were added dropwise with continuous mixing to equal volumes of SRBC and incubated for 30 min at 37°C in a shaking water bath. The antibody-sensitized cells then were washed three times with cold GVB. EA were used at 5×10^9 cells/ml.

SRBC sensitized with IgM were suspended at 2×10^8 cells/ml. C5-deficient mouse serum was diluted 1:5 and added dropwise to an equal volume of EAC. After 30 min of incubation at 37°C in a shaking water bath, the EAC were washed three times, as above, and used at 1×10^8 cells/ml.

Rosetting Assays. The medium was decanted from culture plates and 1-ml vol of EA or EAC were pipetted into the plates. When monolayers had been pretreated with drugs, EA and EAC suspensions were made up both with and without drugs to control for drug effects on the indicator rather than monolayer cells. No drug disrupted rosettes. Plate covers were taped so that the codes of drug treatment and substrate would not be known during the reading of rosettes. In most experiments, the sensitized SRBC were allowed to settle by gravity for 1 h at room temperature (25°C). The plates then were slowly rocked to suspend unattached erythrocytes and gently washed three times. Duplicate plates were read by two observers, and 200 cells were counted per plate. Adherent cells with three or more attached SRBC were scored as rosettes. The percentage of cells forming rosettes was expressed as the mean of duplicates in each experiment. There was no advantage in enumerating attached erythrocytes over simply counting rosettes. No rosettes formed when monolayers were incubated with unsensitized SRBC or with IgM sensitized SRBC.

In some experiments the EA rosetting temperature was 37°C and, after washing, some plates were exposed to one-half strength PBS for 10 s to lyse attached EA. Phagocytosed EA then could be visualized; monocytes with one or more interiorized EA were scored as phagocytic. In other experiments the EA rosetting temperature was 2–4°C after formation of monolayers at 37 or 4°C, as indicated in Results.

Results

Fc and C3 Receptor Activity of MNL Cultured on Surface-Bound Immune Complexes. 90% of MNL adherent to fibrin were monocytes as determined by peroxidase staining and latex particle adherence. In 21 experiments, 87 ± 10% rosetted with EA; and in 6 experiments, 89 ± 8% formed EAC rosettes (Table I).

TABLE I
*Receptor Activity of Adherent MNL**

Percentage of cells	Substrate without surface-bound immune complexes‡	Substrate with surface-bound immune complexes‡
Peroxidase positive	89 ± 4 (4)	65 ± 5 (4)
Forming EA rosettes	87 ± 10 (21)	9 ± 7 (15)
Forming EAC rosettes	89 ± 8 (6)	70 ± 8 (6)

* Monolayers were prepared on fibrin substrates with or without surface-bound HSA-anti-HSA immune complexes, then stained for peroxidase or incubated with rosetting reagents as described in Materials and Methods.

‡ For each experiment, plates were prepared in duplicate, and 200 cells were counted per plate. The data are expressed as the mean percentage of cells ± 1 SD. The number of experiments is given in parentheses.

About 65% of MNL adherent to surface-bound immune complexes were monocytes, but only 9 ± 7% formed EA rosettes. This loss in EA rosetting was seen as early as 30 min after plating of MNL onto complexes. Reduced rosetting persisted through 21 h of culture in 10% FCS; during this time the cells also lost viability (4). When plates were sensitized with $F(ab')_2$ anti-HSA, 78% of the adherent cells formed EA rosettes. EAC rosettes were formed by 70 ± 8% of MNL adherent to complexes (Table I). In one experiment, IgG-sensitized plates were incubated with 20% C5-deficient mouse serum in GVB, washed, and then MNL were applied. C3 rosetting was not reduced. Thus, as has been observed by Douglas (2), MNL cultured on surface-bound immune complexes exhibited a selective loss of EA rosetting.

In one experiment, plates were prepared that contained immune complexes on one-half of the surface. MNL were allowed to settle on these plates and then EA rosetting was measured. 92% of MNL adherent to the portion of the plate without immune complexes formed EA rosettes, but only 7% adherent to the half that contained immune complexes formed rosettes. In addition, when MNL on plain fibrin plates were incubated with soluble HSA-anti-HSA complexes at 2–10 μg/ml for 30 min, then washed, and then incubated with EA, 86% formed rosettes. Therefore, neither solubilized complexes nor soluble factors released by adherent cells accounted for the reduced EA rosetting of monocytes on surface-bound immune complexes.

Effect of Differently Sensitized Plates on Fc Receptor and Phagocytic Activity of Monocytes and Rabbit Alveolar Macrophages. Rabinovitch et al. (8) observed that unstimulated mouse peritoneal macrophages incubated on surface-bound immune complexes displayed reduced Fc-mediated phagocytosis, but attachment of EA was not altered. We compared the abilities of human monocytes and rabbit alveolar macrophages to form EA rosettes and to phagocytose antibody-coated SRBC using substrates that contained 0.10–10 times the standard amounts of antibody (2 μg). Both attachment of EA and phagocytosis of the sensitized cells by human monocytes were reduced as the antibody density was increased from 0.3 to 2 μg per plate (Table II). There was no point where phagocytosis was selectively lost. A slight loss of EA rosetting was observed when activated rabbit alveolar macrophages were in contact with plates that contained 2 μg of antibody, but phagocytosis was reduced by 90% (Table III). Although EA rosetting could be further reduced with a more heavily sensitized plate (20 μg of antibody), it was relatively better preserved with the rabbit alveolar macrophages than with the human peripheral monocytes.

TABLE II

*Effect of Substrate Sensitization on Fc Receptor and Phagocytic Activity of Human MNL**

Antibody/plate	Percentage of cells‡	
	Forming EA rosettes	Phagocytosing sensitized SRBC
μg		
0	68	80
0.3	45	49
0.5	21	44
1	8	16
2	2	5

* Monolayers were prepared on substrates that contained different amounts of immune complexes (as described in Materials and Methods), and then were incubated with EA for 1 h at 37°C. After washing, plates to be scored for phagocytosis were exposed for 10 s to half-normal PBS to lyse adherent, but not interiorized, SRBC.

‡ Adherent cells with three or more SRBC attached were scored as rosettes and cells with one or more interiorized SRBC were scored as phagocytic.

TABLE III

*Effect of Substrate Sensitization on Fc Receptor and Phagocytic Activity of Rabbit Alveolar Macrophages**

Antibody/plate	Percentage of cells‡	
	Forming EA rosettes	Phagocytosing sensitized SRBC
μg		
0	73	29
2	46	3
20	30	3

* Macrophage monolayers were prepared on substrates that contained different amounts of immune complexes (as described in Materials and Methods), and then were incubated with EA for 1 h at 37°C. After washing, plates to be scored for phagocytosis were exposed for 10 s to half-normal PBS to lyse adherent, but not interiorized, SRBC.

‡ Macrophages with three or more SRBC attached were scored as rosettes, and cells with one or more interiorized SRBC were scored as phagocytic.

Effect of Cold, Cytoskeletal Disruption, and Propranolol on Fc Receptor Activities. Monocytes and macrophages have been observed to cap EA (12) and cross-linked cytophilic antibody (13) at 37°C but not at 4°C. We investigated the possibility that the loss of EA rosetting seen with monocytes cultured on adherent complexes represented capping of Fc receptors.

MNL suspensions were chilled to 2–4°C on ice, then pipetted onto substrates that contained fibrin with or without associated complexes. They were allowed to settle for 2 h at 4°C, and then were washed and incubated with EA at 4°C. Afterward, the plates were kept on ice until read (<10 min). Monocytes adhered poorly to plain fibrin when they settled at 4°C, but monolayers formed at 37°C and then chilled to 4°C had the same rosetting activity (88%) as monolayers kept at 25°C (87%). Monocytes adhered well to surface-bound immune complexes at 4°C, but in five experiments, only 4 ± 4% formed EA rosettes. The percentage of monocytes in the MNL adhering to the substrate at 4°C was not changed, as determined by peroxidase staining.

TABLE IV

*Effect of Colchicine, Cytochalasin B, and Propranolol on EA Rosette Formation**

Drug (conc.)‡	Percentage of cells forming EA rosettes§	
	Substrate without adherent complexes	Substrate with adherent complexes
None	87 ± 10	9 ± 7
Cytochalasin B (0.02 mM)	34 ± 4	3 ± 4
Colchicine (0.1 mM)	58 ± 17	1 ± 1
Cytochalasin B (0.02 mM) + Colchicine (0.1 mM)	87 ± 7	2 ± 1
D-L-Propranolol (0.1 mM)	61 ± 15	2 ± 4
(0.2 mM)	28	0

* MNL were incubated with drugs at the indicated concentrations, then monolayers were prepared on fibrin substrates with or without adherent complexes.

‡ MNL were preincubated for 2 h with colchicine or cytochalasin B and for 5 min with propranolol before application to the plates. Conc., concentration.

§ Results are expressed as the mean percentage of EA rosettes ± 1 SD of four plates from two experiments.

Colchicine and cytochalasin B each reduced Fc receptor activity of monocytes cultured on plain fibrin when preincubated 2 h separately with MNL, but not when preincubated together (Table IV). Incubation of cells with these drugs separately or together before addition to substrates inhibited spreading, but did not alter loss of the rosetting activity seen with monocytes cultured on surface-bound complexes (Table IV). In some experiments, MNL were preincubated for 2 h with either colchicine or cytochalasin B or both drugs at 37°C and then were applied to substrates at 4°C. EA rosetting was also performed at 4°C and the same loss of Fc receptors still was observed (data not shown).

The local anesthetic propranolol has been reported to inhibit capping of membrane immunoglobulin by lymphocytes (14). This drug reduced EA rosetting of monocytes on plain fibrin without altering the loss of EA rosetting seen with monocytes on complexes (Table IV). We concluded that the loss of EA rosetting activity observed with cells incubated on surface-bound immune complexes did not represent capping or cytoskeletal modification of Fc receptors.

Neither colchicine, cytochalasin B, nor propranolol affected C3 receptor activity of monocytes cultured on either substrate.

Investigation of the Role of Lysosomal Proteases, Oxygen Radicals, or Membrane Perturbation in the Loss of Fc Receptors. Monocytes cultured on surface-bound immune complexes manifest extensive spreading (2), lysosomal enzyme release (4), and generation of superoxide anion and chemiluminescence (3). We investigated the possibility that lysosomal proteases or products of oxygen metabolism were damaging Fc receptors.

Monocytes settling onto substrates that contain high amounts of HSA exhibit the same secretion of granule enzymes that is seen when monocytes are cultured on substrates that contain immune complexes (4). When 200 μg of HSA was incorporated into substrates of ^{125}I-fibrin, the same degree of lysosomal enzyme activity was elicited by the antigen in the absence of added antibody (data not shown). However, incubation of monocytes on substrates incorporating 200 μg HSA did not reduce Fc receptor activity (87% rosettes).

TABLE V

Effect of Oxygen Radicals, Local Anesthetics, and Indomethacin on Monocyte Fc Receptor Activity

Treatment of cells (conc.)*	Percentage of cells forming EA rosettes	
	Substrate without adherent complexes	Substrate with adherent complexes
None	87 ± 10	9 ± 7
Catalase (60 μg/ml)	87	6
SOD (1 μg/ml)	79	8
Catalase (60 μg/ml) + SOD (1 μg/ml)	88	16
Xanthine oxidase	89	
+ Acetaldehyde (4 mM)	75	
+ Acetaldehyde (10 mM)	54	
+ Acetaldehyde (20 mM)	92	
Ethanol (300 mM)	90	10
Octanol (0.5 mM)	89	4
Lidocaine (4 mM)	94	6
Indomethacin (0.1 μM)	84	4
(1 μM)	90	7
(10 μM)	90	8
(0.2 mM)	85	22
DFP (1 mM)	99	11

* Catalase, SOD, local anesthetics, and DFP were added to MNL suspensions just before addition to plates. The cell suspensions were preincubated in indomethacin for 30 min before formation of the monolayers. Xanthine oxidase and acetaldehyde were added to monocyte monolayers that were further incubated for 1 h before determination of EA rosetting. Conc., concentration.

SOD and catalase reduce levels of superoxide anion and hydrogen peroxide. Incubation of MNL with these enzymes during interaction with surface-bound immune complexes did not affect the loss of Fc rosettes (Table V). The extracellular generation of O_2^- and H_2O_2 by xanthine oxidase and acetaldehyde also did not reduce Fc receptor activity of the cells on plain fibrin (Table V).

Local anesthetics reduce membrane excitation (15), prevent spreading of macrophages (5), inhibit immune-complex-stimulated release of lysosomal enzymes by monocytes (C. G. Ragsdale and W. P. Arend. Manuscript in preparation.), and reduce superoxide anion generation by stimulated macrophages and neutrophils (6, 7). Incubation of MNL with the neutral anesthetics ethanol (300 mM) and octanol (0.5 mM) or with the cationic anesthetic lidocaine (4 mM) did not change receptor activity, expressed either in the absence or presence of surface-bound immune complexes (Table V). Indomethacin (0.1 μM–0.2 mM) or di-isopropylfluorophosphate (DFP) (1 mM) did not affect Fc receptor activity of cells cultured on either substrate.

Effect of 2dG and Cyclic Nucleotides on Fc Receptor Activities. We examined the effects of the glucose analogue 2dG on Fc receptor activity of cells cultured on fibrin with or without adherent immune complexes. 2dG inhibits Fc-mediated phagocytosis by macrophages and exerts this effect within a short time of its addition to cells independently of its effect on ATP levels (16). 2dG also inhibits lysosomal enzyme release of monocytes incubated on complexes (C. G. Ragsdale and W. P. Arend. Manuscript in Preparation.). When 100 mM 2dG was added to MNL just before addition to substrates, 85% of cells incubated on plain fibrin and 9% incubated on adherent complexes formed rosettes.

After prolonged incubation with 2dG, cellular ATP levels fall (16). One consequence

TABLE VI

Influence of Cyclic Nucleotides on Monocyte Fc Receptor Activity

Drug (conc.)*	Percentage of cells forming EA rosettes‡	
	0 μg Antibody/plate	0.5 μg Antibody/plate
None	79 ± 7	23 ± 14
2dG (100 mM)	94 ± 4	80 ± 9
ATP (1 mM)	56 ± 9	9 ± 8
ATP (1 mM) + 2dG (100 mM)	88 ± 3	23 ± 12
8 Br-cAMP (1 mM) + MIBX (0.05 mM)	52 ± 20	15 ± 6
8 Br-cAMP (1 mM) + MIBX (0.05 mM) + 2dG (100 mM)	83 ± 10	41 ± 11

* MNL were preincubated with the drugs at the indicated concentrations (conc.) for 3 h before the formation of monolayers and determination of EA rosetting.

‡ Results are expressed as the mean percent EA rosettes ± 1 SD of four plates from two experiments.

of this fall is that the substrate for generation of cellular cAMP is reduced. We examined the effects of 2dG, ATP, and 8 Br-cAMP on monocyte Fc receptor activity using plates sensitized with 0.5 μg of antibody. MNL were preincubated in suspension for 3 h with no drugs or with 100 mM 2dG, 1 mM ATP with or without 2dG, or 1 mM 8 Br-cAMP and 0.05 mM MIBX with or without 2dG. When monocytes were preincubated in DME-LH, 79% formed EA rosettes on plain fibrin, and 23% formed rosettes on plates that contained 0.5 μg of antibody in immune complexes (Table VI). After preincubation in 2dG, 94% of cells on fibrin formed rosettes, but the EA rosetting of cells cultured on adherent complexes was no longer reduced, as 80% of the cells formed rosettes. That the maintenance of Fc receptor activity seen with 2dG was mediated by cAMP reduction was supported by the fact that ATP or 8 Br-cAMP with MIBX partially reversed the 2dG effect when cells were cultured on complexes. ATP or 8 Br-cAMP with MIBX also reduced EA rosetting by cells on plain fibrin (Table VI). No significant reduction in EA rosetting of monocytes on fibrin was seen with either 8 Br-cAMP or the phosphodiesterase inhibitor MIBX alone (data not shown).

2dG also prevented the immune-complex-induced loss of Fc receptor activity when plates contained 2 μg of antibody (60% formed rosettes). The effect of 2dG could be reversed if 2dG-treated monolayers were washed and incubated a further 3 h in DME-LH (data not shown). These results suggested that the loss of Fc receptors induced by culture of monocytes on surface-bound complexes was mediated by the generation of cAMP within the cells. Similar preincubation of MNL with 8 Br-cGMP did not affect rosetting activity on any substrate.

Discussion

We have confirmed that EA rosetting activity of human monocytes is greatly reduced when the cells are cultured on surface-bound complexes. C3 receptor activity is not reduced when the substrate contains immune complexes with or without added C3. Our results suggest that the cells exhibit a selective loss of Fc receptor number or function and that this loss is mediated by cyclic nucleotides.

Human monocytes respond differently than unstimulated mouse peritoneal macrophages or activated rabbit alveolar macrophages to culture on adherent complexes.

Surface-bound immune complexes caused a selective loss of EA phagocytosis by murine macrophages, but EA adherence was maintained (8). The density of antibody on the culture surface was not known, but it is unlikely that differences observed between monocytes and mouse macrophages resulted from different degrees of substrate sensitization. By using substrates with a density of antibody as low as 0.03 $\mu g/cm^2$ we were unable to find a point where EA phagocytosis by human monocytes was selectively affected; instead, EA rosetting and phagocytosis were reduced in parallel as antibody density increased. In contrast, when rabbit alveolar macrophages were plated onto substrates that contained standard amounts of antibody (2 μg), there was some loss of EA adherence, but phagocytosis was sharply reduced. Substrates with 10 times as much antibody caused more reduction in EA rosetting of the rabbit macrophages. Perhaps the different responses of human monocytes and mouse or rabbit macrophages to surface-bound immune complexes can be attributed to quantitative or qualitative variations in Fc receptors or to alteration in cell function induced by activation or differentiation. Heavily activated rabbit alveolar macrophages possessed more Fc receptors than did lightly stimulated macrophages, although the avidity of binding of monomeric IgG was the same (17). Guinea pig peritoneal macrophages harvested from sterile oil exudates exhibited higher Fc receptor activity than did unstimulated macrophages (18). Two populations of Fc receptors binding monomeric immunoglobulin have been identified on murine macrophages (19). These aspects of Fc receptors have not been studied with human monocytes but differences may exist between these cells and animal macrophages.

The decrease in EA rosette activity of human monocytes cultured on surface-bound complexes was concluded to be a result of loss of Fc receptor function. This loss was not caused by inhibition from solubilized complexes or from the release of soluble substances from the adherent cells. Furthermore, the loss of EA rosetting was not a result of migration or capping of Fc receptors. We found that monocytes on plain fibrin normally formed rosettes at 4°C and that the loss of Fc receptors seen with cells on complexes still occurred at 4°C. The finding of normal Fc receptor function at 4°C differs from that of others (20) and possibly is a result of differences in rosetting techniques. Human monocytes do not cap EA at 4°C, whereas they cap readily at 37°C (12). Propranolol, which inhibits capping of lymphocyte surface immunoglobulin, also did not prevent the loss of EA rosetting with monocytes on complexes. Movement of membrane proteins is mediated by microtubules and microfilaments and is stimulated by external cross-linking of receptors by antibodies or ligands (21). Preincubation of MNL with colchicine or cytochalasin B, or with both drugs together, did not prevent the immune complex-induced loss of Fc receptors. Thus, the loss of EA rosetting activity of MNL on immune complexes was not a result of lateral receptor movement or interiorization mediated by cytoskeletal elements. The Fc receptors were under some cytoskeletal control, however, as colchicine or cytochalasin B reduced EA rosetting with MNL cultured on plain fibrin, as has been observed by others (22, 23).

Monocyte Fc receptors were not destroyed by lysosomal enzymes or by toxic products of oxygen metabolism when the cells were cultured on complexes. Fc receptor activity could not be reduced by incubating MNL with substrates that contained high amounts of antigen, a surface-bound phagocytic stimulus that leads to the same degree of protease activity elicited by adherent immune complexes (4). Inactivation

of superoxide anion and hydrogen peroxide by SOD and catalase did not prevent the loss of EA rosetting by monocytes incubated on complex-containing substrates. Also, the loss of Fc receptor activity could not be induced by extracellular generation of O_2^- and H_2O_2 with xanthine oxidase and acetaldehyde. Local anesthetics that prevent both lysosomal protease release and generation of oxygen radicals did not alter the effect of immune complexes on EA rosetting.

The only treatments that modified the effect of adherent immune complexes on Fc receptor activity were those that changed cellular levels of cAMP. Pretreatment of cells with 2dG prevented the loss of EA rosetting when MNL were cultured on adherent complexes. This effect of 2dG was partially reversed with ATP or by 8 Br-cAMP and MIBX, which suggests that 2dG, by decreasing levels of ATP, reduced the amount of substrate available for generation of cAMP. Reversal of the effect of 2dG by adding 8 Br-cAMP makes unlikely the sole participation of other ATP-dependent pathways in immune complex-induced alteration of EA rosetting. The effect of 2dG was not a result of a loss of monocyte viability because the effect was reversed by removal of 2dG from the medium. Both ATP and 8 Br-cAMP with MIBX also reduced Fc receptor activity of monocytes on plain fibrin.

Studies from other laboratories have described effects of cyclic nucleotides on Fc receptor function that vary with the cell type studied and experimental conditions. Zuckerman and Douglas (24) found that Fc receptor activity of a murine plasmacytoma line was reduced by cholera toxin, but only by amounts 1,000 times higher than those that elevated cellular cAMP levels. In contrast, cholera toxin did not reduce Fc receptor activity of rabbit alveolar macrophages despite the increase in cAMP levels; EA rosetting was reduced 20–30% by aminophylline (24). Measurement of total cell levels of cAMP, however, may not reflect nucleotide levels in critical parts of the cell such as the membrane. Rhodes (25) observed that incubation of guinea pig peritoneal macrophages with dibutyryl cAMP or MIBX blocked increases in Fc receptor activity that otherwise occurred during 3 d of culture. In contrast, some mutant clones of a murine macrophage-like cell line that possessed Fc receptors but lacked EA phagocytosis regained this ability after a 5- to 10-h incubation with 8 Br-cAMP (26). Enhancement of phagocytosis was not accompanied by increases in total cellular cAMP. These observations may not be relevant to our findings with human monocytes, however, as monocyte EA rosetting ability was lost within 30 min of plating of cells onto surface-bound immune complexes.

Adherent immune complexes have been reported to induce other changes in cellular function that may be mediated by cAMP. An inhibition of B-cell mitogenesis by surface-bound complexes was associated with an elevation of cAMP levels (27). Elevation of cellular cAMP by exogenous addition of dibutyryl cAMP also was associated with reduced macrophage release of O_2^- upon stimulation by surface-bound IgG aggregates (28) and by a reduction in antibody-dependent cell-mediated cytotoxicity by MNL (29).

Our studies have not revealed the complete sequence of events that result in loss of EA rosetting by monocytes incubated on surface-bound immune complexes. We hypothesize that the interaction of monocytes with the adherent complexes leads to an activation of adenylate cyclase with an elevation in cellular or membrane cAMP levels, which, in turn, mediate changes in Fc receptor number or function. Prostaglandin generation appeared not essential to this change as indomethacin did not

affect EA rosetting activity. Drugs inhibiting monocyte spreading (local anesthetics, colchicine, and cytochalasin B) did not prevent immune complex-induced loss of EA rosetting, which suggests that total membrane contact with the ligand was not required.

An alteration in Fc receptor activity initiated by an elevation in cellular or membrane cAMP might occur in two ways. Once generated in the cell, cAMP activates protein kinases that may, in turn, mediate phosphorylation of membrane proteins. Thus, the glycoprotein Fc receptor might be altered by direct phosphorylation. Alternatively, protein kinases may mediate activation of phospholipases. Fc receptor function has been shown to be destroyed by treatment of intact cells or isolated receptors with phospholipases (30, 31). cAMP-activated lipases have been described in adipose tissue, and increased lipolysis has correlated better with the degree of protein phosphorylation than with cAMP levels (32). Phospholipases also have been detected in rabbit alveolar macrophages (33) and in human neutrophil granules (34), but in neither instance has activation by cAMP been examined. In addition, phospholipase activity has been implicated in the alterations of membrane lipids induced by phagocytosis (35) and in the release of arachadonic acid from mitogen-stimulated lymphocytes (36). Indomethacin has been reported to inhibit isolated cAMP-dependent protein kinases of ileal mucosa (37), but its effect on the protein kinases of intact cells and other tissues is not known.

The results of our studies may have some relevance to human diseases where monocytes and macrophages come into contact with surface-bound immune complexes, as in rheumatoid arthritis (38) or in some varieties of glomerulonephritis (39, 40). We have observed that the in vitro loss of Fc receptor activity of monocytes cultured on complexes persisted for at least 21 h. Such a loss of Fc receptor activity of monocytes or macrophages in vivo could lead to an impairment in immune clearance or in other protective cellular functions.

Summary

Human monocytes cultured on surface-bound immune complexes exhibited a loss of ability to form rosettes with IgG-sensitized sheep erythrocytes (EA). This loss was not a result of inhibition of Fc receptors by solubilized complexes nor of release of soluble factors by the cells. Loss of EA rosetting was not prevented by culture of monocytes at 4°C, or by treatment with colchicine, cytochalasin B, or local anesthetic agents. These results suggested that the loss was not secondary to capping or interiorization of Fc receptors. The results of other studies indicated that the Fc receptors were not damaged by lysosomal enzymes or oxygen radicals. Maintenance of EA rosetting ability of monocytes cultured on surface-bound immune complexes was seen after a 3-h preincubation of the cells in 100 mM 2-deoxy-D-glucose (2dG). A similar preincubation in ATP or in 8-bromoadenosine 3′:5′-cyclic monophosphoric acid plus the phosphodiesterase inhibitor methyl isobutyl xanthine led to a partial loss of EA rosetting of cells on plain fibrin and to a partial reversal of the effects of 2dG seen with cells on complexes.

We conclude that EA rosetting of monocytes cultured on surface-bound immune complexes is reduced by cyclic nucleotide-mediated effects on Fc receptor number or function.

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