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DIFFERENTIAL REQUIREMENTS FOR CELLULAR CYTOSKELETON IN HUMAN MACROPHAGE COMPLEMENT RECEPTOR- AND Fc RECEPTOR-MEDIATED PHAGOCYTOSIS[1]

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We investigated the requirement for cellular cytoskeleton in CR- and FcR-mediated phagocytosis by human monocyte-derived macrophages (Mϕ). Inhibition of actin microfilament (MF) assembly and stability by cytochalasins B and D completely inhibited Mϕ phagocytosis of sheep E coated with C3b (EC3b), iC3b (EC3bi), and IgG (EIgG) via CR_1, CR_3, and FcR, respectively. Ligand-binding to either CR or FcR was not effected by cytochalasins. Nocodazole (NOC), which prevents microtubule (MT) polymerization, and taxol, which causes random polymerization of MT inhibited Mϕ phagocytosis of EC3b(i) but not EIgG. However, the combination of taxol (5×10^{-4} M) and NOC (2×10^{-6} M) augmented Mϕ CR-mediated phagocytosis. In addition, agents known to increase intracellular cGMP augmented phagocytosis of EC3b(i). Conversely, agents that increase intracellular cAMP inhibited CR-mediated phagocytosis. These agents had no effect on FcR-mediated phagocytosis, and did not effect ligand-binding to CR or FcR. PMA markedly enhanced CR- but not FcR-mediated phagocytosis, and augmentation of CR-mediated phagocytosis by PMA was inhibited by both CD and NOC. In contrast, the synthetic diacylglycerol, 1-oleoyl-2-acetoyl-sn-3-glycerol augmented, and inhibitors of protein kinase C inhibited Mϕ phagocytosis via CR and FcR. These data indicate that for adherently cultured human Mϕ: 1) binding of ligand-coated E to CR or FcR does not require an intact cytoskeleton; 2) intact actin microfilament are required for phagocytosis via CR and FcR; 3) phagocytosis via CR_1 and CR_3 but not FcR is dependent on MT assembly; 4) PMA most likely augments CR-mediated phagocytosis through promotion of MT assembly; and 5) PKC activity is involved in the phagocytic signal generated by both CR and FcR.

Monocytes and Mϕ[3] possess specific membrane receptors that are important in the recognition, phagocytosis, and killing of pathogenic microorganisms. These receptors are CR_1 (1), CR_3 (2, 3), and FcR (4) which are specific for surface-bound C3b, iC3b, and IgG, respectively. In vitro studies have shown that phagocytosis via FcR is a constitutive property of phagocytes and that CR-mediated phagocytosis is regulated developmentally (5–12). CR may be activated for phagocytosis by several agents including phorbol esters (11, 13), cytokines (14), and extracellular matrix proteins (10, 15–19). These agents also may enhance FcR-mediated phagocytosis (13, 15, 17–21).

The process of phagocytosis by monocytes and Mϕ depends not only on the dynamic properties of CR and FcR, but also depends on the integrity of the cellular cytoskeleton. In an electron microscopy study of adherent mouse PM, oriented bundles of MF were observed surrounding ingested latex beads (22). In addition, numerous MT were seen in the region of the oriented 40-Å MF and the 100-Å filaments surrounding phagocytized beads (22). Although the interrelationship of the MT with the MF was difficult to interpret, the observations supported the idea that both MF and MT were involved in the phagocytic process.

However, studies quantifying the ingestion of various particles after exposure of phagocytes to pharmacologic agents that selectively interfere with MF or MT function have not supported this hypothesis. Thus, disruption of MF integrity with CB was found to inhibit human PMN phagocytosis of serum opsonized *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhimurium* (23–25); mouse PM phagocytosis of opsonized *E. coli* and *S. albus*, and latex beads (26, 27); and rabbit alveolar Mϕ phagocytosis of opsonized *S. aureus* (23). In contrast, incubation of phagocytes with colchicine and vineblastine to prevent MT polymerization did not inhibit human PMN phagocytosis of opsonized staphylococci, streptococci, or opsonized LPS-coated paraffin oil droplets (28, 29); mouse PM phagocytosis of opsonized *E. coli* (30); or rabbit PMN phagocytosis of latex particles or paraffin oil droplets emulsified in BSA (31, 32). However, colchicine was reported to inhibit guinea pig PMN and mouse Mϕ phagocytosis of paraffin oil droplets emulsified in BSA (33, 34). Overall, these reports suggest that phagocytosis requires intact actin MF, but does not require MT.

These studies mostly examined the phagocytosis of bacteria and particles opsonized in serum. In these experiments the opsonized particles engage multiple receptors (FcR, CR_1, and CR_3) on the phagocyte's plasma membrane (35, 36). More recent experiments have examined

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[3] Abbreviations used in this paper: Mϕ, macrophage; CB, cytochalasin B; CD, cytochalasin D; NOC, nocodazole; MF, microfilaments; MT, microtubules; OAG, 1-oleoyl-2-acetoyl-sn-3-glycerol; PKC, protein kinase C; PM, peritoneal Mϕ; PMN, polymorphonuclear neutrophil; Hanks'-HEPES, HBSS containing 20 mM HEPES, and 10 μg/ml gentamicin; HBSA, Hanks'-HEPES containing 0.25% BSA; BDVEA, 3.5 mM veronal buffer, pH 7.2 containing 1% BSA, 3.2% dextrose, 20 mM EDTA, and 0.2% azide; VBS^{2+}, 24 mM veronal buffer, pH 7.4, containing 0.15 M NaCl, 0.5 mM $MgCl_2$, and 1.5 mM $CaCl_2$; MTOC, MT organizing center; MAP, MT-associated protein.

the role of MT in phagocytosis mediated by individual membrane receptors. Thus, preincubation of mouse Mϕ with colchicine reduced the phagocytosis of EIgG (34); and incubation of human cultured Mϕ with colchicine and podophylotoxin inhibited PMA-stimulated ingestion of EC3b, EC3bi, and EIgG (11). In other studies, it has been shown that colchicine and vineblastine inhibited the ability of a T cell lymphokine to enable CR on mouse resident PM to become mobile within the plane of the membrane, and mediate phagocytosis (37). Interestingly, colchicine did not inhibit CR mobility on thioglycollate elicited mouse PM. Therefore, intact MT function was not an absolute requirement for CR mobility on mouse PM (37). Thus, these reports do suggest a role for MT in FcR-mediated phagocytosis (11, 34) and CR-mediated phagocytosis (11, 37). In none of these studies was the role of MF and MT in phagocytosis examined concommitantly.

In the present studies, we have used adherently cultured monocyte-derived Mϕ to reexamine the specific roles of MF and MT in promoting binding and ingestion via CR_1, CR_3, and FcR. Use of adherently cultured human Mϕ is advantageous because these cells activate their CR for phagocytosis without the requirement for an exogenous second signal (9, 12). The results of these experiments show that MF are required for phagocytosis by CR and FcR, but that only CR_1 and CR_3 require functional MT. Ligand binding to either CR or FcR does not require an intact cellular cytoskeleton. In addition, the data suggest that PMA augmentation of CR-mediated phagocytosis most likely is via enhancement of MT assembly, and that PKC activation is involved in the phagocytic signal generated by both CR and FcR.

MATERIALS AND METHODS

Reagents. CB, CD, PMA, NOC, dibutryl cAMP, dibutryl cGMP, isoproterenol, imidazole, carbachol (carbamyl choline), bethanechol (carbamyl-β-methylcholine chloride), theophylline, epinephrine, polymyxin B, trifluoperazine, D-sphingosine, staurosporine, palmitoyl-DL-carnitine, and H-7 (1-(5-isoquinolinylsulfonyl)-2-methylpiperazine) were purchased from Sigma Chemical Co., St. Louis, MO. PMA, CB, and CD were dissolved in DMSO at a concentration of 1 mg/ml, and 50-μl aliquots were stored at −20°C until used. NOC (R17934) (methyl (5-(2-thienylcarbonyl)-1 H- benzimidazole-2-yl) carbamate) a synthetic microtubule inhibitor also was dissolved in DMSO at 1 mg/ml, and 50-μl aliquots were frozen at −20°C. Taxol 1 mg/ml in DMSO was a generous gift of Dr. Bruce Giffin, Department of Biology, University of Dayton, Dayton, OH.

Monocyte preparation and culture. Human PBMC cells were prepared under sterile conditions by dextran sedimentation and Ficoll-Hypaque centrifugation as described previously (9, 12). The mononuclear cells were washed in Hanks'-HEPES, and suspended to 3 to 4 × 10^6/ml in Hanks'-HEPES containing 0.1% autologous serum. One ml volumes of mononuclear leukocytes were adhered in 24-well tissue culture plates (Costar, Cambridge, MA) directly to the plastic or to 13-mm diameter glass coverslips for 1 h at 37°C in 5% CO_2-95% air. The adherent monocytes were washed vigorously with Hanks'-HEPES to remove the lymphocytes, and then were cultured in M199 (GIBCO, Grand Island, NY) containing 10% autologous serum and 10 μg/ml gentamicin. Cultured Mϕ were given fresh medium on day 3 or 4, and phagocytosis assays were performed on day 7 (9, 12).

Preparation of C3-coated sheep E. Sheep E bearing C3b (EC3b) and iC3b (EC3bi) were prepared with purified C components as described previously (12, 38, 39). E containing small amounts of C3b first were made by trypsinization of C3. The $EC3b_T$ were incubated with factors B and D to form an alternate pathway C3 convertase, and then further incubated with C3 for 20 min. at 37°C. EC3bi were prepared from EC3b by suspending EC3b (1 × 10^9/ml) in 3.5 mM BDVEA. Factors H and I were added to the EC3b, and the mixture was incubated for 30 min at 37°C. The EC3b and EC3bi were washed twice and stored in BDVEA at 4°C. The bound C3b(i) fragments on the EC3 were quantified in RIA for the uptake of ^{125}I-monoclonal anti-C3c (Bethesda Research Laboratories, Gaithersburg, MD) or ^{125}I-monoclonal anti-C3g (kindly provided by Dr. Peter Lachmann, Medical Research Council, Cambridge, England) as described previously (12, 38, 39). EC3b(i) used in these experiments had 4 to 5 × 10^4 C3 molecules bound per E.

Preparation of EIgG. Sheep E (1 × 10^9 E/ml) in 24 mM veronal buffer, pH 7.4 containing 0.15 M NaCl, 0.5 mM $MgCl_2$, and 1.5 mM $CaCl_2$ (VBS^{++}) were mixed with an equal volume of a 1/300 dilution of IgG anti-E (Cordis Laboratories, Miami, FL), and the mixture was incubated for 30 min at 37°C and 30 min at 4°C (40). EIgG were washed once in VBS^{++} and twice in BDVEA, and then were stored in BDVEA at 4°C until use. The amount of antibody used to coat the E promoted rosette formation and phagocytosis by 50 to 60% of freshly isolated human monocytes (9). In some experiments EIgG were prepared with a 1/3000 dilution of IgG anti-E.

Phagocytosis assay. Binding and ingestion of EC3b(i) and EIgG by Mϕ was quantified as described previously (9, 12). Ligand-coated E were washed in HBSA and standardized to 2 × 10^8 E/ml. E (2 × 10^7) were added to the Mϕ monolayers, and the cultures were incubated for 1 h at 37°C in 5% CO_2-95% air. At the end of the incubation period, unbound E were removed by two washes with HBSA. Bound but uningested E were lysed with an NH_4Cl lysing buffer, the Mϕ were washed once with HBSA, and the monolayers were fixed in 2% glutaraldehyde-1% sucrose in 0.01 M phosphate buffer, pH 7.4. Binding and ingestion was quantified by counting 200 cells per well via phase-contrast microscopy. Results are presented as percent rosettes, the percent of Mϕ that bound three or more E; percent ingestion, the percent of Mϕ that ingested one or more E; and the phagocytic index, the total number of E ingested/100 Mϕ.

In some experiments, phagocytosis was quantified using ^{51}Cr-labeled EIgG as described previously (9). Phagocytosis experiments were performed as described for unlabeled E, except that after lysis of unbound E, monocytes were lysed with 1 ml of 0.05% Triton X-100 (Fisher Scientific, Pittsburg, PA). The wells were washed once with HBSA, the lysate and wash supernate pooled, and radioactivity was quantified in a Packard AUTO-GAMMA 500C (Packard Instruments, Downers Grove, IL). Monocytes that underwent all manipulations except incubation with EIgG were treated with ZAP-OGLOBIN II (Coulter Diagnostics, Hialeah, FL) to lyse the external membrane of the cells. Nuclei then were quantified on a Sysmex Microcell CC-110 counter (American Scientific Products, Columbus, OH). Results are presented as the mean ± SEM of the number of EIgG ingested/Mϕ. All experiments were performed in triplicate.

RESULTS

Requirement for MF in Mϕ CR- and FcR-mediated phagocytosis. Seven-day cultured Mϕ were preincubated for 5 min at 37°C with 5 μg/ml of CB or CD. Binding and ingestion of EC3b, EC3bi, and EIgG then was quantified after 1 h at 37°C. Binding of ligand-coated E to CB- or CD-treated Mϕ was not effected (Fig. 1*A*). In contrast, pretreatment of Mϕ with CB or CD markedly inhibited phagocytosis of EC3b(i) and EIgG (Fig. 1 *B* and *C*). Further experiments showed that CD inhibited Mϕ CR- and FcR-mediated phagocytosis in a concentration-dependent manner (Fig. 2). Phagocytosis was inhibited with as little as 0.05 μg/ml of CD, and complete inhibition was observed with only 1.0 μg/ml of CD.

Requirement for MT in Mϕ CR-mediated phagocytosis. The requirement for MT in Mϕ CR- and FcR-mediated phagocytosis first was investigated using the synthetic MT inhibitor NOC (R17934). NOC increases the concentration of tubulin required for MT assembly and binds to the same site on the tubulin molecule as colchicine (41). It has the advantage, however, of being completely reversible (42), and has a high degree of specificity shown by the absence of non-specific side-effects not related to its antimicrotubular properties (42, 43).

Mϕ were preincubated for 30 min at 37°C with 2.5 μg/ml of NOC, and phagocytosis of ligand-coated E then was quantified after 1 h at 37°C. Pretreatment of Mϕ with NOC inhibited phagocytosis of EC3b and EC3bi, but did not inhibit ingestion of EIgG (Fig. 3). The inhibition of

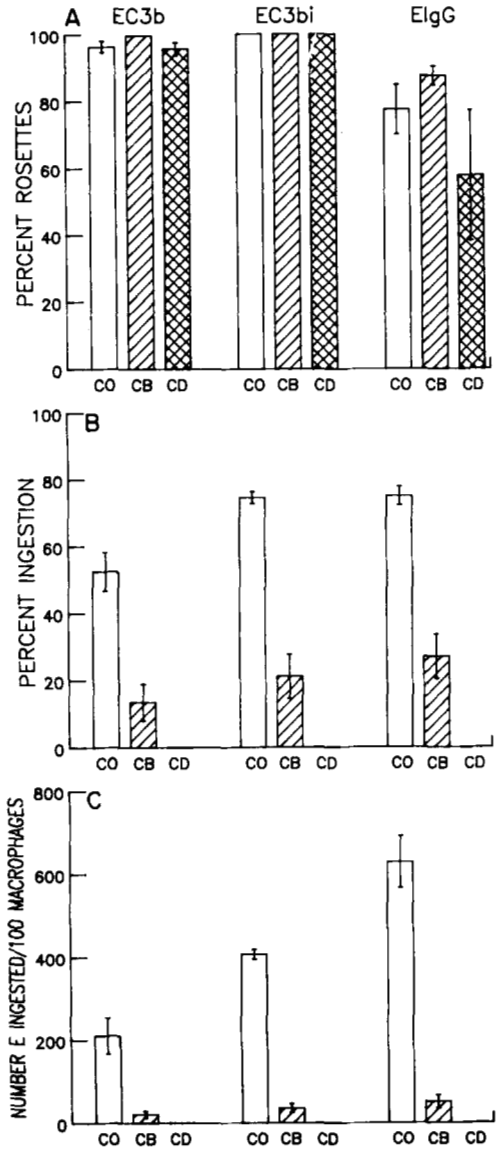

Figure 1. Inhibition of Mϕ CR-and FcR-mediated phagocytosis by CB and CD. Cultured Mϕ were preincubated for 5 min at 37°C with buffer (*CO*) or 5 μg/ml of CB or CD. Ligand-coated E were added, and binding and ingestion were quantified after 1 h at 37°C in 5% CO_2-95% air. Results are presented as the Mean ± SEM (n = 8). *A*, Percent rosettes; *B*, percent ingestion; *C*, number of E ingested/100 mϕ.

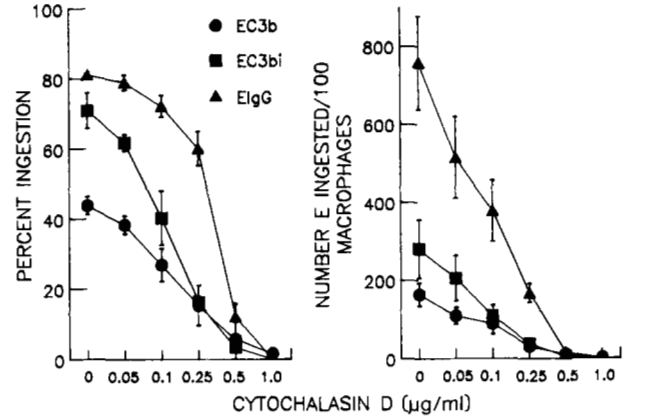

Figure 2. Concentration-dependent inhibition by CD of Mϕ CR- and FcR-mediated phagocytosis. Mϕ were preincubated for 5 min at 37°C with varying concentrations of CD. Ligand-coated E were added and phagocytosis was quantified after 1 h at 37°C. Results are presented as the mean ± SEM (n = 5). The *left panel* shows the percent Mϕ ingesting, and the *right panel* shows the number of E ingested/100 Mϕ.

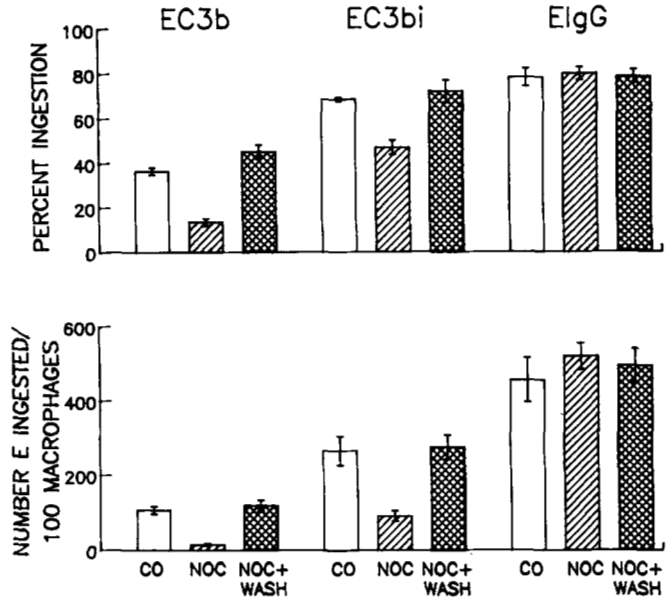

Figure 3. NOC inhibits Mϕ CR- but not FcR-mediated phagocytosis. Mϕ were preincubated for 30 min at 37°C with 2.5 μg/ml of NOC. In some wells the NOC was present during the entire assay (NOC). In other wells, the NOC was removed, and the Mϕ were washed three times with HBSA (NOC + WASH). EC3b, EC3bi, and EIgG were added to the cells and phagocytosis was quantified after 1 h at 37°C. Results are presented as the mean ± SEM (n = 4).

CR-mediated phagocytosis by NOC was completely reversed when Mϕ were washed before the addition of EC3b(i) (Fig. 3). NOC did not effect ligand binding to CR_1, CR_3, or FcR (data not shown). NOC inhibited Mϕ CR_1- and CR_3-mediated phagocytosis in a concentration-dependent manner, with optimum inhibition obtained with 2.5 μg/ml (data not shown). Concentrations of NOC of more than 2.5 μg/ml could not be tested because higher concentrations caused the Mϕ to round up and lift off the glass coverslips or the plastic of the tissue culture dish.

To build an organized MT system, cells must suppress assembly of free MT and initiate MT growth at centrosomes, which are the cell's major MTOC. Taxol lowers the critical concentration of tubulin required for MT assembly, and in living cells, taxol induces massive assembly of free MT and abrogates the organizing capacity of the MTOC (44). The addition of NOC to taxol-treated cells, can restore the assembly-initiating activity of the MTOC and remove free MT (44).

Therefore, Mϕ were preincubated with varying concentrations of taxol or NOC, separately or in combination, for 30 min at 37°C. Phagocytosis of EC3b(i) and EIgG then was quantified after 1 h at 37°C. Taxol and NOC alone consistently inhibited Mϕ CR-mediated phagocytosis (Table I). However, incubation of Mϕ with both taxol (5×10^{-4}M) and NOC (2×10^{-6}M) increased CR_1- and CR_3-mediated phagocytosis by 38 and 58%, respectively. No combination of taxol or NOC effected ingestion of EIgC. These results suggest that polymerization of MT at MTOC is necessary for CR-mediated ingestion.

MT assembly and stability is augmented by agents that increase intracellular cGMP, and is inhibited by agents that increase intracellular cAMP (45–48). Therefore, the requirement for MT in Mϕ CR-mediated phagocytosis was investigated further by quantifying phagocytosis in the presence of pharmacologic agents that affect the intra-

TABLE I

Effect of taxol (TAX) and NOC on Mø CR- and FcR-mediated phagocytosis[a]

Addition	Phagocytic Index[b]		
	EC3b	EC3bi	EIgG
Buffer	148 ± 14	230 ± 30	803 ± 339
NOC (2×10^{-6} M)	41 ± 5	99 ± 14	763 ± 312
TAX (5×10^{-4} M)	51 ± 4	93 ± 15	798 ± 321
NOC (2×10^{-6} M) + TAX (5×10^{-4} M)	205 ± 6	364 ± 33	748 ± 293

[a] Mø were preincubated for 30 min at 37°C with buffer, NOC, TAX, or both, and then phagocytosis of ligand-coated E was quantified after 1 h at 37°C.
[b] Mean ± SEM (n = 5 to 7).

TABLE II

cAMP and cAMP agonists inhibit Mø CR- but not FcR-mediated phagocytosis[a]

Addition	Phagocytic Index[b]		
	EC3b	EC3bi	EIgG
Buffer	403 ± 47	594 ± 43	1241 ± 94
dcAMP (10^{-3} M)	262 ± 50	416 ± 62	1235 ± 131
Epinephrine (10^{-5} M)	248 ± 73	429 ± 46	1242 ± 153
Isoproterenol (10^{-5} M)	236 ± 21	462 ± 32	1237 ± 133

[a] Mø were preincubated for 15 min at 37°C with buffer or with dcAMP, epinephrine, or isoproterenol in the presence of 5×10^{-4} M theophylline, and then phagocytosis of ligand-coated E was quantified after 1 h at 37°C.
[b] Mean ± SEM (n = 3).

TABLE III

cGMP and cGMP agonists augment Mø CR- but not FcR-mediated phagocytosis[a]

Addition	Phagocytic Index[b]		
	EC3b	EC3bi	EIgG
Buffer	83 ± 6	218 ± 8	1227 ± 83
dcGMP (5×10^{-6} M)	130 ± 2	363 ± 24	1139 ± 103
Carbachol (10^{-4} M)	155 ± 14	381 ± 31	1216 ± 60
Bethanechol (10^{-4} M)	119 ± 10	381 ± 18	1221 ± 42

[a] Mø were preincubated for 15 min at 37°C with buffer, or with dcGMP, carbachol, or aminophylline in the presence of 10^{-5} M imidazole, and then phagocytosis of ligand-coated E was quantified after 30 min at 37°C.
[b] Mean ± SEM (n = 3).

cellular concentration of these cyclic nucleotides. Mϕ were preincubated for 15 min with agents that increase intracellular cAMP, or with agents that increase intracellular cGMP, and then ligand-coated E were added for 1 h or 30 min, respectively, at 37°C. Table II shows that preincubation of Mϕ with dibutryl cAMP, epinephrine, and isoproterenol inhibited CR-mediated phagocytosis by 22 to 41%. Conversely, dibutryl cGMP, carbachol, and bethanechol enhanced CR-mediated phagocytosis by 43 to 87% (Table III). Neither adrenergic agents nor cholinergic agents effected Mϕ FcR-mediated phagocytosis (Tables II and III).

In addition to its well known ability to activate PKC (49), PMA has been shown to increase intracellular cGMP (50, 51), and to promote MT assembly in neutrophils and Mϕ by increasing the length and total number of MT associated with centrosomes (MTOC) (52–55). As PMA is a potent phagocyte activator in a number of assays including inducing CR-mediated phagocytosis in suspension cultured Mϕ (11), we sought to determine if PMA would be a stronger stimulus of CR-mediated phagocytosis than the cholinergic agents used in Table III.

Mϕ were incubated simultaneously with EC3b, EC3bi, or EIgG and increasing concentrations of PMA, and phagocytosis was quantified after 1 h at 37°C. Figure 4 shows that incubation of Mϕ with PMA increased CR_1-

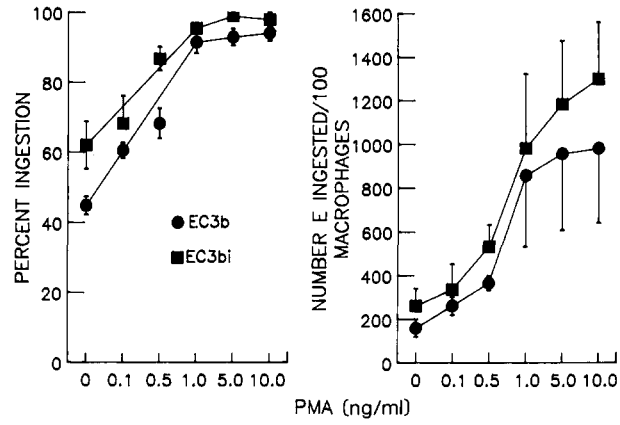

Figure 4. PMA enhances Mϕ CR-mediated phagocytosis in a concentration-dependent manner. Varying concentrations of PMA were added to Mϕ simultaneously with the EC3b(i), and phagocytosis was quantified after 60 min at 37°C. Results are presented as the mean ± SEM (n = 4). The *left panel* shows the percent ingestion, and the *right panel* shows the number of EC3b(i) ingested/100 Mϕ.

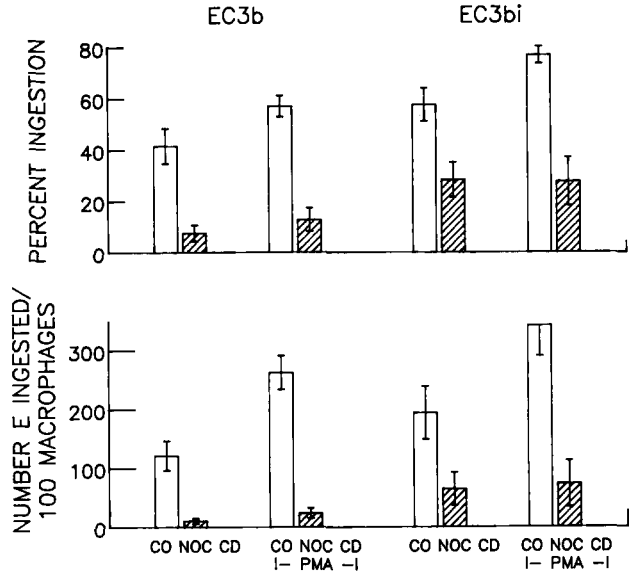

Figure 5. CD and NOC inhibit PMA enhances Mϕ CR-mediated phagocytosis. Mϕ were preincubated with 5 μg/ml of CD for 5 min at 37°C, 2.5 μg/ml of NOC for 30 min, or in buffer for 30 min (*CO*). EC3b and EC3bi then were added to the Mϕ in the presence or absence of 0.5 ng/ml PMA, and phagocytosis was quantified after 1 h at 37°C. Results are presented as the mean ± SEM (n = 4). The *upper panel* shows the percent ingestion, and the *lower panel* shows the number of EC3b(i) ingested/100 Mϕ.

and CR_3-mediated phagocytosis in a concentration-dependent manner. The number of EC3b and EC3bi ingested doubled when Mϕ were stimulated with as little as 0.5 ng/ml of PMA, and the phagocytic index increased four- to sixfold with as little as 10 ng/ml of PMA. PMA (up to 30 ng/ml) did not effect FcR-mediated phagocytosis (data not shown). These results agree with the original experiments of Wright and Silverstein (11).

We next sought to determine if augmentation of CR-mediated phagocytosis by PMA required both MF and MT. Mϕ were preincubated with buffer (control), 2.5 μg/ml of NOC, or 5 μg/ml of CD, and then EC3b and EC3bi were added to the Mϕ with or without 0.5 ng/ml of PMA. Figure 5 shows that PMA increased the number of EC3b and EC3bi ingested/100 Mϕ 2.2- and 1.8-fold, respectively. CD completely blocked CR-mediated phagocytosis, even when the macrophages were stimulated with PMA. In the absence of PMA, NOC inhibited the total number

of EC3b and EC3bi ingested by 91 and 66%, respectively. In the presence of PMA, NOC inhibited phagocytosis of EC3b and EC3bi by 91 and 79%, respectively.

We next considered the possibility that agents which enhanced or inhibited MT function had no effect on FcR-mediated phagocytosis, because the high phagocytic index of the EIgG made it impossible to detect any effects of these agents. Therefore, we prepared EIgG using one-tenth the normal amount of IgG anti-E, and quantified the effect of 10 ng/ml PMA and 2.5 μg/ml of NOC on Mφ phagocytosis of these particles. PMA and NOC were studied because they were the most effective stimulus and inhibitor, respectively, of Mφ CR-mediated phagocytosis. The data in Table IV show that when the phagocytic index of EIgG is reduced to approximately 300, PMA does not enhance, and NOC does not inhibit phagocytosis of EIgG.

Role of PKC in Mφ CR- and FcR-mediated phagocytosis. If the ability of PMA to enhance Mφ CR- but not FcR-mediated phagocytosis was through its ability to promote MT assembly rather than its capacity to stimulate PKC, it would suggest either that PMA did not activate PKC in this system, or, that if PKC was activated, that PKC activation was not important for FcR-mediated phagocytosis. To more directly evaluate the requirement for PKC activity in Mφ CR- and FcR-mediated phagocytosis, Mφ phagocytosis first was quantified in the presence of the synthetic diacylglycerol, OAG, which is the physiologic activator of PKC (56).

In these experiments it was not possible to use phase contrast microscopy to quantify accurately the large number of EIgG that were ingested by OAG-stimulated Mφ. Therefore, EIgG were labeled with ^{51}Cr and phagocytosis of these particles was quantified as described in *Materials and Methods*. Phagocytosis of EC3b and EC3bi were quantified by phase contrast microscopy as usual. In contrast to PMA, OAG caused a concentration-dependent increase in the ingestion of EC3b, EC3bi, and EIgG (Fig. 6).

To further evaluate the role of PKC in Mφ CR- and FcR-mediated phagocytosis, we quantified the effect of PKC inhibitors on Mφ phagocytosis of EC3b(i) and EIgG. Mφ were preincubated for 30 min at 37°C with varying concentrations of agents reported to inhibit PKC, and phagocytosis was quantified after 1 h at 37°C. Sphingosine (57), at concentrations of 25 to 50 μM completely inhibited Mφ CR- and FcR-mediated phagocytosis (Table V); and 10 μM sphingosine was partially inhibitory (data not shown). Concentrations of palmitoylcarnitine (58) from 50 to 75 μM also inhibited Mφ CR- and FcR-mediated phagocytosis (Table V). A total of 100 μM palmitoylcarnitine inhibited FcR-mediated ingestion completely (data not shown). However, four other agents that have been reported to inhibit PKC activity polymyxin B (59), 1-(5-isoquinolinylsulfonyl)-2-methyl' perazine (60), trifluoperazine (61) (at concentrations up to 100 μM), and staurosporine (62) (at concentrations up to 500 nM), failed to inhibit phagocytosis of EC3b(i) and EIgG.

TABLE IV

NOC and PMA do not effect Mø phagocytosis of EIgG prepared with suboptimal amount of IgG anti-E[a]

Addition	Phagocytic Index[b]
Buffer	315 ± 64
Nocodazole	275 ± 95
PMA	352 ± 84

[a] For these experiments, sheep E were prepared with a 1/3000 dilution of rabbit IgG anti-E. Mø were preincubated for 30 min with 2.5 μg/ml of NOC and then phagocytosis of EIgG was quantified after 1 h at 37°C. PMA (10 ng/ml) was added simultaneously with the EIgG.

[b] Mean ± SEM (n = 4).

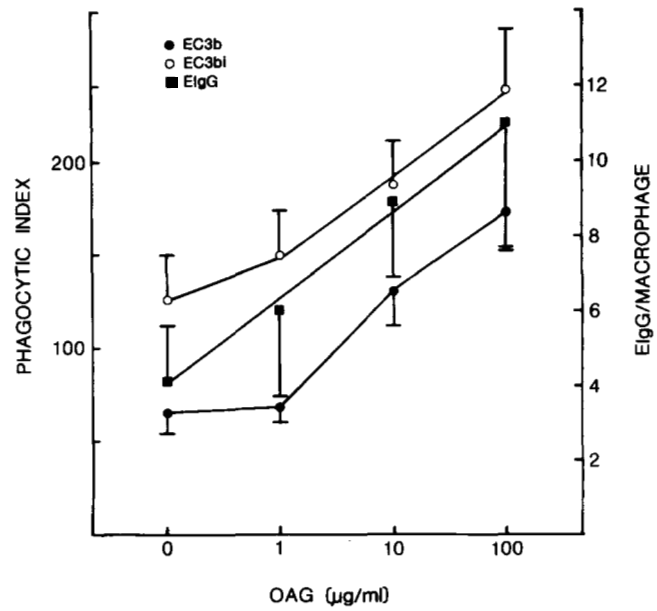

Figure 6. OAG enhances Mφ CR- and FcR-mediated phagocytosis in a concentration-dependent manner. Mφ were preincubated for 15 min at 37°C with varying concentrations of OAG. EC3b, EC3bi, and ^{51}Cr-labeled EIgG then were added to the Mφ, and phagocytosis was quantified after 30 min at 37°C. Results are presented as the mean ± SEM (n = 4 for EC3b(i); n = 5 for EIgG). Phagocytosis of EC3b(i) is presented as the phagocytic index, the total number of E ingested/100 Mφ. Phagocytosis of ^{51}Cr-labeled EIgG is presented as the number of EIgG ingested/Mφ.

TABLE V

Inhibition of Mø CR- and FcR-mediated phagocytosis by inhibitors of PKC[a]

Addition	Phagocytic Index[b]		
	EC3b	EC3bi	EIgG
Buffer	238 ± 20 (12)	524 ± 59 (12)	900 ± 54 (12)
Sphingosine			
50 μM	3 ± 1 (3)	3 ± 0 (3)	2 ± 1 (3)
25 μM	8 ± 3 (6)	15 ± 6 (6)	19 ± 9 (6)
Palmitoyl carnitine			
75 μM	18 ± 8 (6)	19 ± 6 (6)	180 ± 53 (6)
50 μM	83 ± 28 (5)	114 ± 31 (5)	541 ± 87 (5)

[a] Mø were preincubated for 30 min at 37°C with buffer, sphingosine, or palmitoyl carnitine, and then phagocytosis of ligand-coated E was quantified after 1 h at 37°C.

[b] Mean ± SEM (n).

DISCUSSION

Original studies that examined the role of MF and MT in phagocytosis suggested that the functional integrity of actin MF (23–27) but not MT (28–32) were essential for the phagocytic process. A limiting factor in these studies is that the serum opsonized bacteria and other serum opsonized particles used in these investigations engage multiple receptors (CR_1, CR_3, and FcR) on the phagocyte's plasma membrane (35, 36). These receptors may function independently in phagocytosis (9, 11, 12), and, therefore, a possible role for MT might be obscured. Indeed, in contrast to these studies, later experiments using C3b-, iC3b-, and IgG-coated E as the target particles have suggested a role for MT in both CR- and FcR-mediated phagocytosis (11, 34, 37). In experiments with cultured human

Mϕ (11), incubation of Mϕ with colchicine and podophylotoxin resulted in inhibition of ingestion of EC3b, EC3bi, and EIgG. However, because the assays were performed in the presence of PMA, and the effects of PMA on cells are pleiotropic (49–55), the interpretation of these results is difficult. In addition, the requirement for MF was not assessed in these studies.

In the present experiments we reexamined the role of MF and MT in human Mϕ CR- and FcR-mediated phagocytosis. Adherently cultured monocyte-derived Mϕ were used in these experiments because these cells spontaneously activate their CR for phagocytosis during the differentiation process (9, 12). Mϕ were preincubated with various agents that affected the integrity and function of MF and MT, and then phagocytosis of EC3b, EC3bi, and EIgG was quantified.

Inhibition of MF function with CB and CD blocked phagocytosis by CR and FcR. In contrast, disruption of MT with NOC and taxol inhibited only CR-mediated phagocytosis. Incubation of Mϕ with both NOC and taxol, which restores the assembly-initiating activity of the MTOC and removes free MT (44), enhanced CR_1- and CR_3-mediated phagocytosis. NOC and taxol alone or in combination did not effect Mϕ FcR-mediated phagocytosis.

In addition, dibutryl cGMP, and carbachol, bethanechol, and PMA, agents that increase intracellular cGMP and promote MT assembly and stability (45–48, 50, 51), augmented CR_1- and CR_3-mediated ingestion. Conversely, dibutryl cAMP, and epinephrine, and isoproterenol, agents that increase intracellular cAMP and prevent MT assembly (45–48), inhibited CR-mediated phagocytosis. FcR function was not effected by any of these agents.

These results suggest that there is a distinct dichotomy in the requirement for cellular cytoskeketal elements in human Mϕ CR- and FcR-mediated phagocytosis. CR are dependent on both MT and MF for phagocytosis, whereas FcR require only MF.

In contrast to phagocytosis, we observed that ligand binding to Mϕ FcR and CR did not require an intact cytoskeleton. Others have reported that rabbit alveolar Mϕ demonstrated a partial requirement for both MF and MT for optimum EC3b rosette formation (63), and required intact MF for optimum FcR-mediated binding (64). However, maximum inhibition of binding of EC3b or EIgG in these experiments only was 50%. In other studies, human monocyte (65, 66) and neutrophil (67) rosette formation with EIgG was inhibited by CB. Monocytes (66), but not neutrophils (67), also showed some sensitivity to MT poisons with respect to formation of EIgG rosettes. However, the concentrations of colchicine and vinblastine used in these experiments, 10^{-6} and 10^{-5} M, caused the loss of 50% of the adherent monocytes (66).

The differences between our results and these earlier studies are most likely because of differential requirements for cellular cytoskeleton by the different phagocyte populations. Other factors that may have influenced these results include the concentrations of and different inhibitors used, the number of IgG or C3b molecules bound per E, and the different types of assays used to quantify binding.

In agreement with previous studies (11), PMA strongly enhanced CR-mediated phagocytosis, but did not effect FcR-mediated phagocytosis. Mechanism(s) by which PMA might selectively enhance Mϕ CR-mediated phagocytosis include: 1) activation of PKC (49); 2) increasing intracellular cGMP (50, 51), and promoting MT assembly at centrosomes (52–54); or 3) both. If PMA enhancement of Mϕ CR- but not FcR-mediated phagocytosis was mediated by promoting MT assembly, rather than by stimulating PKC, it would suggest either that PMA did not activate PKC in this system, or, that if PKC was activated, that PKC activation was not important for FcR-mediated phagocytosis.

In contrast to PMA, activation of PKC with OAG enhanced Mϕ phagocytosis via CR and FcR. In addition, two agents reported to inhibit PKC activity, sphingosine (57) and palmitoylcarnitine (58), also inhibited Mϕ CR- and FcR-mediated ingestion. Our interpretation of the data, therefore, is that PMA most likely enhances CR-mediated phagocytosis via a direct or indirect effect on MT function. Although it is unclear why only two of six inhibitors of PKC inhibited Mϕ receptor-mediated phagocytosis in this system, overall, the data suggest that PKC is involved in the phagocytic signal generated by both Mϕ CR and FcR.

It is intriguing to speculate that the present observations relate to previous scanning and transmission electron microscopy studies with mouse peritoneal Mϕ (68) and rat Kupffer cells (69). These experiments revealed significant differences in the way the FcR and CR of these cells mediated ingestion of IgG- and C3-coated E. EIgG were ingested after formation of a pseudopod around the entire E. EC3, however, were found to be attached to a single point on the Mϕ membrane, and then were pulled into the interior of the cell without any further contact with the Mϕ membrane.

It is clear from the observations of Reaven and Axline (22) and from other studies (70–73) that there is a dynamic relationship between MT and actin networks. In vitro studies using pure actin filaments and MAP bound to MT have shown that there is an interaction between actin filaments and MT that require MAP (70, 71). These MAP can bind to actin filaments and can induce the reversible formation of actin filament bundles (72). Ultrastructural studies of PMN have shown that binding of ligand-coated particles to membrane receptors induces the rapid assembly of MT from centrioles. MF are recruited to the cytoplasm immediately adjacent to membrane regions containing bound particles. MT are generally excluded from these areas of MF concentration. Nevertheless, the distribution of MF may be regulated in part by the inducible system of cytoplasmic MT (73).

Stimulation of Mϕ CR_1 and CR_3 by EC3b and EC3bi may transmit a signal for the assembly of MT that interact with the cortical actin network to promote phagocytosis. Treatment of Mϕ with PMA and cholinergic agents could enhance this process by increasing intracellular cGMP and promoting MT assembly. In contrast, stimulation of the Mϕ FcR by EIgG may transmit a signal directly to the cortical actin network alone. How these events might be linked to the hydrolysis of inositol phospholipids, the generation of diacylglycerol, and the activation of PKC is unclear. Further investigation of this system may provide clues to the understanding of the biochemical basis of signal transduction, and the linkage of this system to the cellular cytoskeleton.

REFERENCES

1. **Fearon, D. T.** 1980. Identification of the membrane glycoprotein that is the C3b receptor of the human erythrocyte, polymorphonuclear leukocyte, B lymphocyte, and monocyte. *J. Exp. Med. 152:20.*
2. **Beller, D. I., T. A. Springer, and R. D. Schreiber.** 1982. Anti-Mac-1 selectively inhibits the mouse and human type three complement receptor. *J. Exp. Med. 156:1000.*
3. **Wright, S. D., P. E. Rao, W. C. Van Voorhis, L. S. Craigmyle, K. Iida, M. A. Talle, E. F. Westberg, G. Goldstein, and S. C. Silverstein.** 1983. Identification of the C3bi receptor of human monocytes and macrophages by using monoclonal antibodies. *Proc. Natl. Acad. Sci. USA 80:5699.*
4. **Anderson, C. L., and R. J. Looney.** 1986. Human leukocyte IgG Fc receptors. *Immunol. Today 7:264.*
5. **Mantovani, B.** 1975. Different roles of IgG and complement receptors in phagocytosis by polymorphonuclear leukocytes. *J. Immunol. 115:15.*
6. **Scribner, D. J., and D. Fahrney.** 1976. Neutrophil receptors for IgG and complement: their roles in the attachment and ingestion phases of phagocytosis. *J. Immunol. 116:892.*
7. **Newman, S. L. and R. B. Johnston, Jr.** 1979. Role of binding through C3b and IgG in polymorphonuclear neutrophil function: Studies with trypsin-generated C3b. *J. Immunol. 123:1839.*
8. **Ehlenberger, A. G., and V. Nussenzweig.** 1977. The role of membrane receptors for C3b and C3d in phagocytosis. *J. Exp. Med. 145:357.*
9. **Newman, S. L., R. A. Musson, and P. M. Henson.** 1980. Development of functional complement receptors during in vitro maturation of human monocytes into macrophages. *J. Immunol. 125:2236.*
10. **Kaplan, G., and G. Gaudernack.** 1982. In vitro differentiation of human monocytes. Differences in monocyte phenotypes induced by cultivation on glass or on collagen. *J. Exp. Med. 156:1101.*
11. **Wright, S. D., and S. C. Silverstein.** 1982. Tumor-promoting phorbol esters stimulate C3b and C3b' receptor-mediated phagocytosis in cultured human monocytes. *J. Exp. Med. 156:1149.*
12. **Newman, S. L., J. E. Devery-Pocius, G. D. Ross, and P. M. Henson.** 1984. Phagocytosis by human monocyte-derived macrophages. Independent function of receptors for C3b (CR_1) and iC3b(CR_3). *Complement 1:213.*
13. **Wright, S. D., and B. C. Meyer.** 1986. Phorbol esters cause sequential activation and deactivation of complement receptors on polymorphonuclear leukocytes. *J. Immunol. 136:1759.*
14. **Griffin, F. M., Jr., and P. J. Mullinax.** 1985. Effects of differentiation in vivo and of lymphokine treatment in vitro on the mobility of C3 receptors of human and mouse mononuclear phagocytes. *J. Immunol. 135:3394.*
15. **Pommier, C. G., S. Inada, L. F. Fries, T. Takahashi, M. M. Frank, and E. J. Brown.** 1983. Plasma fibronectin enhances phagocytosis of opsonized particles by human peripheral blood monocytes. *J. Exp. Med. 157:1844.*
16. **Wright, S. D., L. S. Craigmyle, and S. C. Silverstein.** 1983. Fibronectin and serum amyloid P component stimulate C3b- and C3bi-mediated phagocytosis in cultured human monocytes. *J. Exp. Med. 158:1338.*
17. **Pommier, C. G., J. O'Shea, T. Chused, K. Yancey, M. M. Frank. T. Takahashi, and E. J. Brown.** 1984. Studies on the fibronectin receptors of human peripheral blood leukocytes. Morphologic and functional characterization. *J. Exp. Med. 159:137.*
18. **Bohnsack, J. F., H. K. Kleinman, T. Takahashi, J. J. O'Shea, and E. J. Brown.** 1985. Connective tissue proteins and phagocytic cell function. Laminin enhances complement and Fc-mediated phagocytosis by cultured human macrophages. *J. Exp. Med. 161:912.*
19. **Newman, S. L., and M. A. Tucci.** 1990. Regulation of human monocyte/macrophage function by extracellular matrix. Adherence of monocytes to collagen matrices enhances phagocytosis of opsonized bacteria by activation of complement receptors and enhancement of Fc receptor function. *J. Clin. Invest. 85:703.*
20. **Gresham, H. D., L. T. Clement, J. E. Lehmeyer, F. M. Griffin, Jr., and J. E. Volanakis.** 1986. Stimulation of human neutrophil Fc receptor-mediated phagocytosis by a low molecular weight cytokine. *J. Immunol. 137:868.*
21. **Parker, C. J., R. N. Frame, and M. R. Elstad.** 1988. Vitronectin (S protein) augments the functional activity of monocyte receptors for IgG and complement C3b. *Blood 71:86.*
22. **Reaven, E. P., and S. G. Axline.** 1973. Subplasmalemmal microfilaments and microtubules in resting and phagocytizing cultivated macrophages. *J. Cell Biol. 59:12.*
23. **Malawista, S. E., J. B. L. Gee, and K. G. Bensch.** 1971. Cytochalasin Breversibly inhibits phagocytosis: functional, metabolic, and ultrastructural effects in human blood leukocytes and rabbit alveolar macrophages. *Yale J. Biol. Med. 44:286.*
24. **Davis, A. T., R. Estensen, and P. G. Quie.** 1971. Cytochalasin B. III. Inhibition of polymorphonuclear leukocyte phagocytosis. *Proc. Soc. Exp. Biol. Med. 137:161.*
25. **Zigmond, S. H., and J. G. Hirsch.** 1972. Effects of cytochalasin B on polymorphonuclear leucocyte locomotion, phagocytosis and glycolysis. *Exp. Cell Res. 73:383.*
26. **Allison, A. C., P. Davies, and S. de Petris.** 1971. Role of contractile microfilaments in macrophage movement and endocytosis. *Nature 232:153.*
27. **Axline, S. G., and E. P. Reaven.** 1974. Inhibition of phagocytosis and plasma membrane mobility of the cultivated macrophage by cytochalasin B. Role of subplasmalemmal microfilaments. *J. Cell Biol. 62:647.*
28. **Malawista, S. E., and P. T. Bodel.** 1967. The dissociation by colchicine of phagocytosis from increased oxygen consumption in human leukocytes. *J. Clin. Invest. 46:786.*
29. **Boxer, L. A., M. Rister, J. M. Allen, and R. L. Baehner.** 1977. Improvement of Chediak-Higashi leukocyte function by cyclic guanosine monophosphate. *Blood 49:9*
30. **Bhisey, A. N., and J. J. Freed.** 1971. Altered movement of endosomes in colchicine-treated cultured macrophages. *Exp. Cell Res. 64:430.*
31. **Ukena, T. E., and R. D. Berlin.** 1972. Effect of colchicine and vinblastine on the topographical separation of membrane functions. *J. Exp. Med. 136:1.*
32. **Oliver, J. M., T. E. Ukena, and R. D. Berlin.** 1974. Effects of phagocytosis and colchicine on the distribution of lectin-binding sites on cell surfaces. *Proc. Nat. Acad. Sci. USA 71:394.*
33. **Stossel, T. P., R. J. Mason, J. Hartwig, and M. Vaughan.** 1972. Quantitative studies of phagocytosis by polymorphonuclear leukocytes: use of emulsions to measure the initial rate of phagocytosis. *J. Clin. Invest. 51:615.*
34. **Walter, R. J., R. D. Berlin, J. R. Pfeiffer, and J. M. Oliver.** 1980. Polarization of endocytosis and receptor topography on cultured macrophages. *J. Cell Biol. 86:199.*
35. **Newman, S. L., and L. K. Mikus.** 1985. Deposition of C3b and iC3b onto particulate activators of the human complement system. Quantitation with monoclonal antibodies to human C3. *J. Exp. Med. 161:1414.*
36. **Verbrugh, H. A., P. K. Peterson, B. T. Nguyen, S. P. Sission, and Y. Kim.** 1982. Opsonization of encapsulated *Staphylococcus aureus*: the role of specific antibody and complement. *J. Immunol. 129:1681.*
37. **Griffin, F. M., and P. J. Mullinax.** 1981. Augmentation of macrophage complement receptor function in vitro. III. C3b receptors that promote phagocytosis migrate within the plane of the macrophage plasma membrane. *J. Exp. Med. 154:291.*
38. **Ross, G. D., J. D. Lambris, J. A. Cain, and S. L. Newman.** 1982. Generation of three different fragments of bound C3 with purified factor I or serum. I. Requirements for factor H vs CR_1 cofactor activity. *J. Immunol. 129:2051.*
39. **Ross, G. D., S. L. Newman, J. D. Lambris, J. E. Devery-Pocius, J. A. Cain, and P. J. Lachmann.** 1983. Generation of three different fragments of bound C3 with purified factor I or serum. II. Location of binding sites in the C3 fragments for B and H, complement receptors, and bovine conglutinin. *J. Exp. Med. 158:334.*
40. **Kabat, E. A., and M. M. Mayer.** 1961. Complement and complement fixation. In *Experimental Immunochemistry, 2nd ed.* Charles C. Thomas, Springfield, IL, p. 133.
41. **Hoebeke, J., G. Van Nijen, and M. De Brabander.** 1976. Interaction of oncodazole (R17934), a new anti-tumoral drug, with rat brain tubulin. *Biochem. Biophys. Res. Commun. 69:319.*
42. **De Brabander, M. J., R. M. L. Van de Veire, F. E. M. Aerts, M. Borgers, and P. A. J. Janssen.** 1976. The effects of methyl[5-(2-thienylcarbonyl)-1H-benzimidazole-2-yl] carbamate, (R17934; NSC 238159), a new synthetic anti-tumoral drug interfering with microtubules, on mammalian cells. *Cancer Res. 36:905.*
43. **De Brabander, M., R. Van de Veire, F. Aerts, G. Geuens, M. Borgers, L. Desplenter, and J. De Cree.** 1975. R17934: a new anti-cancer drug interfering with microtubules. Effects on neoplastic cells cultured in vitro and in vivo. In *Microtubules and Microtubule Inhibitors.* M. Borgers and M. De Brabander, eds. ASP Biological and Medical Press B.V., Amsterdam, p. 509.
44. **De Brabander, M., G. Geuens, R. Nuydens, R. Willebrords, and J. De Mey.** 1982. Microtubule stability and assembly in living cells: The influence of metabolic inhibitors, taxol and pH. *Cold Spring Har. Symp. Quant. Biol. 46:227.*
45. **Goldstein, I., S. Hoffstein, J. Gallin, and G. Weissmann.** 1973. Mechanisms of lysosomal enzyme release from human leukocytes: microtubule assembly and membrane fusion induced by a component of complement. *Proc. Natl. Acad. Sci. USA 70:2916.*
46. **Zurier, R. B., G. Weissmann, S. Hoffstein, S. Kammerman, and H. H. Tai.** 1974. Mechanisms of lysosomal enzyme release from human leukocytes. II. Effects of cAMP and cGMP, autonomic agonists, and agents which affect microtubule function. *J. Clin. Invest. 53:297.*
47. **Weissmann, G., I. Goldstein, S. Hoffstein, and P. Tsung.** 1975. Reciprocal effects of cAMP and cGMP on microtubule-dependent release of lysosomal enzymes. *Ann. N.Y. Acad. Sci. 253:750.*
48. **Oliver, J. M.** 1976. Impaired microtubule function correctable by cyclic GMP and cholinergic agonists in the Chediak-Higashi syndrome. *Am. J. Pathol. 85:395.*
49. **Castagna, M., Y. Takai, K. Kaibuchi, K. Saro, U. Kikkawa, and Y. Nishizuka.** 1982. Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor promoting phorbol esters. *J. Biol. Chem. 257:7847.*
50. **Estensen, R. D., H. R. Hill, P. G. Quie, N. Hogan, and N. D. Goldberg.**

1973. Cyclic GMP and cell movement. *Nature 245:458.*

51. **Goldberg, N. D., M. K. Haddox, R. Estensen, J. G. White, C. Lopez, and J. W. Hadden.** 1974. Evidence of a dualism between cyclic GMP and cyclic AMP in the regulation of cell proliferation and other cellular processes. In *Cyclic AMP, Cell Growth, and the Immune Response.* W. Braun, L. M. Lichtenstein, and C. W. Parker, eds. Springer-Verlag, New York, p. 247.
52. **Schliwa, M., K. B. Pryzwansky, and G. G. Borisy.** 1983. Tumor promoter-induced centrosome splitting in human polymorphonuclear leukocytes. *Eur. J. Cell Biol. 32:75.*
53. **Phaire-Washington, L., E. Wang, and S. C. Silverstein.** 1980. Phorbol myristate acetate stimulates pinocytosis and membrane spreading in mouse peritoneal macrophages. *J. Cell Biol. 86:634.*
54. **Phaire-Washington, L., S. C. Silverstein, and E. Wang.** 1980. Phorbol myristate acetate stimulates microtubule and 10-nm filament extension and lysosomal redistribution in mouse macrophages. *J. Cell Biol. 86:641.*
55. **Goldstein, I. M., S. T. Hoffstein, and G. Weissmann.** 1975. Mechanisms of lysosomal enzyme release from human polymorphonuclear leukocytes. Effects of phorbol myristate acetate. *J. Cell Biol. 66:647.*
56. **Nishizuka, Y.** 1986. Studies and perspectives of protein kinase C. *Science 233:305.*
57. **Hannun, Y. A., C. R. Loomis, A. H. Merril, and R. M. Bell.** 1986. Sphingosine inhibition of protein kinase C activity and of phorbol dibutyrate binding in vitro and in human platelets. *J. Biol. Chem. 261:12604.*
58. **Nakai, T., S. Mita, S. Yamamoto, T. Nakadate, and R. Kato.** 1984. Inhibition by palmitoylcarnitine of adhesion and morphological changes in HL-60 cells induced by 12-O-tetradecanoylphorbol-13-acetate. *Cancer Res. 44:1908.*
59. **Mazzei, G. J., N. Katoh, and J. F. Kuo.** 1982. Polymyxin B is a more selective inhibitor of phospholipid-sensitive Ca^{2+}-dependent protein kinase than for calmodulin-sensitive Ca^{2+}-dependent protein kinase. *Biochem. Biophys. Res. Commun. 109:1129.*
60. **Hidaka, H., M. Inagaki, S. Kawamoto, and Y. Sasaki.** 1984. Isoquinolinesulfonamides, novel and potent inhibitors of cyclic nucleotide dependent protein kinase and protein kinase C. *Biochemistry 23:5036.*
61. **Ikeda, M., W. J. Deery, M. S. Ferdows, T. B. Nielsen, and J. B. Field.** 1987. Role of cellular Ca^{2+} in phosphorylation of 21 K and 19 K polypeptides in cultured thyroid cells: Effects of phorbol ester, trifluoperazine, and 8-diethylamino-octyl-3,4,5-trimethoxybenzoate hydrochloride. *Endocrinology 121:175.*
62. **Tamaoki, T., H. Nomoto, I. Takahashi, Y. Kato, M. Morimoto, and F. Tomita.** 1986. Staurosporine, a potent inhibitor of phospholipid/Ca^{2+} dependent protein kinase. *Biochem. Biophys. Res. Commun. 135:397.*
63. **Atkinson, J. P., J. M. Michael, H. Chaplin, Jr., and C. W. Parker.** 1977. Modulation of macrophage C3b receptor function by cytochalasin-sensitive structures. *J. Immunol. 118:1292.*
64. **Atkinson, J. P., and C. W. Parker.** 1977. Modulation of macrophage Fc receptor function by cytochalasin-sensitive structures. *Cell. Immunol. 33:353.*
65. **Herskovitz, B., I. V. D-Guerry, R. A. Cooper, and A. D. Schreiber.** 1977. Effect of cytochalasin B on human monocyte binding and sphering of IgG-coated human erythrocytes. *Blood 49:289.*
66. **Passwell, J. H., E. Schneeberger, and E. Merler.** 1978. Cellular requirements for the formation of EA rosettes by human monocytes. *Immunology 35:863.*
67. **Lawrence, W. D., C. H. Packman, J. M. Rowe, and M. A. Lichtman.** 1981. Attachment of particle-bound IgG and complement to human neutrophils. *Blood 58:772.*
68. **Kaplan, G.** 1977. Differences in the mode of phagocytosis with Fc and C3 receptors in macrophages. *Scand. J. Immunol. 6:797.*
69. **Munthe-Kass, A. C., G. Kaplan, and R. Seljelid.** 1976. On the mechanism of internalization of opsonized particles by rat Kupffer cells in vitro. *Exp. Cell Res. 103:201.*
70. **Griffith, L. M., and T. D. Pollard.** 1978. Evidence for actin filament-microtubule interaction mediated by microtubule-associated proteins. *J. Cell Biol. 78:958.*
71. **Griffith, L. M., and T. D. Pollard.** 1982. The interaction of actin filaments with microtubules and microtubule-associated proteins. *J. Biol. Chem. 257:9143.*
72. **Sattilaro, R. F., W. L. Dentler, and E. L. LeCluyse.** 1981. Microtubule-associated proteins (MAPs) and the organization of actin filaments *in vitro. J. Cell Biol. 90:467.*
73. **Oliver, J. M.** 1978. Cell biology of leukocyte abnormalities—membrane and cytoskeletal function in normal and defective cells. *Am. J. Pathol. 93:221.*