ACTIVATION OF MOUSE PERITONEAL MACROPHAGES BY MONOCLONAL ANTIBODIES TO MAC-1 (COMPLEMENT RECEPTOR TYPE 3)

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Macrophage activation, enhancement of the ability of macrophages to kill microbial pathogens (1, 2), is associated with complex modifications in the cells' secretory, endocytic, locomotory, cytotoxic, and antigenic properties (3). Phenotypic changes correlated with activation include increased release of hydrogen peroxide, decreased secretion of protein, increased binding of particles coated with IgG, decreased ingestion of these and other particles, and increased expression of Ia antigens. At least three biochemically defined cytokines have been characterized as macrophage activators: interferon-γ (IFN-γ) (4), tumor necrosis factor (TNF-α)[1] (5, 6), and lymphotoxin (TNF-β) (6). We know little about the structures and modes of action of receptors for these cytokines. Experiments with crosslinking agents suggest that molecules of ~95 kD may be associated with both IFN-γ receptors (7) and TNF-α/β receptors (8).

Macrophages express a family of structurally and functionally related surface glycoproteins: lymphocyte function–associated antigen-1 (LFA-1), Mac-1, and p150/95 (9). Each of these molecules consists of an antigenically distinct α chain (M_r = 150–190 kD) noncovalently associated with an identical β chain (M_r = 95 kD) as an $\alpha_1\beta_1$ dimer (9, 10). Congenital inability to express the β chain results in marked impairment of surface expression of all three heterodimers (11), severe defects in leukocyte accumulation in vivo and adherence in vitro, and an increase in life-threatening infections (12). In vitro studies indicate that at least two of these proteins act as receptors for cell-bound ligands. Mac-1 mediates the attachment and phagocytosis of particles coated with C3bi by granulocytes and macrophages, and thus is identical to complement receptor type three (CR3) (13, 14). LFA-1 mediates the attachment of macrophages to tumor cells (15) and the attachment of cytotoxic T cells to targets (9, 16). In addition, recent findings implicate Mac-1 in binding by phagocytes of diverse microbes (17–21) and bacterial cell wall lipopolysaccharide (LPS) (21).

In the foregoing studies, monoclonal antibodies to Mac-1 have helped clarify

TABLE I
Monoclonal Antibodies

Clone	Reactivity	Isotype	Reference
M1/70	α Chain of Mac-1 (CR3)	IgG2b	16
M18/2	β Chain of LFA-1*	IgG2a	11
FD441.8	α Chain of LFA-1	IgG2b	22
F4/80	gp 160	IgG2b	23
B21.2	Ia^{b,d}	ND	24
2.4G2	FcR II‡	IgG2b	25
HO-13-4	Thy-1.2	IgM	26
R4-6A2	Murine rIFN-γ	IgG1	27

* Also recognizes β chain of Mac-1 and p150/95.
$\ddagger$ FcR for IgG1 and IgG2b monomers and for immune complexes.

its functions by affording immediate inhibition of rapid cell responses, such as binding and ingestion. In contrast, in the experiments described below, we focused on delayed effects. We found that four features of activation of mouse peritoneal macrophages induced by recombinant IFN-γ (rIFN-γ) were elicited within 1–2 d of exposure to submicrogram concentrations of mAb to Mac-1. Moreover, F(ab')$_2$ of anti-Mac-1 mAb, or plating of macrophages on C3bi-coated surfaces, inhibited the activation of macrophages by rIFN-γ. These findings assign a new function of Mac-1 (CR3): the ability, when suitably ligated, to evoke various features of macrophage activation. These observations also raise the possibility that Mac-1 participates in the activation of macrophages by cytokines.

Materials and Methods

Mice. Female mice 8–12 wks old were purchased from the Trudeau Institute, Saranac Lake, NY (ICR and CD$_2$F$_1$ strains) and Charles River Laboratories, Wilmington, MA (CD$_1$ strain).

Macrophage Culture. Resident macrophages were washed from the peritoneal cavity with RPMI 1640 (KC Biologicals, Inc., Lenexa, KS). After centrifugation at 170 g for 10 min, the cell pellets were resuspended in complete medium: RPMI 1640 containing 10% heat-inactivated FCS (HyClone Laboratories, Logan, UT) and 2 mg/100 ml of gentamycin sulfate (Schering Corp., Kenilworth, NJ). The level of contaminating LPS in complete medium was <10 pg/ml as determined by a chromogenic *Limulus* amebocyte lysate test (M. A. Bioproducts, Walkersville, MD) with LPS from *Escherichia coli* 0111:B$_4$ (List Biological Laboratories, Campbell, CA) as a standard. When erythrocytes were visible, the cell pellet was treated with 0.2% NaCl for 30 s. Adherent macrophage monolayers were obtained by plating the cells in 96-well plastic trays (Corning Glass Works, Corning, NY) at 1.5–2 × 10^5 cells/well, or on 13-mm glass coverslips with 10^6 cells/coverslip for 2 h at 37 °C in 5% CO$_2$, 95% air. Nonadherent cells were removed and freshly prepared complete medium was added with the indicated experimental reagents.

Immunologic Reagents. The mAbs used are listed in Table I. IgG was purified from hybridoma supernatants using anti–rat IgG coupled to agarose beads (HyClone) according to the supplier's instructions. F(ab')$_2$ of M1/70 was a generous gift from Dr. E. J. Brown, Washington University, St. Louis, MO. Mouse rIFN-γ synthesized in *E. coli* (28), and rabbit antiserum (titer, 6.4 × 10^5 neutralizing units/ml) against recombinant mouse TNF-α were the gifts of Genentech, Inc., South San Francisco, CA. rIFN-γ had a specific activity of 5.2 × 10^7 U/mg protein as assayed by protection of mouse L cells against encephalomyocarditis virus in comparison to the National Institutes of Health IFN-γ standard, a purity of >99.96% by PAGE, and LPS contamination of <0.025 ng/mg

protein. Rabbit antiserum to this preparation of pure mouse rIFN-γ was raised by us as described elsewhere (Shparber, M., A. Ding, and C. Nathan, manuscript in preparation) and had a neutralizing titer of 1:1,000, as judged by its ability to inhibit completely the enhancement of mouse peritoneal macrophage H_2O_2 releasing capacity in response to 100 antiviral units/ml of rIFN-γ, when the antiserum and rIFN-γ were added to the macrophages simultaneously.

H_2O_2 Release. H_2O_2 secretion was detected fluorometrically by horseradish peroxidase (HRP)-catalyzed oxidation of fluorescent scopoletin to a nonfluorescent product as described in detail for macrophages in 96-well plates (29). The protein of cells adherent at the start of the assay was measured by the method of Lowry et al. (30) with bovine serum albumin as a standard. Specific release was calculated after subtracting H_2O_2 and protein values for cell-free samples and expressed as nmol H_2O_2/mg cell protein/120 min.

Ia Induction. B21.2 (Table I) was iodinated with carrier-free $Na^{125}I$ (New England Nuclear, Boston, MA) by the Iodogen method (31). Quantitative binding studies were performed at 0°C using coverslips with CD_2F_1 macrophages cultured for 48 h in the presence or absence of 100 U/ml rIFN-γ or 1 μg/ml of M1/70. Triplicate coverslips were exposed to 0.25 ml of iodinated B21.2 at a saturating level (0.3 μg/ml) for 2 h, then washed by dipping in six beakers of 150 ml ice-cold 0.9% NaCl. Cells were solubilized in 0.3 ml of 0.1 N NaOH for γ scintigraphy (Autogamma 5220; Packard Instrument Co., Downers Grove, IL) and for protein assay.

Protein Secretion. Quadruplicate coverslips of macrophages were incubated for 48 h in the presence or absence of rIFN-γ or mAbs, washed three times in 0.9% NaCl, and incubated 4 h in 0.25 ml/coverslip of methionine-free RPMI plus 50 μCi/ml of [^{35}S]-methionine, sp act 1,000 Ci/mmol (New England Nuclear). The conditioned medium was pooled and precipitated with 7% ice-cold TCA in the presence of 75 μg/ml of *Micrococcus leisodeikticus* (Sigma Chemical Co., St. Louis, MO) as a carrier. The pellet was washed twice with 5% TCA and solubilized in 50 μl of 0.0625 M Tris buffer containing 1% of SDS. Radioactivity was determined in a scintillation counter. Monolayers of macrophages were solubilized with 0.3 ml of 0.1 N NaOH and the protein contents were assayed by the method of Lowry et al. (30).

T Cell Depletion. Equal volumes of peritoneal cells (7 × 10⁶ per 1.5 ml), 1:5 dilution of anti-Thy-1.2 mAb hybridoma supernatant, and complement (baby rabbit serum, Cedarlane Laboratory, Hornby, Ontario, Canada) were incubated together at 37°C for 1 h in a water bath. All reagents were diluted in RPMI 1640 with 0.3% bovine serum albumin and 25 mM Hepes buffer, pH 7.2 (32). The cells were washed twice in RPMI 1640 before adherence.

Preparation of Zymosan, Heat-killed Bacteria and IgG-coated Glass Beads. Suspensions of zymosan (ICN, Plainview, NJ) were prepared by the method of Bonney et al. (33). *Listeria monocytogenes* (Trudeau Institute, Saranac, NY) were grown overnight to log-phase in trypticase soy broth (BBL; Becton Dickinson Co., Cockeysville, MD). After immersion in a boiling water bath for 10 min, they were washed twice with PBS, suspended at 5 × 10⁹ cells/ml in PBS, and stored in aliquots at −70°C. IgG-coated glass beads were prepared as described by Aderem et al. (34). When used as triggering agents in the H_2O_2 assay, particles were substituted for phorbol myristate acetate (PMA) in the assay mixture.

Binding and Phagocytosis. Sheep erythrocytes were coated with rabbit IgG (EIgG) or human C3bi (EC3bi) as described (35). Monolayers of phagocytes on 13-mm glass coverslips were washed and overlaid with 3 × 10⁵ E. After a 45 min incubation at 37°C, the coverslips were dipped in PBS to remove unbound E, and attachment was scored in a phase-contrast microscope. Data are presented as attachment index, the number of E bound per 100 phagocytes. Phagocytosis was scored in a similar fashion after uningested E were lysed by brief exposure to distilled water.

Preparation of Murine C3bi-coated Plates. Plastic surfaces were coated with C3bi by the action of whole mouse serum on an IgM-coated surface as described (36). In brief, a plastic plate was coated consecutively with poly-L-lysine, dinitrophenol (DNP) and monoclonal anti-DNP IgM (a gift of Dr. V. Nussenzweig). Then freshly prepared mouse serum
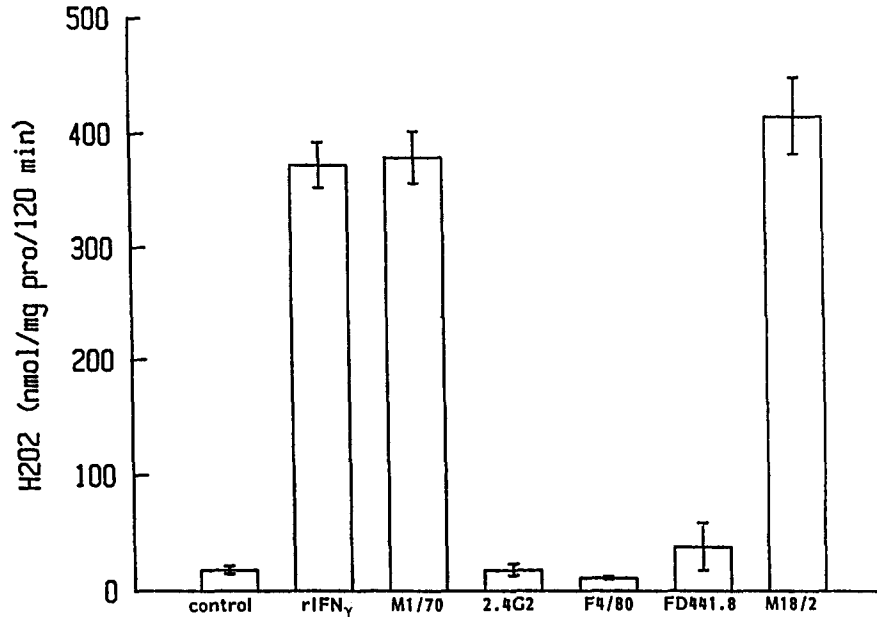
FIGURE 1.   In vitro activation of resident mouse peritoneal macrophages with mAbs to CR3. Macrophages were incubated with hybridoma supernates M1/70, 2.4G2, F4/80, FD441.8 (each 50% vol/vol), M18/2 IgG (1 μg/ml) or rIFN-γ (100 U/ml) for 48 h. Stimulation of H_2O_2 release by 100 ng/ml of PMA was assayed. The results are representative of seven separate experiments. Bars are means $\pm$ SE for triplicates.

was added and incubated at 37°C for 30 min. Control wells were filled with PBS instead of serum in the last step.

Results

Activation of Macrophage H_2O_2-releasing Capacity by mAb for Mac-1.   Resident peritoneal macrophages from normal mice release little H_2O_2 when triggered with PMA, parasites, or other stimuli (37, 38). However, macrophages activated in vivo by inflammatory or infectious agents (39), and both in vivo and in vitro by rIFN-γ (4, 40), display an enhanced capacity to release H_2O_2 upon subsequent triggering. Fig. 1 compares PMA-triggered H_2O_2 release from resident peritoneal macrophages incubated for 48 h with five mAbs directed against macrophage surface antigens (Table I). M1/70, which recognizes the α subunit of Mac-1, was as effective as rIFN-γ in enhancing macrophage H_2O_2-releasing capacity. In seven such experiments, M1/70 induced 13 $\pm$ 4-fold as much H_2O_2 releasing capacity as seen in medium alone (mean $\pm$ SE). At the dilutions used, M1/70 contained <0.1 ng/ml LPS. LPS at this concentration was unable to mimic the effect of M1/70 (data not shown). Also effective was M18/2, directed to the β chain of the LFA-1 and Mac-1 family. In four such experiments, M18/2 resulted in 14 $\pm$ 4-fold enhancement of H_2O_2-releasing capacity compared to control. However, other isotype-matched mAbs were ineffective, including those directed to the α chain of LFA-1 (FD441.8) and to FcR (2.4G2).

M18/2 was used in the above experiments as pure IgG, indicating that its action was not attributable to other components of hybridoma-conditioned
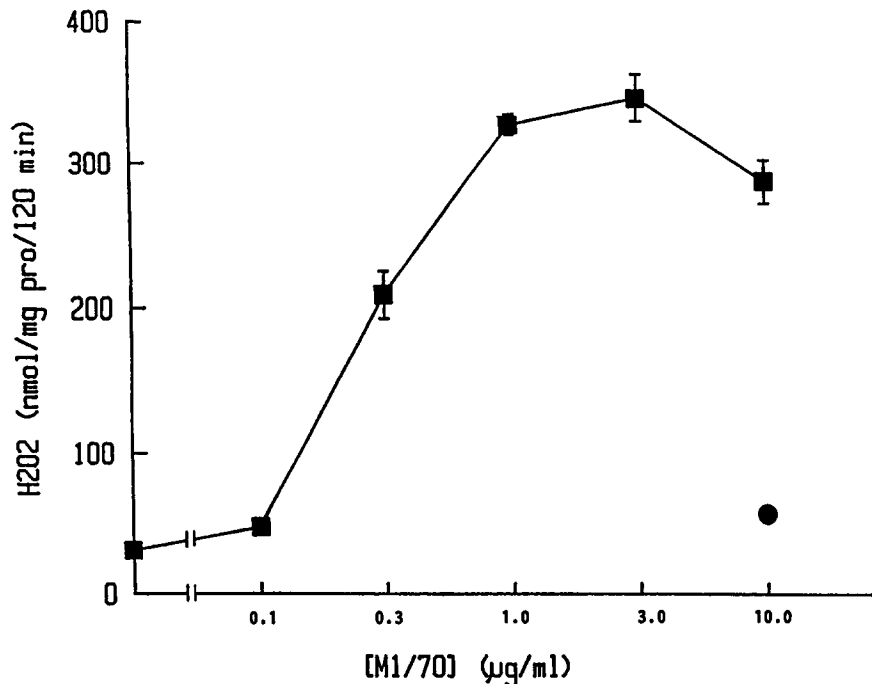
FIGURE 2. Dose response of M1/70-mediated activation. Monolayers of macrophages were cultured in varying concentrations of purified M1/70 IgG (■) or 10 μg/ml of 2.4G2 IgG (●) for 48 h before H_2O_2 assay. The results are representative of six separate experiments. Values are means ± SE for triplicates. Where no error bars are shown, they are smaller than the symbol.

medium. Similarly, Fig. 2 shows that purified M1/70 IgG was as active as M1/70 hybridoma supernatant. At 1 μg/ml, M1/70 IgG was as effective in potentiating the release of H_2O_2 as the optimal concentration of rIFN-γ. In six such experiments, the concentration of M1/70 IgG resulting in 50% of the maximal activation of macrophage H_2O_2-releasing capacity averaged 0.18 ± 0.03 μg/ml. M1/70 IgG at 1 μg/ml was used in all further experiments.

The action of M1/70 was not mediated by its Fc domain. A monoclonal antibody against FcR (2.4G2) did not affect release of H_2O_2 (Fig. 2), nor did it block the action of M1/70 (data not shown). Moreover, $F(ab')_2$ of M1/70 enhanced H_2O_2-releasing capacity (Fig. 3). Unexpectedly, M1/70 $F(ab')_2$ markedly inhibited the activating effect of rIFN-γ when both were added simultaneously (Fig. 3).

Because a stable preparation of soluble C3bi is not available for testing, to observe whether interaction of CR3 with its natural ligand would enhance H_2O_2 releasing capacity, we plated macrophages on plastic surfaces coated with C3bi. 48 h later, the plates were washed and tested for H_2O_2 in response to PMA. The H_2O_2-releasing capacity of such cells was no greater than for controls, and in fact, such cells responded less well to rIFN-γ (Fig. 4).

Time Course of M1/70-mediated Activation. rIFN-γ requires 1–2 d to induce substantial H_2O_2-releasing capacity and antiprotozoal activity in vitro (4). Activation mediated by M1/70 followed a similar time course (Fig. 5).
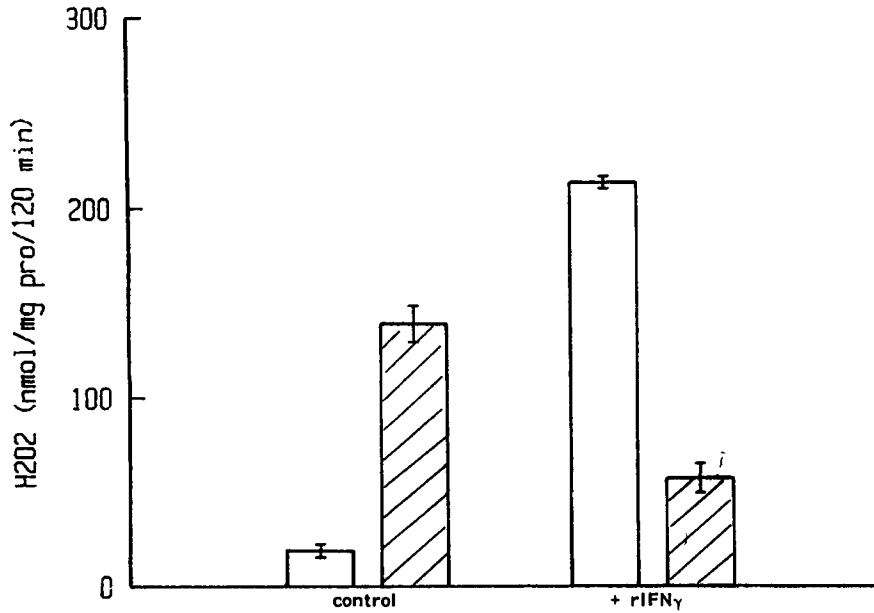
FIGURE 3.   Effect of F(ab')$_2$ of M1/70 on H$_2$O$_2$ release. Cells were incubated in the absence (*open*) or presence (*shaded*) of 1 μg/ml of F(ab')$_2$ with or without rIFN-γ (100 U/ml) for 48 h before assay. The results are representative of four separate experiments and are means $\pm$ SE for triplicates.
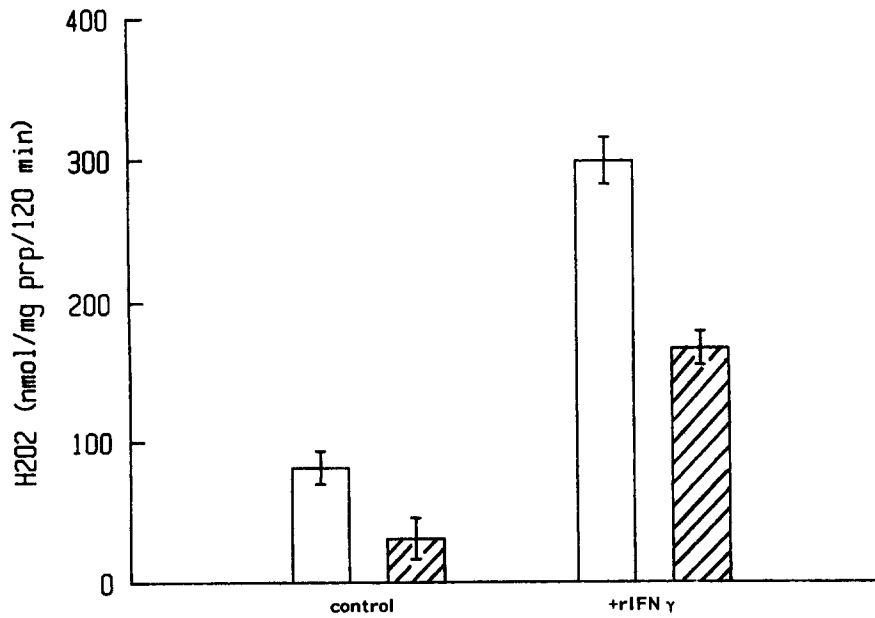

FIGURE 4.   Effect of C3bi-coated surface on H$_2$O$_2$ release. Cells were plated on IgM- (*open*) or IgMC3bi- (*shaded*) wells. Other conditions were the same as Fig. 3. The results are representative of three separate experiments and the data are means $\pm$ SE for triplicates.
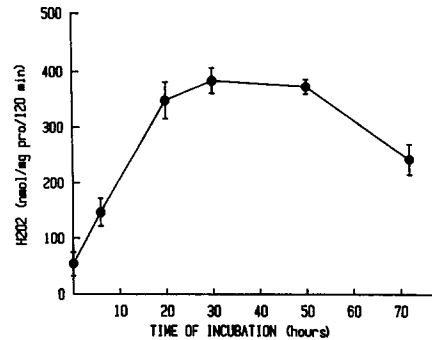
FIGURE 5. Time course of M1/70 activation. Complete medium with 1 μg/ml of purified M1/70 was added to macrophage monolayers at different time points. All H_2O_2 release assays were performed together 72 h after plating the cells. Time of incubation indicates the duration between addition of antibody and the assay. The results are representative of five separate experiments and the values are means ± SE for triplicates.

TABLE II

Induction of Ia Antigen on Resident Macrophages

Exp.	Percent of control (cpm/μg protein)*	
	M1/70-treated[‡]	rIFN-γ-treated[§]
1	164 ± 12	284 ± 13
2	155 ± 12	173 ± 12
3	251 ± 44	534 ± 18
4	213 ± 18	393 ± 30
5	209 ± 12	ND
6	175 ± 22	140 ± 9
Mean ± SE[‖]	195 ± 14	305 ± 65

* Control coverslips were preincubated for 48 h in medium alone. Values are means ± SE for triplicates. If expressed as molecules of anti-Ia (× 10^{-8}) per microgram cell protein, the results would be 2.31 ± 0.20 (controls); 4.50 ± 0.49 (M1/70-treated); and 7.10 ± 1.6 (rIFN-γ-treated).
[‡] 1 μg/ml for 48 h.
[§] 100 U/ml for 48 h.
[‖] Mean ± SE of the mean values for the individual experiments. p value: M1/70 vs. control, $p < 0.05$; rIFN-γ vs. control, $p < 0.1$; rIFN-γ vs. M1/70, $p > 0.1$ (Student's t-test for paired means).

Induction of Ia Antigen. Like IFN-γ (24, 41), M1/70 induced Ia antigens on resident peritoneal macrophages. In fact, the extent of induction by the cytokine and by the mAb did not differ to a statistically significant degree (Table II).

Inhibition of Protein Secretion. Activation of macrophages by rIFN-γ has been shown to inhibit overall protein synthesis (42). We found that exposure to rIFN-γ also decreased the overall secretion of protein (Table III). Treatment of macrophages with M1/70 induced the same effect. In fact, the patterns of individual proteins, as reflected in autoradiograms after SDS-PAGE, closely resembled that reported with macrophages activated in vivo (not shown) (43 and Shparber et al., manuscript in preparation).

Inhibition of Particle-induced H_2O_2 Release and Phagocytosis. Even though rIFN-

TABLE III

Secretory Proteins from M1/70-treated Macrophages

Exp.	Percent of control (cpm/μg protein)*	
	M1/70-treated[‡]	rIFN-γ-treated[§]
1	40	67
2	47	48
3	73	62
Mean ± SE	53 ± 8.2	59 ± 4.6

* Control coverslips were incubated with medium alone.
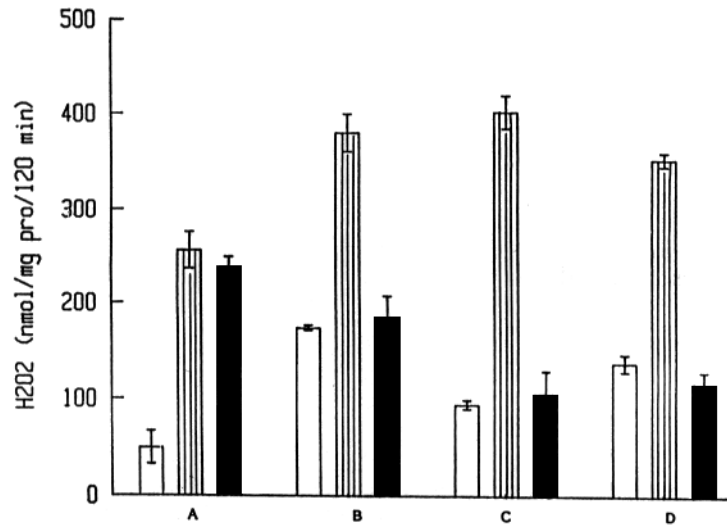[‡] 1 μg/ml for 48 h.
[§] 100 U/ml for 48 h.

FIGURE 6. H_2O_2 release of macrophages triggered with different stimuli. Resident peritoneal macrophages were cultured in the absence (□) or the presence of 100 U/ml rIFN-γ (▥) or 1 μg/ml M1/70 (■) for 48 h. H_2O_2 release was assayed upon treatment with (A) 100 ng/ml PMA, (B) 200 μg/ml zymosan, (C) 2 mg/ml IgG-coated glass beads, or (D) 10^8 heat-killed listeria. The results are representative of three separate experiments. Values are means ± SE for triplicates.

γ enhances FcR expression and binding of EIgG to macrophages, it decreases the ingestion of these particles (44–46). Likewise, activation of macrophages in vivo inhibits their phagocytosis of a variety of particles (47, 48). Nonetheless, activation of macrophages both in vitro and in vivo generally increases the amount of H_2O_2 they release in response to contact with particles. In contrast, M1/70-treated macrophages released no more H_2O_2 than controls when challenged with unopsonized zymosan, IgG-coated glass beads, or heat-killed listeria, even though the response to PMA was increased an average of fivefold in the same set of experiments (Fig. 6). The inhibition seen with zymosan and bacteria might be attributed to the involvement of CR3 in their binding (18, 19). However, this would not explain the inhibition seen with IgG-coated beads.

The effect of M1/70 on the morphology of the cells may in part explain the

above results. Thus, in contrast to the spreading induced by rIFN-γ, M1/70 induced marked cell-cell aggregation (Fig. 7). The clusters arose through aggregation rather than proliferation, because such clusters contained no more cells and incorporated no more [³H]thymidine than controls (data not shown). Aggregation of macrophages might restrict their ability to make contact with particles.

However, M1/70 also suppressed the phagocytic capacity of macrophages plated sparsely so that aggregates did not form. As shown in Table IV, under these conditions, EIgG were bound better by M1/70-treated macrophages than by control cells, but their uptake was almost completely suppressed. This was similar to the effect of rIFN-γ, but more marked.

Thus, M1/70 interfered with the macrophage response to particles in at least three ways: blockade of a receptor (CR3) involved in binding of some particles, induction of cell clustering in densely plated cultures, and inhibition of signalling by FcR without inhibition of its capacity to bind.

Evidence against Mediation of M1/70's Effects by Endogenous IFN-γ or TNF-α. All actions of M1/70 described so far, except for its induction of cell clustering, resembled those of rIFN-γ. Thus, we considered the possibility that M1/70 induced the release of IFN-γ in the adherent peritoneal cell cultures, and that IFN-γ mediated its effects. Since the most likely source of IFN-γ was contaminating T cells, we first tested the effect of depleting T cells with anti-Thy-1.2 mAb and complement under conditions where 99% of thymocytes were lysed. Table V shows the results from three such experiments. Depletion of T cells did not affect the ability of M1/70 to activate macrophages.

Nonetheless, because NK cells or macrophages could also be sources of IFN-γ (49–52) or TNF-α (53, 54), we tested the effects of antibodies that neutralize IFN-γ or TNF-α. Neither mAb nor polyclonal antiserum raised to murine rIFN-γ blocked M1/70-mediated enhancement of H_2O_2-releasing capacity, although both antibodies abrogated the effect of exogenous rIFN-γ (Fig. 8). Similarly, rabbit antiserum against murine rTNF-α at 100 neutralizing units/ml did not have any effect on M1/70-mediated macrophage activation (data not shown).

Discussion

Cell differentiation is controlled in large part by interactions of cells with extracellular molecules. The latter may include specific components of the extracellular matrix or neighboring cells. It has not been clear, however, whether the cell surface receptors known as adhesion molecules play a role in signalling changes in phenotype. We show here that mAbs that ligate the adhesion-promoting receptor, Mac-1 (CR3), on resident mouse peritoneal macrophages elicited differentiative changes associated with macrophage activation.

These changes included enhanced capacity to secrete H_2O_2, decreased secretion of proteins, increased expression of Ia antigen, and decreased phagocytosis of a variety of particles, including IgG-coated erythrocytes. Decreased phagocytosis by M1/70-treated macrophages was so marked that we could not assess the ability of anti-Mac-1–treated macrophages to kill intracellular pathogens (not shown). In this respect, anti-Mac-1 mAbs differed from rIFN-γ. Otherwise, the changes in macrophages induced by mAbs to Mac-1 closely resembled those induced by rIFN-γ, as well as by TNF-α and TNF-β (6) in type, extent, and time
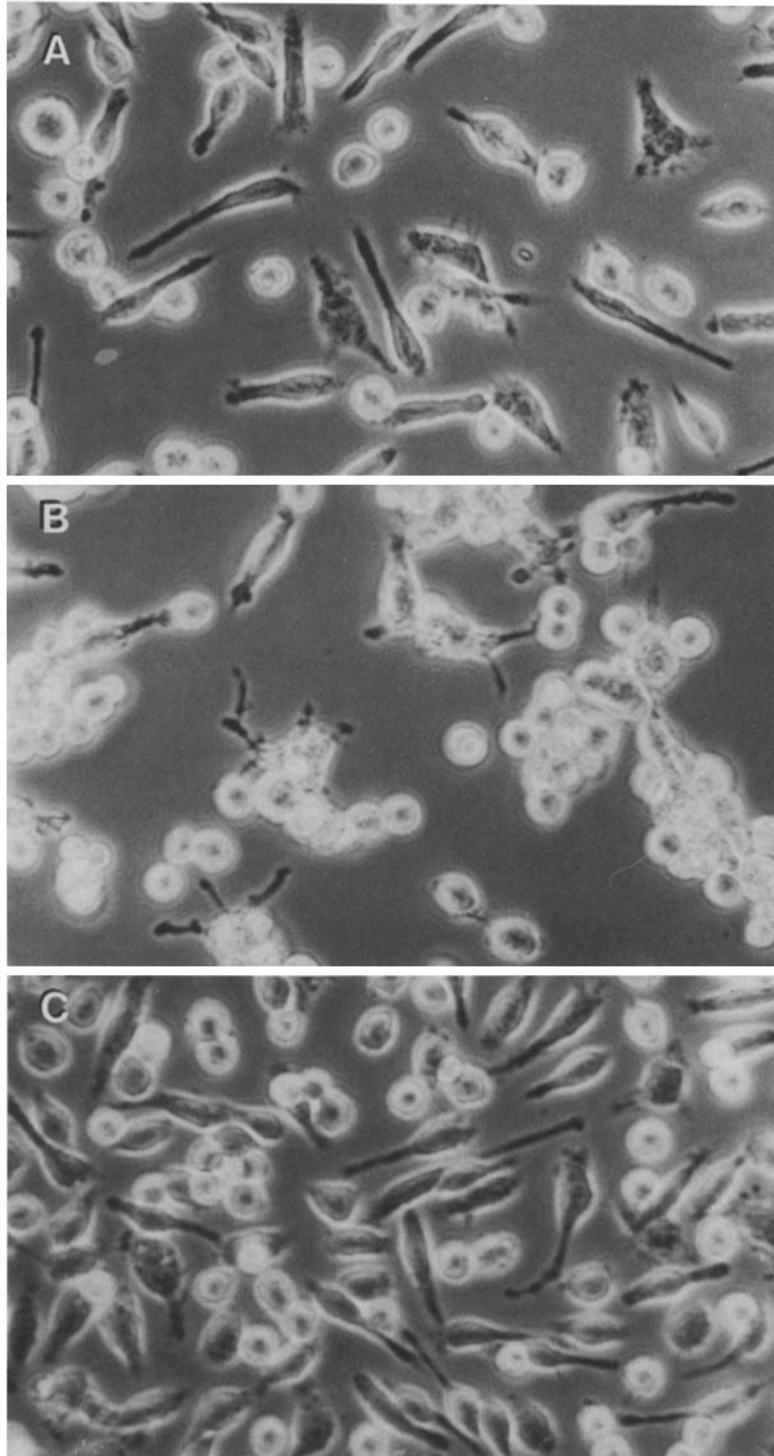
FIGURE 7.   Aggregation of M1/70-treated macrophages. Cells were incubated with (A) medium alone, (B) 1 μg/ml of M1/70, or (C) 100 U/ml of rIFN-γ for 48 h at 37°C.

TABLE IV
Effect of M1/70 on Attachment and Phagocytosis of Opsonized Erythrocytes

Treatment of macrophages	Attachment index		Phagocytic index	
	EC3bi	EIgG	EC3bi	EIgG
Control	547	596	24	312
M1/70*	172	746	4	4
rIFN-γ‡	268	1024	7	122

* 1 μg/ml for 48 h.
‡ 100 U/ml for 48 h.

TABLE V
Effect of Depletion of T Cells

Exp.	Incubation with:	H_2O_2 produced (nmol/mg protein/120 min) after pretreatment* with:			
		None	Antibody	C'	Antibody + C'
1	Medium	20	67	33	47
	M1/70‡	158	136	118	168
2	Medium	4	12	12	12
	M1/70	186	142	199	132
3	Medium	33	50	27	46
	M1/70	168	229	207	242

Results are means for triplicates.
* 1 h at 37°C, concentrations indicated in Materials and Methods.
‡ 1 μg/ml for 48 h.

course. Equally surprising, F(ab')₂ of M1/70, or modulation of Mac-1 by plating macrophages on C3bi-coated surfaces, inhibited macrophage activation by rIFN-γ.

The mAb appeared to work by binding Mac-1 itself, rather than crossreacting with other molecules or FcR. Thus mAbs were effective when directed to either the α chain or the antigenically dissimilar β chain of Mac-1, but not when reactive with the α chain of LFA-1, nor with two other macrophage surface antigens of similar abundance. Anti-FcR mAb neither mimicked nor blocked the effect of M1/70, nor was the Fc portion of M1/70 necessary.

Ligation of Mac-1 did not appear to activate macrophages by inducing the release of IFN-γ or TNF-α in the cultures. T cells, which may have contaminated the cultures, do not express Mac-1 and thus are unlikely to respond to anti-Mac-1 mAbs. In any case, treatment with anti-Thy-1.2 plus complement had no effect (Table V). NK cells, which both express Mac-1 and secrete IFN-γ (49, 50) and TNF-α-like substances (54), are exceedingly rare in adherent peritoneal cell populations from normal, untreated mice (55). In any case, M1/70-induced activation was not inhibited by neutralizing monoclonal or polyclonal antibodies to murine IFN-γ or TNF-α. The lack of inhibition of M1/70-induced activation by antibodies to TNF-α does not rule out a possible role for nonsecreted TNF-
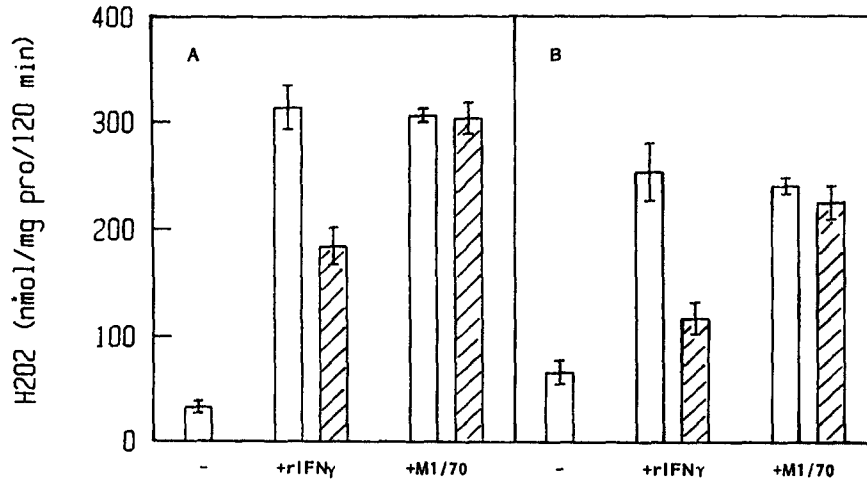
FIGURE 8.   Effects of anti-rIFN-γ antibodies on activation by M1/70. Cells were incubated with 100 U/ml of rIFN-γ or 1 μg/ml of M1/70 in the absence (*open*) or presence (*shaded*) of (*A*) mAb R4-6A2 (50%, vol/vol), (*B*) antiserum (1:2,500). PMA-dependent H_2O_2 release was then determined. The results are representative of four separate experiments and are means ± SE for triplicates.

α in this phenomenon. Since microbes and microbial products now known to bind Mac-1 are potent inducers of TNF-α release from macrophages, it would be of interest to examine induction of TNF-α mRNA in response to anti-CR3 mAbs.

Our observations with F(ab')$_2$ of M1/70 and with solid-phase C3bi suggest that CR3-reactive ligands may either induce or inhibit macrophage activation. The factors determining the outcome are unknown, but may include the degree or time course of CR3 crosslinking or clustering. These considerations may pertain to the conflicting observations on induction or inhibition of macrophage activation by microbial products now known to bind to CR3. For example, LPS can both enhance macrophage respiratory burst capacity (56) and inhibit the enhancement of respiratory burst capacity by other activators (A. Ding and C. Nathan, manuscript in preparation). Similarly, microbial cell wall preparations both elicit (57) and inhibit (58) various features of activation. Further studies of the interaction of these agents with CR3 may help explain these seemingly paradoxical observations. At present, we have no proof that macrophage activation can ensue from the binding of C3bi to CR3, although this seems likely.

The present findings suggest the existence of an intimate but undefined relationship between Mac-1 and cytokines that activate macrophages. Four such relationships are under consideration. (*a*) The ability of anti-Mac-1 mAbs to elicit several features of macrophage activation in common with cytokines may reflect the independent transduction of similar signals by mAb-ligated Mac-1 and by cytokine-ligated receptors. (*b*) Receptors for cytokines might be structurally similar to Mac-1. Mac-1 is clearly not identical with IFN-γ receptors or TNF-α receptors, given the different distribution and abundance of these molecules on various cell types. However, it is possible that a β chain similar to that of Mac-1 is associated with IFN-γ receptors and TNFα receptors, and that mAbs to the β

chains and certain epitopes on α chains (such as M1/70) could mimic the binding of IFN-γ or TNF-α. (c) Ligation of Mac-1 might cause cytokine receptors to transduce signals in the absence of their ligands, by analogy with interactions recently reported for colony-stimulating factors (59). (d) Ligated cytokine receptors might transduce their macrophage-activating signals via an interaction with Mac-1. mAb to Mac-1 could mimic this interaction and bypass the cytokines. These speculations do not make clear whether F(ab')$_2$ of M1/70 or engagement of CR3 by C3bi on the vessel surface suppressed rIFN-γ-induced activation by making Mac-1 inaccessible to rIFN-γ, or by delivering a prevailing negative signal.

It is now evident that a thorough understanding of the physiologic functions of Mac-1 will require further characterization not only of Mac-1, but also of the receptors for macrophage activating factors.

Summary

Several features of activation of mouse peritoneal macrophages were elicited by 1–2-d exposure to submicrogram concentrations of anti-Mac-1 (M1/70), a rat monoclonal antibody that reacts with the α chain of complement receptor type 3 (Mac-1). The changes induced included enhanced capacity to secrete H_2O_2 when triggered with PMA, decreased secretion of proteins, increased expression of Ia antigen and decreased phagocytosis of particles. These changes closely resembled those induced by rIFN-γ in type, extent, and time course. The concentration of M1/70 IgG resulting in 50% of the maximal activation of macrophage H_2O_2-releasing capacity averaged 0.18 ± 0.03 μg/ml. This activation was not blocked by anti-FcR mAb, and could be reproduced with M18/2, a mAb against β chain of Mac-1, suggesting that a direct ligation of Mac-1 with mAb was responsible for the activation. Neither depletion of T cells nor addition of neutralizing Abs to IFN-γ or TNF-α prevented M1/70-mediated macrophage activation. Moreover, F(ab')$_2$ of M1/70, or plating of macrophages on C3bi-coated surfaces, inhibited the activation of macrophages by rIFN-γ. These findings suggest that Mac-1 (CR3) may play an important role in macrophage activation.

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