

INTERFERON- $\gamma$  DEPRESSES BINDING OF LIGAND BY C3b  
AND C3bi RECEPTORS ON CULTURED HUMAN  
MONOCYTES, AN EFFECT REVERSED BY FIBRONECTIN

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Receptors for C3 bind ligand and generate intracellular signals that lead to the engulfment of C3-coated particles. Recent experiments suggest that both the binding and the subsequent signal transduction activities of C3 receptors can be regulated. Cultured human monocytes (MO)<sup>1</sup> express receptors for C3b (CR1) and C3bi (CR3) that bind ligand-coated particles but do not signal the cell to initiate phagocytosis. After stimulation of the MO with PMA (1), or after interaction of the MO with surfaces coated with fibronectin (Fn, reference 2), CR1 and CR3 readily generate signals leading to phagocytosis. Thus PMA and Fn regulate the signaling capacity of CR1 and CR3 in these cells. In polymorphonuclear leukocytes, both the binding and the signaling activities are coordinately regulated. Brief (15 min) stimulation with PMA increases binding and signaling activities of CR1 and CR3, but prolonged (60 min) incubation with PMA eliminates both binding and signaling (3). Since the PMA-mediated decline in the binding capacity of CR1 and CR3 is not accompanied by a loss of cell surface receptors, it appears that PMA regulates the binding and signaling activities of existing cell surface receptors.

In this report, we show that treatment of human monocytes and MO with recombinant IFN- $\gamma$  causes CR1 and CR3 to lose the ability to bind ligand. IFN- $\gamma$ -treated cells express normal levels of cell surface CR1 and CR3 as measured with anti-receptor antibodies, and the inhibition of ligand binding by CR1 and CR3 is reversed in minutes by interaction of the phagocytes with Fn-coated surfaces. Therefore, cultivation with IFN- $\gamma$  causes a reversible change in the nature of these receptors, which prevents them from interacting with ligand. Although Fn enables CR1 and CR3 on IFN- $\gamma$ -treated MO to bind ligand, these receptors fail to promote phagocytosis (signaling). For signaling to occur, IFN- $\gamma$ -treated cells require exposure to both Fn and PMA. PMA enables only signaling

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<sup>1</sup> *Abbreviations used in this paper:* Fn, fibronectin; HSA, human serum albumin; E, sheep erythrocyte; MO, monocytes cultured in Teflon beakers for 3-7 d; PD, PBS without divalent cations; phorbol dibutyrate; PDFCS, PD containing 1% FCS.

but does not influence binding. These data indicate that the capacity of receptors to bind ligand and to initiate a response are independently regulated.

### Materials and Methods

**Reagents.** PMA and aprotinin were obtained from Sigma Chemical Co., St. Louis, MO. Recombinant IFN- $\gamma$  and IFN- $\alpha$  were a gift of Hoffman-LaRoche Inc., Nutley, NJ, and the mAb, B133.3 directed against IFN- $\gamma$  (4), was a generous gift of Dr. B. Perussia, Wistar Institute, Philadelphia, PA. Levels of immunoreactive IFN- $\gamma$  were measured by a sensitive RIA (Centocor Inc., Malvern, PA). mAb 3D9 directed against CR1 (5), was provided by Drs. J. Oshea and E. Brown, National Institutes of Health, Bethesda, MD; mAb LeuM5 against p150/95 (6) was provided by Dr. C. Y. Wang, Sloan Kettering, New York; and mAb TS 1/22 directed against LFA-1 (7) was provided by Dr. T. A. Springer, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA. OKM1 and OKM10 directed against CR3, and 3C10 directed against a 55 kD protein unique to monocytes and macrophages were as previously described (8, 9). Affinity-purified F(ab)<sub>2</sub> fragments of goat anti-mouse IgG were purchased from Boehringer Mannheim Diagnostics, Inc., Houston, TX. Immunoglobulins were radioiodinated to a specific activity of  $0.4\text{--}2 \times 10^7$  cpm/ $\mu\text{g}$  by the "iodogen" procedure (10). Biotin-*N*-hydroxysuccinimide ester was purchased from Bethesda Research Laboratories, Bethesda, MD and was used to biotinylate OKM10 according to the manufacturers instructions. Colloidal gold conjugated to streptavidin was a kind gift of Janssen Pharmaceutica, Beerse, Belgium.

**Cells.** Human monocytes were purified from buffy coats on Percoll gradients and were cultured with 12.5% human serum in Teflon beakers as previously described (1). MO were iodinated with lactoperoxidase, extracted with Triton X-100, precipitated with OKM1 or TS 1/22, and separated on gels as previously described (8). Sheep erythrocytes (E) coated with C3b (EC3b), C3bi (EC3bi), or IgG (EIgG) were prepared as described (1). The number of C3/E was determined using iodinated Fab fragments of a monoclonal anti-C3, and the number of IgG/E was determined using directly iodinated IgG anti-E, as described previously (1). Unless otherwise stated, the EC3b, EC3bi, and EIgG were coated with  $\sim 80,000$  ligands/sheep erythrocyte.

**Attachment and Phagocytosis of Ligand-coated Erythrocytes.** Monolayers of MO were prepared as follows. Cells were suspended at  $0.75 \times 10^6$ /ml in PBS (137 mM NaCl; 2.7 mM KCl; 0.9 mM CaCl<sub>2</sub>; 0.5 mM MgCl<sub>2</sub>; 8 mM phosphate, pH 7.4) containing 3 mM glucose, 0.5 mg/ml human serum albumin (HSA [Worthington Biochemical Corp., Freehold, NJ]), and 0.3 U/ml aprotinin. Terasaki tissue culture plates (Miles Laboratories Inc., Naperville, IL) were coated with HSA (1 mg/ml), Fn (100  $\mu\text{g}/\text{ml}$ , Greater New York Blood Center), or OKM10 (50  $\mu\text{g}/\text{ml}$ ) by a 60 min incubation at 20°C. The culture surfaces were washed, 5  $\mu\text{l}$  MO suspension was added per well, and cells were allowed to spread at 37°C for 45 min.

Attachment of ligand-coated E to monolayers of MO was determined by adding  $5 \times 10^5$  E/well and incubating for 45 min at 37°C. The attachment of E to MO was scored by phase contrast microscopy as previously described (1). Measurement of phagocytosis was made in a similar fashion and was scored after lysing uningested E by a brief exposure to distilled water. Results are reported as attachment index or phagocytic index, i.e., the number of E bound or phagocytosed per 100 phagocytes.

**Expression of Receptor Protein on the Cell Surface.** Monolayers of adherent MO prepared as described above were immersed in cold PBS without divalent cations (PD) containing 1% FCS (PDFCS). Saturating amounts of mAbs (5  $\mu\text{g}/\text{ml}$ ) were then added to duplicate wells and the plates were incubated at 0°C for 50 min. The monolayers were thoroughly washed and incubated with 5  $\mu\text{g}/\text{ml}$  <sup>125</sup>I-F(ab)<sub>2</sub> anti-IgG for an additional 50 min at 0°C. After washing, individual wells were cut from the Terasaki plate and cell-bound radioactivity was counted in a gamma counter. Background binding was measured in wells without primary antibody. Background binding varied from 75–150 cpm/well, and the binding to cells ranged from 1,500 cpm/well (3D9) to 20,000 cpm/well (OKM10). Control experiments showed that MO remained attached to the culture wells throughout the incubations.

*Electron Microscopy.* Control of IFN- $\gamma$ -treated MO were plated on plastic surfaces coated with HSA or OKM10 for 60 min at 37°C. The location of CR3 was then determined by methods to be described in detail elsewhere (Detmers, P. A., et al., manuscript in preparation). In brief, the cells were fixed in 0.2% glutaraldehyde, 1% ethyldimethylaminopropyl carbodiimide (11), quenched with 0.5 mg/ml NaBH<sub>4</sub> in PBS, and incubated sequentially with PDFCS, biotinylated OKM10 (5  $\mu$ g/ml in PD containing 1 mg/ml HSA), and streptavidin-conjugated, 5-nm colloidal gold, with washes after the last two incubations. Labeled cells were fixed in glutaraldehyde and osmium tetroxide (12), dehydrated in a graded series of ethanol, and removed from the plastic surfaces by addition of propylene oxide (13). Monolayers pelleted by centrifugation in a microcentrifuge were embedded in Epon, sectioned, stained with uranyl acetate and lead citrate, and viewed at 80 kV on a 100CX microscope (JEOL USA, Peabody, MA). Control preparations included: (a) omission of biotinylated OKM10 and (b) addition of a 70-fold excess on nonbiotinylated OKM10 with the biotinylated OKM10. No labeling with streptavidin gold was observed in either of these two cases (data not shown).

## Results

*IFN- $\gamma$  Decreases the Capacity of MO To Bind C3b and C3bi-coated Erythrocytes.* IFN- $\gamma$  (100 U/ml) was added at intervals to cultures of monocytes, and after 5 d the binding of EC3b and EC3bi to the MO was assessed. IFN- $\gamma$  causes a sharp decline in binding of both EC3b and EC3bi, which is visible within 18 h and is maximal within 48 h (Fig. 1). Addition of IFN- $\gamma$  to MO 1 h before assay had no effect on binding (data not shown). The effect of IFN- $\gamma$  does not result in a generalized depression in the capacity of MO to bind particles since the binding of E1gG is increased by treatment with IFN- $\gamma$  (Fig. 1, references 14 and 15). A depression in the binding of EC3b and EC3bi occurs when IFN- $\gamma$  is added to freshly isolated monocytes or to 5-d cultures (data not shown), suggesting that responsiveness to IFN- $\gamma$  is not developmentally regulated. A single dose of IFN- $\gamma$  depresses binding of EC3b and EC3bi for 48 h, but receptor activity returns to normal levels after an additional 3 d of culture (Fig. 1). We have found that MO deplete >90% of the added IFN- $\gamma$  within 48 h (Table I), suggesting that reversal of the effects of IFN- $\gamma$  may be caused by consumption of the added IFN- $\gamma$ . This hypothesis is supported by the finding that the binding activity of CR1 and CR3 remains depressed in cultures to which fresh IFN- $\gamma$  was added at 48 h intervals (Fig. 1). These observations suggest that the effect of IFN- $\gamma$  on CR1 and CR3 is reversible and requires the continued presence of IFN- $\gamma$ .

Doses of IFN- $\gamma$  as low as 25 U/ml cause a sharp decline in receptor activity, and maximal depression is observed at 100 U/ml (Fig. 2). This dose response range is comparable to that observed for IFN- $\gamma$ -induced increases in Fc receptors (14), Ia antigens (16, 17), and peroxide-producing capacity (18). The action of our recombinant IFN- $\gamma$  preparations derives from their IFN- $\gamma$  content, since a neutralizing monoclonal anti-IFN- $\gamma$  antibody (4) completely blocks its action (Fig. 2). Depression of the binding activity of CR1 and CR3 is not a property shared by all interferons since IFN- $\alpha$  had no effect on the binding of EC3b or EC3bi to MO (data not shown).

The addition of IFN- $\gamma$  to cultures of monocytes does not change the number of MO harvested after 2–5 d (data not shown), suggesting that IFN- $\gamma$  does not cause either cell proliferation or cell death. The entire population of monocytes appears to respond uniformly to IFN- $\gamma$  since all cells in IFN- $\gamma$ -treated cultures

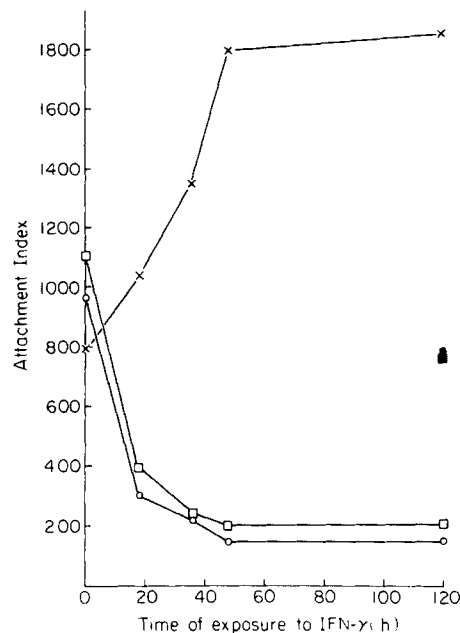


FIGURE 1. Attachment of ligand-coated erythrocytes to monocytes cultured for various periods with IFN- $\gamma$ . Monocytes were cultured for 120 h in RPMI 1640 containing 12.5% normal human serum. At various times before the end of the cultivation, 100 U/ml IFN- $\gamma$  was added to the cultures. The attachment of EC3b (○), EC3bi (□), or EIgG (×) was then measured as described in Materials and Methods. Monocytes given IFN- $\gamma$  at the start of the culture period showed strong depression of the binding of EC3b and EC3bi after 48 h (data not shown), but during further cultivation for 3 d, the capacity to bind EC3b and EC3bi was recovered (●, ■). MO consume >85 U/ml of IFN- $\gamma$  within 48 h (Table I), and thus to maintain IFN- $\gamma$  levels for extended periods, fresh IFN- $\gamma$  was added at 48 h intervals (○, □; 120 h time point). Results are representative of three separate experiments.

TABLE I  
Consumption of IFN- $\gamma$  by MO

IFN- $\gamma$ added	MO present	Immunoreactive IFN- $\gamma$ present after 48 h
0	0	0.05 ( $\pm$ 0.04) U/ml
0	$10^6$ /ml	0.42 ( $\pm$ 0.38) U/ml
100 U/ml	0	96 ( $\pm$ 22) U/ml
100 U/ml	$10^6$ /ml	5 ( $\pm$ 11.7) U/ml

Identical cultures that contained  $10^6$  monocytes/ml or medium alone were established. After cultivation for 3 d, IFN- $\gamma$  was added as shown, and after an additional 48 h of culture, the levels of immunoreactive IFN- $\gamma$  in the cell-free culture supernatant was determined by RIA. The units of IFN- $\gamma$  added were calculated from the antiviral titer of our rIFN- $\gamma$  stock, and the units of immunoreactive IFN- $\gamma$  were calculated from parallel assays of standards supplied by Centocor Inc. Data represent the average of three experiments  $\pm$  SD.

exhibited depressed binding of EC3b and EC3bi. IFN- $\gamma$ -treated MO exhibit a highly uniform, round, well-spread appearance that contrasts with the heterogeneous collection of spindle-shaped and irregularly spread cells present in untreated cultures (data not shown).

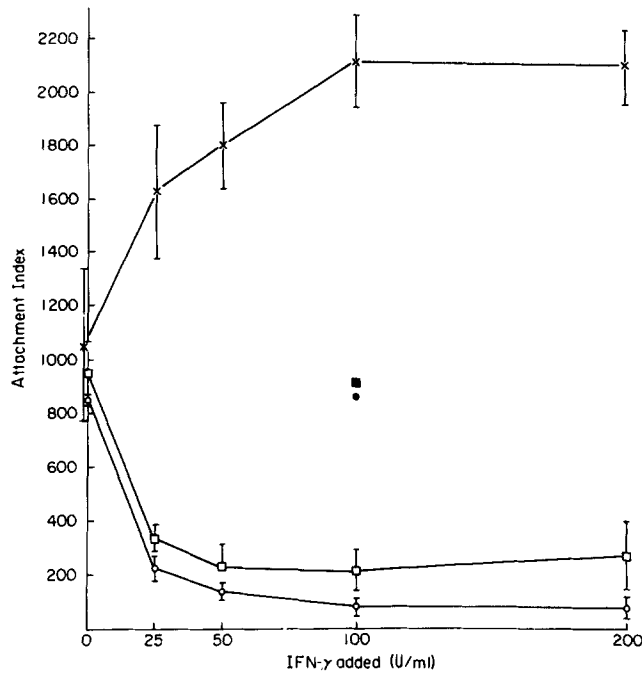


FIGURE 2. Attachment of ligand-coated erythrocytes to MO exposed to different doses of IFN- $\gamma$ . Monocytes were cultured for 3 d, then various doses of IFN- $\gamma$  were added. After an additional 48 h of culture, the attachment of EC3b (O), EC3bi (□), or EIgG (X) was measured. Results are averaged from three experiments, and error bars depict standard deviations. ● and ■ depict an experiment in which the IFN- $\gamma$  was incubated for 30 min with a 1.5-fold excess of the neutralizing anti-IFN- $\gamma$  antibody, B133.3 (4), before addition to the phagocytes.

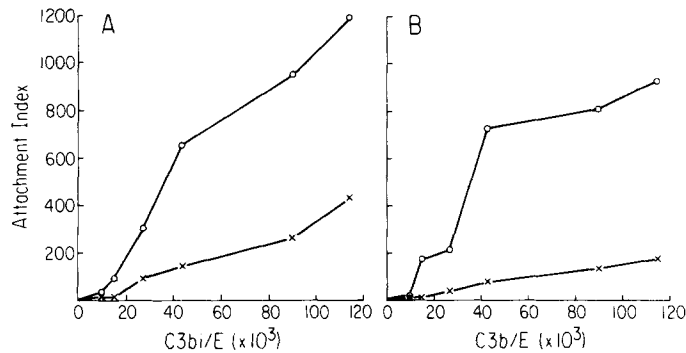


FIGURE 3. Effect of ligand density on the attachment of ligand-coated erythrocytes to control and IFN- $\gamma$ -treated MO. Erythrocytes were coated with various numbers of C3bi (A) or C3b (B) as described in Materials and Methods. The attachment of these erythrocytes was then measured using MO cultured for 5 d in the absence (O) or presence (X) of 100 U/ml IFN- $\gamma$  (added at 48 h interval).

The effect of ligand density on the binding of EC3b and EC3bi to IFN- $\gamma$ -treated MO was next measured. E were prepared bearing different numbers of C3b or C3bi, and the attachment of these particles was determined using MO cultured for 5 d in the presence or absence of IFN- $\gamma$  (Fig. 3). Half-maximal binding of E to control MO was observed with about 38,000 C3b or C3bi/E. In

TABLE II  
*Expression of Antigens in IFN- $\gamma$ -treated Phagocytes*

mAb	Antigen	Expression (relative to untreated MO)		
		Mean $\pm$ SD	n*	Significance <sup>‡</sup>
OKM1	CR3	0.99 $\pm$ 0.24	8	
OKM10	CR3	1.12 $\pm$ 0.23	4	
3D9	CR1	0.76 $\pm$ 0.21	7	
TS1/22	LFA-1	2.08 $\pm$ 0.49	9	$p < 0.001$
LeuM5	p150,95	1.03 $\pm$ 0.18	7	
9.3F10	HLA class II	2.05 $\pm$ 0.62	5	$p < 0.005$
3C10	p55	0.55 $\pm$ 0.19	5	$p < 0.001$

Monocytes were cultured for 5 d in the presence or absence of 100 U/ml IFN- $\gamma$ . The binding of mAbs to monolayers of MO was then measured as described in Materials and Methods. Antigen expression on IFN- $\gamma$ -treated MO is presented relative to expression on untreated MO.

\* Number of experiments performed.

<sup>‡</sup> Probability that expression on IFN- $\gamma$ -treated MO is not altered, based on a *t* test of paired samples.

contrast, half-maximal binding of EC3b or EC3bi to IFN- $\gamma$ -treated MO could not be observed even with 120,000 C3/E. The inhibition of CR1 activity by IFN- $\gamma$  appears more complete than CR3 since moderate binding of EC3bi (one third maximal) can be observed with very high levels of C3bi per E (Fig. 3).

*IFN- $\gamma$  Does Not Decrease the Number or Molecular Weight of C3 Receptors.* Though treatment of MO with IFN- $\gamma$  sharply decreases the capacity of MO to bind EC3b and EC3bi, the number of C3b and C3bi receptors detected with monoclonal anti-receptor antibodies is not radically altered. IFN- $\gamma$ -treated MO expressed normal levels of CR3 antigens as measured by two separate anti-CR3 antibodies (Table II), and expression of CR1 antigen was lowered by only 25%. To observe whether all the cells in the population respond similarly to IFN- $\gamma$ , we measured expression of CR3 using a FACS. These studies showed a unimodal distribution of CR3 on both control and IFN- $\gamma$ -treated cells (Fig. 4). The antigens detected by our mAbs appear to be intact receptors since CR3 immunoprecipitated from <sup>125</sup>I surface-labeled, IFN- $\gamma$ -treated MO exhibited electrophoretic mobilities identical with those from untreated MO (Fig. 5).

*Effect of IFN- $\gamma$  on the Expression of Other Cell Surface Antigens.* The expression of several other MO antigens was measured on control and IFN- $\gamma$ -treated cells. LFA-1, p150,95, and CR3 are  $\alpha_1\beta_1$  heterodimers composed of an  $\alpha$  chain that is unique and a  $\beta$  chain that is common to all three proteins (19, 8). The expression of p150,95 was unaffected by IFN- $\gamma$  (Table II), but expression of LFA-1 was increased approximately twofold (Table II and Fig. 5). Induction of LFA-1 by IFN- $\gamma$  on murine macrophages has been recently reported (20). We also confirmed the observations of several other investigators (16, 17) that the expression of Ia antigens is increased by IFN- $\gamma$ . The expression of 3C10, a marker for monocytes and macrophages (9), is decreased approximately twofold by IFN- $\gamma$ .

*The Ligand-binding Activity of CR1 and CR3 in IFN- $\gamma$ -treated MO Is Restored by Fn.* When IFN- $\gamma$ -treated MO were allowed to spread for 45 min on surfaces coated with Fn, the binding activity of CR1 and CR3 was fully restored (Table

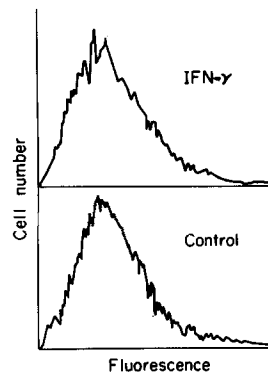


FIGURE 4. Expression of CR3 on control and IFN- $\gamma$ -treated MO. Monocytes were cultured for 4 d, then 100 U/ml IFN- $\gamma$  was added as indicated. After an additional 48 h of culture, the MO were washed and incubated at 0°C for 60 min with 2  $\mu$ g/ml OKM1. Cells were then washed, incubated with fluoresceinated F(ab)<sub>2</sub> fragments of goat anti-mouse Ig (Scandic, Vienna, Austria), then washed again. The fluorescence of the cells was measured with an Ortho Diagnostic Systems Inc. FACS II flow cytometer (Raritan, NJ) using a linear fluorescence scale.

III). This action of Fn was observed over a wide range of doses of IFN- $\gamma$  (25–200 U/ml) and times of incubation with IFN- $\gamma$  (18–120 h). The binding of IFN- $\gamma$ -treated MO to Fn-coated surfaces did not alter the number of CR1 or CR3 on the cell (Table III). Thus, Fn enhances the capacity of CR1 and CR3 to bind ligand through an alteration in the function of existing receptors, not by a change in the number of cell-surface receptors. Interestingly, the effect of Fn on the capacity of CR1 and CR3 to mediate binding could not be mimicked with PMA (Table III).

To test whether the effect of Fn on the binding activity of CR1 and CR3 is reversible, IFN- $\gamma$ -treated MO were removed with EDTA from Fn-coated surfaces (21) and were plated on fresh HSA- or Fn-coated surfaces. IFN- $\gamma$ -treated MO removed from Fn lose the ability to bind EC3b and EC3bi when they are replated for 45 min on control HSA-coated substrates (Fig. 6). However, binding activity can be restimulated by plating these MO on fresh Fn-coated surfaces. Thus, activation of the binding activity of CR1 and CR3 by Fn requires its continuous presence and is fully reversible.

*Effect of IFN- $\gamma$  on the Capacity of Receptors To Promote Phagocytosis.* Previous work had shown that treatment of MO with PMA enables CR1 and CR3 to promote phagocytosis (reference 1 and Table IV). In IFN- $\gamma$ -treated MO, however, PMA stimulated neither binding (Table III) nor phagocytosis (Table IV) mediated by CR1 or CR3. Spreading of IFN- $\gamma$ -treated cells on Fn-coated surfaces stimulated the capacity of CR1 and CR3 to bind ligand (Table III), but did not enable these receptors to promote phagocytosis (Table IV). Phagocytosis was only initiated by CR1 and CR3 when IFN- $\gamma$ -treated MO were simultaneously treated with Fn and PMA (Table IV). This observation suggests that PMA and Fn have distinct effects on CR1 and CR3; Fn enables binding and PMA enables signaling.

We have also observed that although IFN- $\gamma$ -treated MO bind EIgG more avidly than untreated control cells (Figs. 1 and 2), phagocytosis of EIgG is

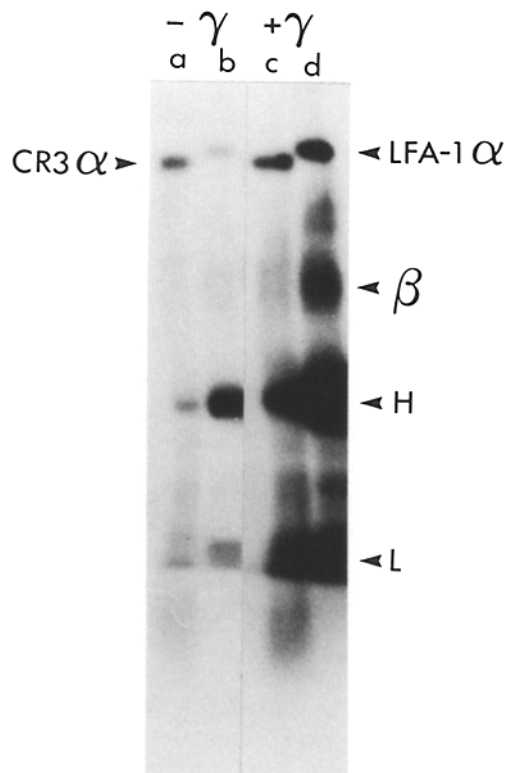


FIGURE 5. The molecular weight of CR3 is not altered by IFN- $\gamma$ . Monocytes were cultured for 5 d in the absence (*a* and *b*) or presence (*c* and *d*) of 100 U/ml IFN- $\gamma$ . Cells were then iodinated, extracted, and immunoprecipitated with anti-CR3 (OKM1, *a* and *c*) or anti-LFA-1 (TS1/22, *b* and *d*) using protein A-Sepharose. IFN- $\gamma$  causes increased expression of LFA-1, but does not change the molecular weight of either CR3 or LFA-1  $\alpha$  chains. The protein A used in the procedure also precipitates IgG heavy and light chains (*H* and *L*) that are adsorbed to the MO. IFN- $\gamma$  causes a sharp increase in the amount of IgG adsorbed to the MO, probably through increased expression of Fc receptors.

depressed two- to threefold in IFN- $\gamma$ -treated cells (Table IV). The capacity of IFN- $\gamma$ -treated MO to phagocytose EIgG is partially restored by the combined action of Fn and PMA (Table IV).

*IFN- $\gamma$  Does Not Alter The Mobility of CR3.* In murine macrophages, the signaling capacity of C3 receptors correlates with their mobility in the membrane; receptors that do not promote phagocytosis do not diffuse to the substrate-attached domain of phagocytes spread on C3-coated surfaces (22, 23). To observe the mobility of CR3 on IFN- $\gamma$ -treated MO, cells were plated on surfaces coated with monoclonal anti-CR3 (OKM10). Previous studies showed that the CR3 on untreated MO is redistributed by such substrates so that the apical surface of MO becomes depleted of receptors (8). The distribution of CR3 on IFN- $\gamma$ -treated MO could not be determined with a rosette assay because these MO do not bind EC3bi. We therefore tagged receptors with biotinylated OKM10 and streptavidin-colloidal gold as described in Materials and Methods, and used electron microscopy to determine the cellular distribution of CR3. Fig. 7*b* shows



TABLE III  
*The Inactivity of Complement Receptors on IFN- $\gamma$ -treated MO Is Reversed by Fn*

MO	Surface	Attachment index			Receptor expression	
		EC3b	EC3bi	EIgG	CR1	CR3
Control	HSA	790	770	946	100	100
IFN- $\gamma$	HSA	160	113	1,520	96	91
Control	Fn	792	664	929	98	94
IFN- $\gamma$	Fn	814	750	1,618	95	87
IFN- $\gamma$ + PMA*	HSA	87	86	1,040	ND	ND

Monocytes were cultured for 5 d in the absence (control) or presence of 100 U/ml IFN- $\gamma$ . Cells were then allowed to spread for 45 min on plastic culture surfaces derivatized with HSA or Fn, and the attachment index of the indicated ligand-coated erythrocyte was determined as described in Materials and Methods. Expression of cell surface receptors were measured in parallel preparations using quantitative binding of the mAbs OKM1 and 3D9 as described in Materials and Methods, and data are given as the percent of antigen expression in control cells spread on HSA-coated surfaces. The experiment shown here is representative of five separate experiments.

\* 30 ng/ml PMA was added during the spreading of cells on an HSA-coated surface.

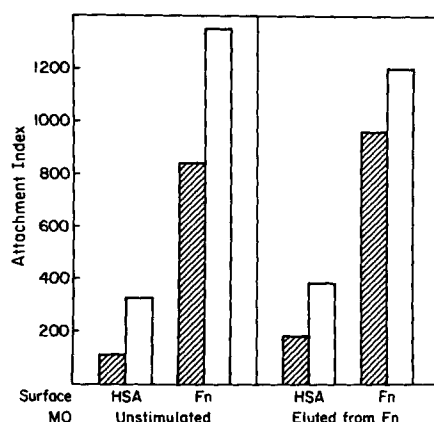


FIGURE 6. Activation of the binding activity of CR1 and CR3 by Fn is reversible. Monocytes were cultured for 3 d, then 100 U/ml IFN- $\gamma$  was added and cultivation was continued for 48 h. The IFN- $\gamma$ -MO were allowed to spread for 45 min at 37°C on surfaces coated with HSA or Fn, and their capacity to bind EC3b (open bars) and EC3bi (hatched bars) was measured (left panel). A parallel preparation of IFN- $\gamma$ -treated MO spread on Fn was eluted from the Fn-coated surface with EDTA (22), washed, and replated on fresh HSA- or Fn-coated surfaces, and the binding of EC3b and EC3bi was again measured.

that IFN- $\gamma$ -treated cells plated on HSA-coated surfaces express abundant CR3 on the apical surface. Thus, the dysfunction of CR3 in IFN- $\gamma$ -treated cells is not caused by gross redistribution of receptors. Cells spread on OKM10-coated surfaces express little if any CR3 on the apical surface (Fig. 7, *c* and *d*), indicating that the receptors diffused to the substrate-attached portion of the cell and were trapped by OKM10. Since the extent of down-modulation of CR3 is comparable in both control and IFN- $\gamma$ -treated cells, we believe that IFN- $\gamma$  does not cause gross alteration of the mobility of CR3. Finally, we have observed that CR3 exists in clusters on both the control and IFN- $\gamma$ -treated MO. Further studies are

TABLE IV  
*Phagocytosis of Ligand-coated Erythrocytes in IFN- $\gamma$ -treated MO*

MO	Treatment	Phagocytic Index		
		EC3b	EC3bi	EIgG
Control	—	16	59	410
Control	FN*	206	232	478
Control	PMA <sup>‡</sup>	272	317	263
Control	PMA + Fn <sup>§</sup>	205	209	259
IFN- $\gamma$	—	4	37	167
IFN- $\gamma$	Fn	39	67	222
IFN- $\gamma$	PMA	39	63	188
IFN- $\gamma$	PMA + Fn	347	555	341

Monocytes were cultured for 5 d in Teflon beakers. Where indicated, 100 U/ml IFN- $\gamma$  was added 48 h before the experiment. Monolayers of phagocytes were prepared and the phagocytosis of the indicated ligand-coated erythrocyte was measured as described in Materials and Methods. Data are from an experiment representative of three separate experiments.

\* Phagocytosis was measured using monolayers of MO spread on Fn-coated surfaces.

<sup>‡</sup> 30 ng/ml PMA was added during the incubation of MO with the ligand-coated erythrocytes.

<sup>§</sup> Phagocytosis was measured in the presence of 30 ng/ml PMA using MO spread on Fn-coated surfaces.

underway to determine whether the size and spacing of the clusters are influenced by IFN- $\gamma$ .

### Discussion

Here we show that cultivation of human monocytes or macrophages with recombinant IFN- $\gamma$  causes a striking decrease in the capacity of CR1 and CR3 to bind particles coated with the corresponding ligand. The dysfunction of CR1 and CR3 induced by IFN- $\gamma$  appears distinct from previously described dysfunctions of receptors in macrophages, in that *binding* of ligand is affected. Human and mouse macrophages possess complement receptors that fail to initiate phagocytosis (1, 22), but only IFN- $\gamma$ -treated MO express receptors that fail to bind ligand.

The biochemical mechanisms by which the binding activity of CR1 and CR3 are regulated by IFN- $\gamma$  are not clear. Reduced binding activity is not the result of irreversible modifications such as proteolysis since the molecular weight of CR3 is not altered (Fig. 5), and the dysfunction is rapidly reversed when the MO spread on surfaces coated with Fn (Table III). Reduced binding activity is also not associated with decreased mobility of the cell surface receptors since they continue to diffuse readily in IFN- $\gamma$ -treated MO (Fig. 7). Finally, reduced binding activity is not associated with changes in the number of cell surface receptor molecules since the number of CR1 and CR3 is unchanged by treatment of MO with IFN- $\gamma$  or with Fn (Tables II and III). We thus propose that depression of binding activity of CR1 and CR3 by IFN- $\gamma$  is caused by reversible conformational changes in the receptors. Such conformational changes could result from post-translational modifications such as phosphorylation or methylation, or from the binding of an inhibitory subunit or an allosteric effector.

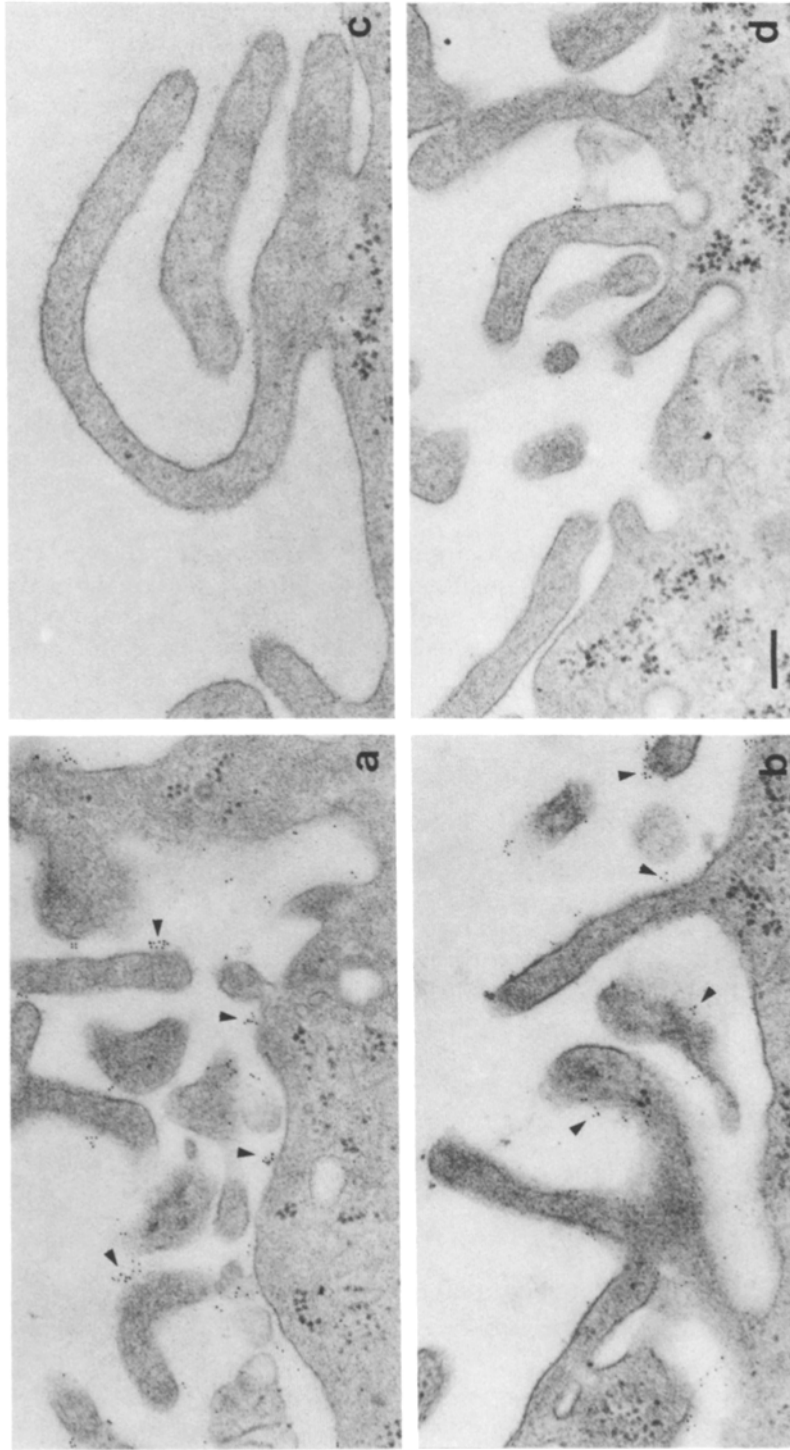


FIGURE 7. Mobility of CR3 on control and IFN- $\gamma$ -treated macrophages. Paired beakers of monocytes were cultured for 3 d, 100 U/ml IFN- $\gamma$  was added to one beaker, and cultivation was continued for 48 h. The cells were then allowed to spread for 45 min at 37°C on plastic surfaces coated with HSA (a and b) or OKM10 (c and d), and cell surface CR3 was labeled with biotinylated OKM10 and streptavidin gold. Both control (a) and IFN- $\gamma$ -treated MO (b) express CR3 on their apical surface when spread on an HSA-coated surface (arrowheads in a and b indicate some of the labeled areas). However, neither control (c) nor IFN- $\gamma$ -treated cells (d) express CR3 on their apical surface when allowed to spread on an OKM10-coated surface. 40,000 $\times$ . Bar = 0.2  $\mu$ m.

FIGURE 7. Mobility of CR3 on control and IFN- $\gamma$ -treated macrophages. Paired beakers of monocytes were cultured for 3 d, 100 U/ml IFN- $\gamma$  was added to one beaker, and cultivation was continued for 48 h. The cells were then allowed to spread for 45 min at 37°C on plastic surfaces coated with HSA (a and b) or OKM10 (c and d), and cell surface CR3 was labeled with biotinylated OKM10 and streptavidin gold. Both control (a) and IFN- $\gamma$ -treated MO (b) express CR3 on their apical surface when spread on an HSA-coated surface (arrowheads in a and b indicate some of the labeled areas). However, neither control (c) nor IFN- $\gamma$ -treated cells (d) express CR3 on their apical surface when allowed to spread on an OKM10-coated surface. 40,000 $\times$ . Bar = 0.2  $\mu$ m.

Regulation of ligand binding activity has been observed for several other receptors. The binding affinity of epidermal growth factor receptors is depressed in cells treated with platelet-derived growth factor (24), and the affinity of receptors for  $\beta$ -adrenergic ligands (25) and FMLP (26) can be depressed by prior treatment of cells with ligand (homologous desensitization) or with several pharmacologic agents. Regulation of binding activity may thus represent a general mechanism for controlling a cell's responsiveness to a ligand. The observation that receptors may exist in a state incapable of binding ligand also indicates that rosette assays and other measures of ligand binding may be poor indicators of the presence or absence of receptors on a cell.

Spreading of IFN- $\gamma$ -treated MO on Fn-coated surfaces enables CR1 and CR3 to bind ligand, but the receptors remain incapable of signaling phagocytosis (Tables III and IV). Treatment of IFN- $\gamma$ -treated MO with PMA enables CR1 and CR3 to generate signals for phagocytosis, but only if binding is also enabled by Fn (Table IV). These observations suggest that the two functions of complement receptors, binding of ligand and signaling of phagocytosis, may be controlled at separate points in IFN- $\gamma$ -treated cells; control of binding is exerted by Fn and control of signaling by PMA.

Our observations show that the capacity of IFN- $\gamma$ -treated MO to bind C3-coated cells is under physiological regulation; binding does not occur until the cells receive an additional stimulus (Fn). Control mechanisms similar to those observed with CR1 and CR3 could potentially regulate other cell-cell adhesion events, such as the interaction of cytolytic T cells with targets, the interaction of activated macrophages with tumor cells, and the interaction of T cells with accessory cells. Since several of such cell-cell adhesion events are mediated by LFA-1, and since LFA-1 is structurally homologous to CR3 (8, 19), we suggest that cell-cell adhesion events mediated by LFA-1 may be controlled by changes in the binding activity of LFA-1.

In contrast with the observations on complement-coated erythrocytes, the binding of EIgG is strongly enhanced in IFN- $\gamma$ -treated MO (Fig. 1 and 2). These data are consistent with the well-documented increase in the expression of a high affinity FcR caused by IFN- $\gamma$  (14, 15). IFN- $\gamma$ -treated MO, however, show decreased capacity to phagocytose EIgG (Table IV). These data suggest that the high-affinity FcR may not efficiently promote phagocytosis. Consistent with this hypothesis are the observations that >70% of the uptake of EIgG is inhibited when the low-affinity FcR is removed from MO (27), and clearance of IgG-coated erythrocytes is decreased 98% by antibodies against a low-affinity receptor (28). These observations suggest that phagocytosis of IgG-coated particles by MO may be promoted primarily by a low-affinity FcR, not by the high-affinity FcR induced by IFN- $\gamma$ .

### Summary

Cultivation of human monocytes with recombinant IFN- $\gamma$  causes a 5–10-fold depression in their binding of EC3b or EC3bi. This effect is observed within 18 h and is expressed for 5 d in the presence of 100 U/ml IFN- $\gamma$ . The capacity of IFN- $\gamma$ -treated phagocytes to bind EC3b and EC3bi is fully restored if the phagocytes are allowed to spread for 45 min on surfaces coated with Fn. IFN- $\gamma$ -

treated cells express normal levels of cell surface C3b and C3bi receptors as measured with monoclonal anti-receptor antibodies, and spreading on Fn does not alter receptor number. We conclude that cultivation with IFN- $\gamma$  causes a change in the nature of these receptors that prevents them from interacting with ligand. Immunoelectron microscopy shows that C3bi receptors are expressed on the apical surface of the IFN- $\gamma$ -treated MO and that these receptors exhibit normal capacity to migrate in the plane of the membrane. Thus, the nature of the change caused by IFN- $\gamma$  is not related to changes in receptor number, location, or mobility. While spreading of IFN- $\gamma$ -treated cells on Fn enables C3 receptors to bind ligand, it does not enable them to promote phagocytosis. Treatment of cells with PMA alone does not affect binding or phagocytosis, but treatment of cells with both Fn and PMA enables cells to phagocytose EC3b and EC3bi. These data indicate that the binding and signaling activities of C3 receptors are separately regulated. Fn enables receptors to bind ligand and PMA enables them to signal phagocytosis.

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