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Biochemical Models of Interferon-γ-Mediated Macrophage Activation: Independent Regulation of Lymphocyte Function Associated Antigen (LFA)-1 and I-A Antigen on Murine Peritoneal Macrophages[1]

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IFN-γ can induce the expression of both class II histocompatibility antigens (Ia) and the lymphocyte function associated (LFA)-1 antigen on murine peritoneal macrophages. We have examined the molecular changes which lead to altered expression of these two cell surface proteins to determine whether they are regulated by similar or independent mechanisms. While I-A antigen expression can be induced or enhanced by treatment of macrophages with either phorbol diesters and/or the Ca^{2+} ionophore A23187, these agents had no effect upon expression of LFA-1 under similar conditions. Macrophages from the A/J strain mouse exhibit a deficiency in their sensitivity to IFN-γ which is seen in our studies as an inability of IFN-γ to elevate I-A antigen expression. However, expression of I-A could be modulated in these cells by treatment with either phorbol diesters or A23187. In contrast, IFN-γ could induce LFA-1 antigen on A/J derived macrophages and this was not affected by either phorbol or A23187. Thus these two antigens, despite coordinate expression in response to IFN-γ in normal mouse strains, are clearly regulated independently. These results suggest that IFN-γ generates at least two independent molecular events in macrophages which ultimately modulate the expression of cell surface proteins important to the performance of activated functions.

INTRODUCTION

Development of the multiple functional capabilities of murine mononuclear phagocytes requires the acquisition of requisite cellular and biochemical capacities [for review see (1)]. Such changes can be induced both *in vivo* following local injection of various phlogistic agents or *in vitro* in response to defined extracellular signals (1–4). The most thoroughly studied of these signals is known as macrophage activating factor (MAF)[3]

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[3] Abbreviations used: MAF, macrophage-activating factor; IFN-γ, interferon-γ; LFA, lymphocyte function associated; PMA, phorbol 12-myristate 13-acetate; TG, thioglycollate; MAb, monoclonal antibody; RIA, radioimmunoassay; LPS, lipopolysaccharide.

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(5, 6). Several laboratories have convincingly demonstrated that interferon-γ (IFN-γ) is one potent MAF (7–10). IFN-γ induces a broad range of biochemical and functional changes in macrophages [for review see (1)]. These include (i) priming for tumor cytolysis (6, 8), (ii) the modulation of the NADP(H)-oxidase complex necessary for execution of the respiratory burst (9), (iii) elevated expression of several cell surface antigens and proteins including class II histocompatibility (Ia) antigens [for review see (11, 12)], the lymphocyte function associated antigen (LFA)-1 (13), and receptors for the Fc region of immunoglobulins (14), and (iv) reduction in expression of several surface proteins including the transferrin receptor, the mannose receptor, and the antigen recognized by the monoclonal antibody F4/80 (15, 16). However, little is known about the intracellular events involved in these changes.

In this regard, Celada *et al.* (17) have recently identified a specific cell surface receptor for IFN-γ on macrophages and have linked this receptor to the development of antitumor activity. In addition, we have shown that IFN-γ can induce a three- to fivefold increase in the potential activity of a Ca^{2+}, phospholipid-dependent protein kinase activity (protein kinase c) (18). This indicates that this enzyme may play a significant role in the response to IFN-γ. In support of this possibility, we have also observed that several aspects of IFN-γ action can be reproduced by treatment of cells with either phorbol diesters, which bind to and stimulate protein kinase c (19, 20), the Ca^{2+} ionophore A23187, or a combination of these two agents. These latter data suggest that in addition to protein kinase c, changes in intracellular Ca^{2+} levels may also participate in the response to IFN-γ.

In previous work we observed that treatment with bacterial lipopolysaccharide (LPS), resulted in induction of LFA-1 antigen expression (13). However, LPS has an inhibitory effect on Ia antigen expression (21). Thus we wished to determine whether treatment with PMA and/or A23187 could recapitulate the effects of IFN-γ on the expression of I-A *and* LFA-1 antigens. While IFN-γ clearly alters the expression of both antigens we wished to determine if the biochemical events involved would be identical for both antigens. These studies have employed two distinct experimental systems. First we have asked whether treatment with phorbol diesters and/or A23187 could mimic the effects of IFN-γ on expression of both antigens. Second, we have asked whether macrophages derived from the A/J mouse strain, which is deficient in response to IFN-γ, would exhibit elevation of either antigen in response to treatment with IFN-γ. The results of such studies clearly demonstrate that these two cell surface proteins are independently regulated in IFN-γ-treated macrophages.

MATERIALS AND METHODS

Mice. Inbred female, specific-pathogen-free C57BL/6J and A/J mice were obtained from The Trudeau Institute, Inc. (Saranac Lake, N.Y.) and from the Jackson Laboratory (Bar Harbor, Me.), respectively. Mice of 8 to 16 weeks of age were used. The animals were kept in the Animal and Laboratory Isolation Facility at Duke University under conditions designed to minimize infection with environmental pathogens which may alter the endogenous level of macrophage activity (22).

Reagents. BCG, Phipps strain 1029, was purchased from The Trudeau Institute. Brewer's thioglycollate (TG) broth, prepared according to manufacturer's instructions, was obtained from Difco Manufacturing Company, Detroit, Michigan. Phorbol 12-

myristate 13-acetate (PMA) was purchased from Consolidated Midlands Corporation, and the calcium ionophore (A23187) was obtained from Sigma Chemical Company. All these compounds were prepared in DMSO, aliquoted, and stored at −70°C until use. The phorbol esters were prepared at 1 mg/ml, while stock concentrations of A23187 were stored at 2×10^{-3} *M*. Recombinant inteferon-γ produced by COS-7 cells transfected with the murine IFN-γ gene expressed under control of the SV40 promoter (23) was generously supplied by Dr. Patrick Gray from Genentech, Inc. and was stored at −70°C until use. Macrophage-activating factor (MAF) was a generous gift from Dr. Robert Schreiber (Scripps Clinic). This material was produced by a T-cell hybridoma (clone 24/G1) (7).

Macrophages. The elicitation, identification, and functional analysis of macrophages in various stages of development have been described in detail (1) and routinely assessed in our laboratory. In brief, inflammatory macrophages were obtained 3 days after intraperitoneal (ip) injection of 1 ml of sterile TG. BCG-activated macrophages were obtained from mice primed with intradermal (id) injection of 75 mg of Ribi cell vaccine, 5×10^6 organisms ip and then 1×10^7 organisms 3 days before peritoneal lavage. The peritoneal exudate cells (PEC) were obtained by peritoneal lavage with 10 ml of cold Hanks' balanced salt solution supplemented with 10 units of heparin/ml. The PEC were centrifuged at 250*g* for 5 min, resuspended in Eagle's minimum essential medium (EMEM) + 10% fetal bovine serum (Sterile Systems, Inc., Salt Lake City, Utah). Aliquots of 2×10^5 macrophages in 100 μl of medium were plated in flat-bottom microtiter plates (Linbro 7600605). The plates were incubated at 37°C in 5% CO_2 for 2 hr and the monolayers were washed (×3) to remove nonadherent cells. We routinely found the cell monolayers to contain >95% macrophages as determined by morphology with Wright's stain or a histochemical assay for nonspecific esterase.

Treatment of macrophages in vitro. Macrophage monolayers were incubated at 37°C with 0.15 ml of EMEM + 10% FBS containing various agents for 14 to 18 hr. Following this exposure period, macrophage monolayers were subjected to radioimmunoassay and were analyzed for cytolytic activity.

Monoclonal antibody. The MAb in this study were previously described (24–26). The M17/4 (αLFA-1, IgG_{2a}) MAb was partially purified from culture supernatant by ammonium sulfate (2.2 *M*) precipitation and dialyzed extensively against 0.1 *M* Tris–HCl buffer, pH 7.8.

The M5/114 (αI-A^b, IgG_{2b}) was used as culture supernatant. Supernatant containing MAS-053 MAb produced by clone YE2/36HLK was purchased from Accurate Chemical, Westbury, New York. This rat MAb (IgG_{2a}) recognizes a monomorphic determinant on HLA-DR and mouse I-A antigens (27).

Radioimmunoassay (RIA). This method was previously described (13, 28). Briefly, monolayers of macrophages were incubated for 30 min at 4°C with 100 μl/well of 2% normal mouse serum, 8% normal horse serum, and 0.02% sodium azide in PBS. The fluid was aspirated and 25 μl of MAb diluted in PBS containing 8% horse serum and 0.02% sodium azide (referred to as "diluent") was added for 2 hr in the cold. Following two washes with 150 μl of cold diluent, 25 μl (1.5×10^5 cpm) of ^{125}I-labeled $F(ab')_2$ of goat anti-rat IgG_{HL} (Cappel Labs, Malvern, Pa.) was added per well. Radioiodination of antibodies was performed by the chloramine-T technique. Specific activity ranged from 2.8 to 4.2 μCi/μg. Following 2 hr in the cold, the plates were washed (150 μl ×3) and the monolayers were dissolved by the addition of 100 μl of 0.25% sodium dodecyl sulfate per well. The content of each well was then transferred into a tube and counted

in a Packard gamma scintillation spectrometer. Triplicate wells were used per each point tested; standard deviation of the mean for the triplicates did not exceed 15%.

To reduce nonspecific binding of MAb, we used a solution composed of 2% normal mouse serum and 8% normal horse serum for preincubation of macrophages prior to the application of MAb. This procedure resulted in binding of 100 to 400 cpm when 2% normal rat serum was used as a negative control. Thus RIA described here does not measure binding via Fc receptors. No correlations between expression of a certain antigen and MAb isotype could be made [Fig. 4 and Ref. (28)].

Cytolysis. As described previously (29), macrophages were resuspended in RPMI 1640 (Microbiological Associates) with 10% FBS, penicillin, streptomycin and glutamine at 2×10^6 macrophages/ml and 0.1 ml added to wells of a 96-well tissue culture plate (6-mm wells, Linbro Plastics, Hamden, Conn.). Following a 1 hr incubation at 37°C in 5% CO_2, the monolayers were washed and the appropriate activating agent in RPMI 1640 media was added.

The P815 target cells were labeled with 300 μCi of $Na_2\ ^{51}CrO_4$ (sp act, 50 to 400 mCi/mg, New England Nuclear, Boston, Mass.) for 60 min at 37°C. After two washes, cells were resuspended in RPMI 1640 + 10% FBS and aliquots containing 5×10^4 P815 in RPMI 1640 + 10% FBS were added to wells containing macrophage monolayers. In some wells 10 ng/ml bacterial lipopolysaccharide (phenol extract, *Escherichia coli,* serotype 026:B6, Sigma Chemical Co., St. Louis, Mo.) was added. Control wells containing only P815 targets were prepared to assess the spontaneous release of label. The total incorporated radioactivity was determined following lysis of an aliquot of targets with 0.5% sodium dodecyl sulfate. The plates were returned to 37°C with 5% CO_2 for 16 hr. The released radioactivity was determined by carefully removing a 0.1 ml aliquot from each well and counting in a gamma spectrometer. Lysis was calculated with the formula:

$$\% \text{ Specific lysis} = \frac{\begin{array}{r}(\text{cpm released in presence of macrophages}) \\ -\ (\text{cpm released in absence of macrophages})\end{array}}{\begin{array}{r}(\text{total incorporated cpm}) \\ -\ (\text{cpm released in absence of macrophages})\end{array}} \times 100.$$

The spontaneous release did not exceed 18% of the total incorporated radioactivity. Each group was run with at least triplicate samples.

RESULTS

Differential Effects of PMA and A23187 on Expression of LFA-1 and Ia Antigens

Enhanced expression of both LFA-1 and Ia antigens on macrophages in response to MAF (i.e., IFN-γ) has been previously reported (11, 13). Furthermore, we have earlier shown that certain macrophage responses to IFN-γ can be reproduced *in vitro* by treatment of these cells with appropriate doses of either PMA or A23187 or both (33). Indeed, it has also been reported that PMA can modulate the expression of Ia antigen on B lymphocytes (30). We have therefore determined whether LFA-1 and I-A antigen expression on macrophages could be modulated by treatment with these agents. C57BL/6-derived, TG-elicited macrophages were incubated for 16 hr with various doses of PMA or A23187 and the expression of both Ia and LFA-1 antigens was assessed by RIA (Fig. 1). This experiment demonstrates that both agents are capable of enhancing the basal expression of I-A antigen (Fig. 1A) in a dose-dependent

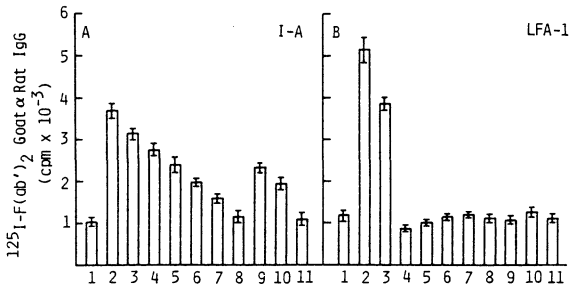

FIG. 1. Ia but not LFA-1 can be induced by PMA and A23187. TG-induced, C57BL/6-derived macrophages (2×10^5) were treated for 16 hr with (1) medium; (2) MAF1:100; (3) IFN 20 units/ml; (4) A23187 1×10^{-6} *M*; (5) A23187 5×10^{-7} *M*; (6) A23187 2×10^{-7} *M*; (7) A23187 1×10^{-8} *M*; (8) A23187 2×10^{-9} *M*; (9) PMA 50 ng/ml; (10) PMA 10 ng/ml; (11) PMA 2 ng/ml. Expression of I-A^b (A) and LFA-1 (B) was then analyzed in RIA using 1:10 dilution of M5/114 (αI-A^b) and 1:100 dilution of M17/4 (αLFA-1) MAb. Results are expressed as the mean of cpm of ^{125}I-F(ab')$_2$ of goat anti-rat IgG bound to macrophages in triplicate wells. This experiment was repeated three times with similar results.

fashion. Under the same conditions, neither agent was able to alter LFA-1 expression (Fig. 1B). In the same experiment, both MAF (1:100 dilution) or 100 U/ml of recombinant IFN-γ resulted in augmented expression of both antigens.

As a further examination of this phenomenon, we measured the effect of PMA or A23187 on I-A and LFA-1 expression in BCG-elicited macrophages. These cells, in contrast to those elicited by TG-broth, express elevated levels of both antigens when freshly explanted but exhibit decay of antigen density following overnight incubation which can be prevented by treatment with MAF or IFN-γ (see Fig. 2). When these cells are treated with PMA or A23187, similar results to those presented in Fig. 1 were obtained (see Fig. 2). While levels of both antigens are reduced after culture (between 20 and 55% in various experiments), only I-A antigen expression could be stimulated or maintained by treatment with either PMA or A23187 (Fig. 2B). LFA-1 expression was not affected (Fig. 2A).

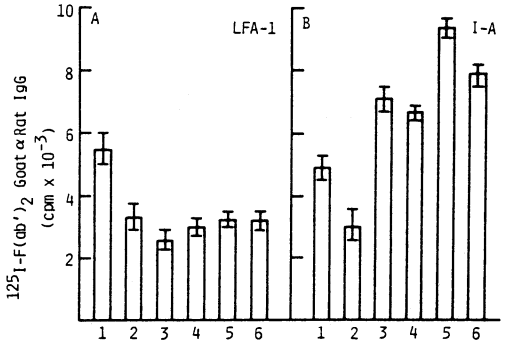

FIG. 2. PMA and A23187 can augment expression of I-A but not LFA-1 on BCG-activated macrophages. Two $\times$ 10^5 BCG-activated macrophages were incubated for 14 hr. with various agents and analyzed for LFA-1 (A) and I-A^b (B) expression. Group 1: BCG macrophages without incubation (freshly explanted); Group 2: BCG macrophages treated with medium; Group 3: BCG macrophages incubated with 1×10^{-6} *M* A23187; Group 4: BCG macrophages incubated with 2×10^{-7} *M* A23187; Group 5: BCG macrophages incubated with 50 ng/ml PMA; Group 6: BCG macrophages treated with 10 ng/ml PMA. This experiment was repeated twice with similar results.

The induction of I-A expression could be observed between 8 and 18 hr of incubation with PMA and A23187 (Fig. 3). A similar time course has been reported for the IFN-γ induced elevation of LFA-1 antigen on macrophages (13). Longer exposure of cultures to either agent resulted in macrophage toxicity (data not shown).

PMA and A23817 Act Cooperatively to Prime for Tumor Cytolysis But Not For LFA-1 Expression

In work to be reported in detail elsewhere, we have observed that the ability of IFN-γ to prime macrophages for tumor cytolysis could be reproduced by simultaneous treatment with both PMA and A23187 (Somers *et al.*, manuscript submitted for publication). This raised the possibility that, though LFA-1 expression might not be mimicked by treatment with either PMA or A23187 alone, it might instead require cooperation. This possibility was tested by measuring both cytolytic priming and LFA-1 expression on the same population of macrophages. While TG elicited macrophages were primed for cytolysis (i.e., they killed P815 tumor cells only in the presence of LPS) following treatment with both agents (but not with either alone) only MAF or recombinant IFN-γ were able to modulate the expression of LFA-1 (Table 1). Thus, LFA-1 expression is regulated independently from priming for cytolytic function and from I-A antigen expression. It is noteworthy that no cooperativity between PMA and A23187 for I-A induction could be observed in our system (data not shown).

A/J-Derived Macrophages Can Be Induced to Express LFA-1 But Not Ia Antigens

Macrophages derived from A/J strain mice have been documented to exhibit defective tumoricidal function (31, 32). This deficit appears to be a consequence of reduced sensitivity to MAF or IFN-γ (6). Since LFA-1 and I-A antigen expression appear to fall under independent regulation (see above), we wished to determine whether the deficit in the response to IFN-γ seen in the A/J-derived macrophages would influence the behavior of both antigens in identical fashion. Macrophages from both A/J and C57BL/6 mice were incubated for 16 hr with recombinant IFN-γ

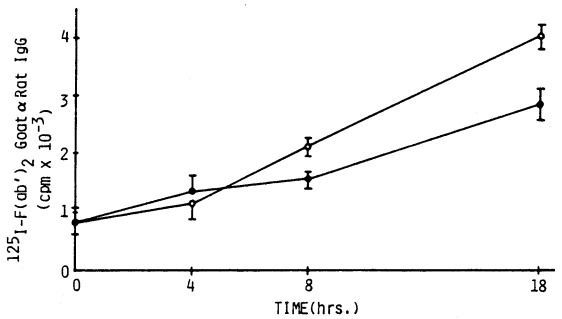

FIG. 3. Time dependence of I-A induction by PMA and A23187. TG-induced C57BL/6-derived macrophages (2×10^5) were incubated with A23187 1×10^{-6} (○) and PMA 50 ng/ml (●) for various times. Expression of I-A^b was then analyzed in RIA using 1:10 dilution of M5/114 (αI-A^b).

TABLE 1

A23187 and PMA Can Cooperatively Prime Macrophages For Cytolysis But Cannot Induce LFA-1[a]

Group	Treatment	LFA-1 expression[b] (cpm bound ± SD)	% Specific lysis of P815 targets[c]	
			No LPS	LPS added
1	Medium	782 ± 39	0	3
2	MAF(1:100)	4041 ± 282	0	43
3	PMA 10 100 ng/ml	701 ± 47	−2	0
4	PMA (10 ng/ml	826 ± 41	−1	4
5	A23187 1×10^{-6} *M*	389 ± 120	0	7
6	A23187 1×10^{-7} *M*	539 ± 68	0	4
7	3 + 5	479 ± 39	−1	17
8	3 + 6	595 ± 106	−2	0
9	4 + 5	559 ± 57	5	34
10	4 + 6	630 ± 22	2	8

[a] TG-elicited macrophages (2×10^5) were incubated for 14 hr with PMA, A23187, or both agents together and analyzed for their ability to lyse P815 targets and to express LFA-1 antigen.

[b] LFA-1 expression is given as the mean of cpm bound of ^{125}I-F(ab')$_2$ Goat anti-rat IgG to macrophages in triplicate wells ± standard deviation. M17/4 MAb was used at 1:100 dilution.

[c] Percentage specific lysis of P815 (5×10^4) targets at 4:1 macrophage to target ratio, in the presence (10 ng/ml) or absence of LPS. The experiment was repeated three times with similar results.

(Fig. 4) and analyzed for expression of both antigens by RIA as described above. IFN-γ was able to induce a substantial enhancement of LFA-1 expression in both types of macrophages. Furthermore neither PMA nor A23187 was able to alter expression of this protein in either macrophage type. On the other hand, expression of Ia antigen was modulated by IFN-γ only in the C57BL/6 derived macrophages; the response to IFN-γ with respect to this antigen in A/J was clearly deficient at all doses of IFN-γ employed. In contrast to the situation with the LFA-1 antigen, treatment of A/J-derived macrophages with either PMA or A23187 resulted in moderate increase in I-A antigen expression.

The A/J strain deficiency is quantitative in that increasing doses of IFN-γ can, in some circumstances, overcome the defect. We exploited this information to ask whether the increased expression of LFA-1 induced by IFN-γ in the A/J strain required a higher concentration of IFN-γ than that seen in the C5BL/6 derived macrophages. Figure 5 shows that in both strains, the dose–response curves exhibited similar profiles. In all cases the effects of IFN-γ on expression of LFA-1 and I-A antigen could be distinguished. Therefore, despite the fact that these changes both result from treating cells with the same agent, they must be governed by different mechanisms.

DISCUSSION

Activation of macrophages by IFN-γ to perform such complex functions as tumor cytolysis or antigen presentation is accompanied by altered expression of certain key proteins which are necessary for the execution of the particular function in question

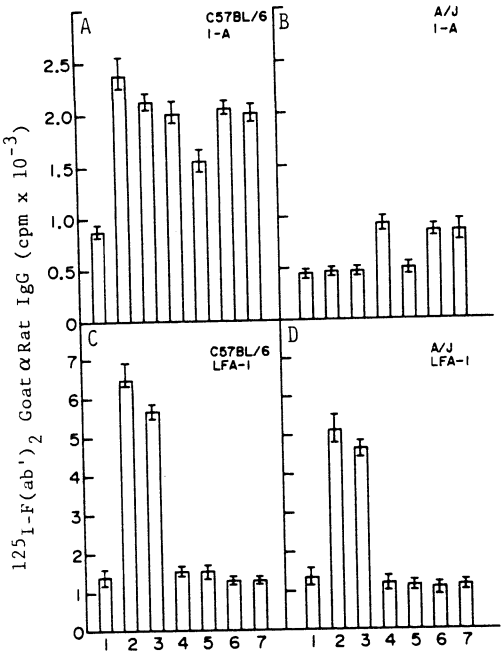

FIG. 4. IFN-γ can induce LFA-1 but not I-A on A/J derived inflammatory macrophages. TG-induced macrophages (2×10^5) from C57BL/6 mice (A) and from A/J mice (B) were incubated for 18 hr with various agents and analyzed for I-A expression with undiluted supernatant of MAS-053 MAb (see Materials and Methods) (1) Medium, (2) IFN-γ (100 μ/ml), (3) IFN-γ (20 μ/ml), (4) PMA (50 ng/ml), (5) PMA (10 ng/nl), (6) A23187 (1×10^{-6} *M*), (7) A23187 (2×10^{-7} *M*). In the same experiment induction of LFA-1 was also analyzed on C57BL/6 (C)- and A/J (D)-derived TG-induced macrophages. This experiment was repeated twice with similar results.

[for review see (1)]. For example, expression of both I-A and LFA-1 antigens is modulated in response to treatment *in vitro* with IFN-γ [(11, 12, 13) and Fig. 1]. Previous reports have demonstrated that some aspects of the response of murine macrophages

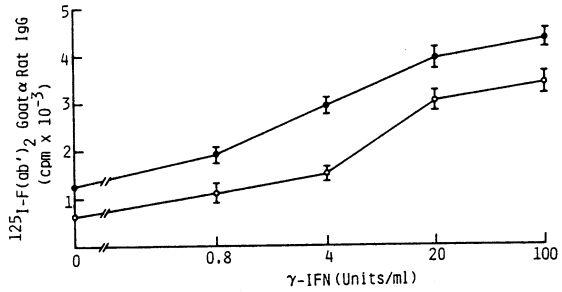

FIG. 5. Dose dependence of LFA-1 induction on IFN-γ. TG-elicited macrophages (2×10^5) from C57BL/6 (○) and A/J (●) mice were incubated for 18 hr with various amounts of recombinant IFN-γ. Expression of LFA-1 was determined with 1:100 dilution of M17/4 antibody. This experiment was repeated twice with similar results.

to IFN-γ can be reproduced by treatment of the cells with phorbol diesters and/or Ca^{2+} ionophores (18, 33). As further study of how the IFN-γ signal alters the biochemical and functional characteristics of macrophages we have asked whether the change in the expression of I-A or LFA-1 antigens occurs by a similar mechanism involving either changes in intracellular Ca^{2+} or stimulation of protein kinase c (the molecular target of phorbol diesters). While I-A antigen expression is enhanced in macrophages derived from either C57BL/6 or A/J mice which have been treated with PMA or A23187, these agents have no effect upon expression of LFA-1 antigen. In contrast, IFN-γ treatment of macrophages derived from the deficient A/J mouse strain leads to induction of LFA-1 without changing expression of I-A antigen. These results document, that, while IFN-γ treatment does modulate the expression of both proteins, the mechanisms involved can be easily distinguished.

Preliminary evidence suggesting that LFA-1 and I-A antigen are regulated independently was provided by data published in a previous report (13). While LFA-1 antigen can be induced in macrophages treated with small amounts of LPS (100 pg/ml), I-A antigen expression is inhibited rather than induced when cells are treated with LPS even in the presence of IFN-γ (21). While these findings do not establish that IFN-γ modulates the expression of these two cell surface proteins by independent mechanisms, it does indicate that they can respond in opposite ways to one signal while responding coordinately to another signal.

Several recently reported observations have supported roles for both increased intracellular Ca^{2+} levels and protein kinase c activity in the response of peritoneal macrophages to IFN-γ (18, 33). For example, reduced expression of the cell surface receptor for transferrin on macrophages could be mimicked by treatment of the cells with either A23187 or PMA (33). Furthermore, IFN-γ treatment modulated the response of macrophages to phorbol diesters by increasing the *potential* activity of the Ca^{2+}, phospholipid-dependent enzyme, protein kinase c (18, 34). Finally, macrophages primed with IFN-γ also exhibit a more intense protein phosphorylation response when triggered with LPS (Weiel *et al.*, manuscript submitted for publication). The ability to modulate the expression of I-A antigen on macrophages by treatment with either PMA or A23187 is, therefore, consistent with these observations and provides further support for an activation mechanism involving these events. Similar results have been reported in splenic B lymphocytes (30). The finding that LFA-1 expression does not vary in macrophages treated with either A23187, PMA, or the two agents together suggests that a signal independent of either Ca^{2+} or protein kinase c is also generated following treatment with IFN-γ.

Macrophages derived from the A/J mouse strain are well known to have a quantitative deficiency in tumoricidal activation that has been localized to the priming effect of MAF or IFN-γ (31, 32). This deficiency in response to IFN-γ is confirmed by the studies reported here concerning I-A antigen expression. The observation that enhanced I-A expression can be produced in the A/J mouse by utilizing either PMA or A23187 suggests that the defect in this mouse strain occurs prior to the action of either of these agents. The ability of IFN-γ to stimulate enhanced expression of LFA-1 in A/J macrophages indicates that the deficiency in these cells is probably not a consequence of reduced expression of the IFN-γ receptor. Furthermore, it provides additional evidence demonstrating that the pathway involved in regulating LFA-1 expression is independent of that regulating I-A expression.

One possible simplifying explanation of the observations reported here would be that the modulation of I-A and LFA-1 antigens results from entirely different signals. For example, I-A expression might be modulated by IFN-γ and LFA-1 expression might be effected by a contaminant present in the preparation of IFN-γ which has yet to be identified. While we cannot entirely rule this possibility out on the basis of current data, it appears very unlikely in light of the fact that we have employed not only MAF derived from a T-cell hybridoma shown to produce high levels of IFN-γ, but also recombinant IFN-γ derived from cDNA clones expressed in cultured mammalian cells (23). Thus the effects of IFN-γ reported here are likely due to the action of a single molecule.

The results presented here provide additional support for a model of macrophage activation which involves changes in intracellular Ca^{2+} and protein kinase c. However, by examination of the expression of LFA-1 on macrophages in comparison to that of I-A antigens, we can now distinguish additional changes (as yet unidentified) which participate in the macrophage response to IFN-γ. It is possible that the IFN-γ receptor is coupled to several immediate signal transduction mechanisms which then operate independently. Alternatively, IFN-γ may be coupled to a single second messenger system which itself sets in motion multiple changes which are independent of one another. These observations support the notion that IFN-γ induces a pleiotropic response in macrophages (35). The independent regulation of I-A and LFA-1 antigens may now serve as a model system in which to further explore this phenomenon.

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