

REGULATION OF MURINE MACROPHAGE Ia ANTIGEN  
EXPRESSION BY A LYMPHOKINE WITH  
IMMUNE INTERFERON ACTIVITY

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The initiation of many antigen-specific, T-dependent immune responses requires the participation of accessory cells that express Ia antigens (1, 2). Recent evidence indicates that the Ia antigen expression of macrophages, a principle class of accessory cells, is regulated by lymphokines: Steinman et al. (3) have reported that culture supernatants of *Trypanosoma cruzi*-activated spleen cells enhanced the synthesis and expression of Ia antigens by murine macrophages in vitro. Steeg et al. (4) have observed that culture supernatants of concanavalin A-stimulated spleen cells (Con A supernatant)<sup>1</sup> induced Ia<sup>-</sup> murine thioglycollate-elicited peritoneal exudate macrophages to express Ia antigens in vitro. Macrophages incubated with lymphokine-containing culture supernatants developed the capacity to initiate the mixed leukocyte reaction (4) and the antigen-specific activation of helper T lymphocytes (5, 6). Finally, Scher et al. (7) have reported that intraperitoneal injections of culture supernatants of *Listeria monocytogenes*-stimulated lymphocytes induced peritoneal exudates that are enriched in Ia<sup>+</sup> macrophages.

Identification of the lymphokine(s) that modulates macrophage Ia antigen expression has been hampered by the plethora of lymphokine activities present in relatively low titers and high-protein contents of the lymphokine-containing culture supernatants. This paper characterizes the macrophage Ia antigen regulatory mediator present in the Con A supernatant and demonstrates that the macrophage Ia antigen regulatory mediator shares antigenic (8) and biochemical characteristics with immune interferon (IFN- $\gamma$ ). Furthermore, independently prepared IFN- $\gamma$ , purified to 10<sup>7</sup> U/mg protein specific activity, both induced and maintained macrophage Ia antigen

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<sup>1</sup> *Abbreviations used in this paper:* CI, cytotoxicity index; Con A supernatant, concanavalin A-stimulated spleen cell supernatant; control supernatant; Con A-supplemented spleen cell supernatant; CSF, macrophage-granulocyte colony-stimulating factor; ETAF, epidermal cell-derived thymocyte-activating factor; Ia<sup>+</sup>, Ia antigen expressing; Ia<sup>-</sup>, Ia antigen deficient; IFN, interferon; IL-1, interleukin 1; IL-2, interleukin 2; MAF, macrophage-activating factor; MIF, migration inhibitory factor; pI, isoelectric point; PMA, phorbol myristic acetate.

expression in vitro. Finally, we present evidence that a number of other lymphokines present in the Con A supernatant have no macrophage Ia antigen regulatory activity.

### Materials and Methods

**Induction of Macrophage Ia Antigen Expression.** P388D<sub>1</sub> macrophages (9) were maintained in RPMI 1640 (Flow Laboratories, Inc., Rockville, MD) containing 5% fetal calf serum (lot 100308; Sterile Systems Inc., Logan UT) and antibiotics (medium). The cells were maintained at a density of  $5 \times 10^5$  cells/ml in roller bottles (Costar, Data Packaging, Cambridge, MA) at 37°C on a shaking apparatus. To induce Ia antigen expression,  $1 \times 10^4$  macrophages in 0.2 ml enriched McCoys 5-A medium for granulocyte/macrophage culture (10) containing serial dilutions of Con A supernatant were incubated in wells of microtiter plates (Costar Data Packaging) for 2–3 d at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> in air. The percentage of Ia<sup>+</sup> macrophages was determined by removal of the culture supernatants and incubation of the macrophages with monoclonal anti-I-A<sup>d</sup> (clone 25-9-17, kindly provided by Dr. David Sachs, National Institutes of Health, or B6AF<sub>1</sub> anti-B10.D2, kindly provided by Dr. John Kappler, Dept. of Medicine, National Jewish Hospital) in 50 µl RPMI 1640 for 1 h at 4°C, followed by a second removal of the culture supernatants and incubation of the cultures with 50 µl of Low Tox M rabbit complement (Cedarlane Laboratories, Hicksville, NY) at a 1:10 or 1:15 final dilution for 1 h at 37°C. Viability was determined by trypan blue exclusion, with at least 100 cells per microtiter well scored. Each treatment was assayed in triplicate, with an additional complement control (typically <5% nonspecific cytotoxicity), and the data are expressed as the mean cytotoxicity index (CI) (4) with 95% confidence limits, based on arcsine square transformation.

**Interferon Assay.** Interferon (IFN) levels were quantified by a modification (11) of the viral plaque reduction assay of Wagner (12). The IFN titer (expressed as units per milliliter) is defined as the reciprocal of the log culture supernatant dilution resulting in a 50% plaque reduction as compared with control cultures, calculated by linear regression. The IFN assay was calibrated using WHO international IFN reference standard G-002-9-04-511 (Research Resources Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health).

**Lymphokines.** Con A-stimulated spleen cell supernatants were prepared using C3H/HeN (Ia<sup>k</sup>) spleen cells and adsorbed with Sephadex G-10 as previously described (4). For chromatography, supernatants were concentrated by ultrafiltration using immersible CX-10 ultrafiltration units (Millipore Corp., Bedford, MA). Partially purified IFN-γ (sp ac  $10^7$  U/mg protein) was prepared from staphylococcal enterotoxin A-stimulated spleen cell culture supernatants by sequential controlled pore glass, Con A Sepharose, and AcA54 gel filtration chromatography as described (13). Other reagents tested for macrophage Ia antigen regulatory activity included (a) L cell IFN, the culture supernatant of polyinosinic acid:polycytidylic acid-stimulated L929 fibroblasts (14), was tested as both a crude supernatant and in partially purified form, by sequential ammonium sulfate precipitation, Affigel 202 chromatography, and polyuridylic acid Sepharose chromatography (15); (b) IFN-γ containing phorbol myristic acetate (PMA)-stimulated BFS T lymphocyte cell line supernatants were pH 2 treated, trypsin treated, and partially purified by isoelectric focusing as described (11); (c) interleukin 2 (IL-2) and macrophage-granulocyte colony-stimulating factor (CSF) were prepared from serum-free culture supernatants of PMA-stimulated EL4 thymoma cells by absorption with activated charcoal, ammonium sulfate precipitation, and phenyl Sepharose chromatography as described (16); (d) macrophage-activating factor (MAF), as defined by the capacity to induce macrophages to kill tumor cells (17), was obtained from serum containing culture supernatants of PMA-stimulated EL4 thymoma cells and was partially purified by AcA54 gel filtration chromatography;<sup>2</sup> (e) epidermal cell-derived thymocyte-activating factor (ETAF) was obtained from unstimulated culture supernatants of PAM 212 cells and was partially purified by Sephacryl S-200 superfine gel filtration chromatography as described (18).

<sup>2</sup>Meltzer, M. S., W. R. Benjamin, and J. J. Farrar. Macrophage activation for tumor cytotoxicity: induction of macrophage tumoricidal activity by lymphokines from EL-4, a continuous T cell line. Manuscript submitted for publication.

*Gel Filtration Chromatography.* 2 ml of a 20×-concentrated Con A supernatant was passed over a 2.6- × 100-cm Sephacryl S-200 superfine (Pharmacia Fine Chemicals, Piscataway, NJ) gel filtration column at a rate of 20 ml/h at 4°C. The column was previously equilibrated in 0.10 M NaCl, 0.05 M Tris, pH 7.5 buffer, and calibrated with blue dextran (exclusion marker), bovine serum albumin (67,000  $M_r$ ), ovalbumin (43,500  $M_r$ ), and cytochrome *c* (12,384  $M_r$ ). 4-ml fractions were collected and brought to 1% final concentration fetal calf serum (FCS) after OD<sub>280</sub> determinations. The fractions were then filter sterilized and assayed for macrophage Ia antigen regulatory activity (at final concentrations of 10<sup>-1</sup>, 10<sup>-2</sup>, and 10<sup>-3</sup>) and IFN antiviral activity.

*Isoelectric Focusing.* A 50-ml Con A supernatant was focused using a 100-ml preparative isoelectric focusing column (LKB Laboratories, Gaithersburg, MD) as previously described (11). The 2-ml fractions were assayed for macrophage Ia antigen regulatory activity (at final concentrations of 1:5, 1:10, and 1:50) and IFN antiviral activity.

*Phenyl Sepharose Chromatography.* A 1-ml 10×-concentrated Con A supernatant was fractionated by phenyl Sepharose chromatography as previously described (16). The 2-ml fractions were assayed for macrophage Ia antigen regulatory activity (at final concentrations of 10<sup>-1</sup>, 10<sup>-2</sup>, and 10<sup>-3</sup>) and IFN antiviral activity.

*Polyuridylic Acid Sepharose Chromatography.* A 0.5-ml, 20×-concentrated Con A supernatant was fractionated by polyuridylic acid (poly U) Sepharose chromatography as described (19, 20). The 2-ml fractions were assayed for macrophage Ia antigen regulatory activity (at final dilutions of 10<sup>-1</sup>, 10<sup>-2</sup>, and 10<sup>-3</sup>) and IFN antiviral activity.

*Chromatography Controls.* As a control for possible nonspecific binding to Sepharose, Sepharose (CL-4B; Pharmacia Fine Chemicals) was swollen and equilibrated in each chromatography buffers. 1 ml of 10×-concentrated Con A supernatant was dialyzed for 24 h at 4°C against 100 vol of the appropriate buffer, applied to the Sepharose column, and eluted with the appropriate buffer. Fractions were brought to 1% FCS, dialyzed for 24 h against 50 vol of phosphate-buffered saline (PBS), filter sterilized, and assayed for macrophage Ia antigen regulatory activity (at 1:5, 1:10, and 1:40 final concentrations) and IFN antiviral activity.

*Antibody Neutralization Experiments.* Two conventional antisera were used in this study: (a) goat anti-mouse type I IFN antiserum (Research Resources Branch, National Institute of Allergy and Infectious Diseases) neutralizes IFN- $\alpha$  and IFN- $\beta$ , but not IFN- $\gamma$ ; (b) rabbit anti-mouse IFN- $\gamma$  antiserum prepared against staphylococcal enterotoxin A-induced IFN- $\gamma$  partially purified to 10<sup>7</sup> U/mg protein specific activity (21) was subsequently absorbed against spleen cells and FCS as described (22). This antiserum neutralizes mitogen and mixed lymphocyte reaction-induced IFN, but not virally induced IFN (21). In addition, when Con A supernatants were absorbed for 1 h at 4°C with sufficient antisera to neutralize twice the number of IFN units present, significant reductions in the dose-response curves for the interleukin 1 (IL-1), interleukin 2 (IL-2), and CSF bioassays (22) as well as MIF (R. Moulton, personal communication) and MAF (M. Meltzer, personal communication) assays were not obtained, as compared with absorption of a Con A supernatant with a control normal rabbit serum. For macrophage Ia antigen regulatory activity experiments, 1-ml aliquots of a 1:40 dilution of Con A supernatant were incubated with antiserum or control serum (starting with sufficient antiserum to neutralize double the IFN units contained in the preparation) at 4°C for 1 h. The culture supernatants were then assayed for macrophage Ia antigen regulatory activity and IFN activity.

*Additional Treatments.* To determine the pH stability of the macrophage Ia antigen regulatory lymphokine, 1-ml Con A supernatants were dialyzed for 24 h at 4°C against 100 vol of 0.1–0.2 M Tris-glycine, sodium acetate, sodium citrate, potassium phosphate, and borate buffers with pHs ranging from 2 to 10. The supernatants were then dialyzed for an additional 24 h at 4°C against 100 vol of PBS, filter sterilized, and assayed for macrophage Ia antigen regulatory activity (at 1:5, 1:10, and 1:50 final concentrations) and IFN antiviral activity.

The heat stability of the macrophage Ia antigen regulatory activity was determined by incubating 1-ml Con A supernatants at either 37 or 56°C for 1 h, and cooling immediately.

To determine the sensitivity of the macrophage Ia antigen regulatory activity to trypsin, 0.7 ml of Con A supernatant was treated with either 10 U of bovine pancreas trypsin or RNase attached to cross-linked beaded agarose (Sigma Chemical Co., St. Louis, MO) as previously

described (11). The supernatants were tested for Ia antigen regulatory activity (at 1:10, 1:50, and 1:250 final concentrations) and for IFN antiviral activity.

## Results

*Assay for Lymphokine Induction of Macrophage Ia Antigen Expression.* A modified assay for lymphokine induction of macrophage Ia antigen expression was developed (8), using the Ia<sup>-</sup> P388D<sub>1</sub> macrophage cell line as a target cell rather than thioglycollate-induced peritoneal exudate macrophages. In the experiment shown in Table I, P388D<sub>1</sub> macrophages (Ia<sup>d</sup>) were incubated for 2 d with dilutions of Con A supernatant or a control supernatant prepared from C3H/HeN (Ia<sup>k</sup>) spleen cells, and the percentage of cells expressing I-A<sup>d</sup> antigens was determined. As shown, incubation of the macrophages with Con A supernatant induced endogenously derived Ia antigen expression in a dose-dependent manner. The sensitivity of this assay is a CI of 6, and its precision is 7%. The use of the P388D<sub>1</sub> cell line as a target cell in this assay is advantageous (*a*) as a source of macrophages free of other contaminating cell types, (*b*) as a homogeneous source of Ia<sup>-</sup> cells, and (*c*) because P388D<sub>1</sub> cells can be grown in suspension culture, permitting cell recovery without cell surface damage resulting from trypsinization or scraping.

*Characterization of the Con A Supernatant Macrophage Ia Antigen Regulatory and IFN- $\gamma$  Activities.* Using this assay, the macrophage Ia antigen regulatory mediator(s) in the Con A supernatant has been analyzed to determine its biochemical and antigenic characteristics, which are summarized in Table II. Also shown are the characteristics of the Con A supernatant IFN antiviral activity, quantified by a viral plaque reduction assay. Pluses (+) in this table indicate that the Con A supernatant, as treated, had significant biological activity in the indicated assay and that the activity was detectable in a dose-dependent manner.

Table II indicates that both the Con A supernatant macrophage Ia antigen regulatory and IFN- $\gamma$  activities were trypsin sensitive, sensitive to overnight dialysis to pH 2 and 3 buffers, resistant to dialysis to pH 4–10 buffers, and resistant to heating at 56°C for 1 h.

Gel filtration chromatography of the Con A supernatant is shown in Fig. 1. Both the macrophage Ia antigen regulatory and IFN- $\gamma$  activities eluted from Sephacryl

TABLE I  
*Con A Supernatant Induction of P388D<sub>1</sub> Macrophage Ia Antigen Expression*

Supernatant dilution	Mean CI (95% confidence limits)	
	Con A supernatant*	Control supernatant‡
0	0	0
1:20	56.8 (54.5, 59.1)§	0
1:80	64.4 (56.5, 72.3)§	2.9 (2.2, 3.7)
1:320	12.8 (10.7, 14.9)	0
1:1,280	0.5 (0, 2.3)	0

P388D<sub>1</sub> (Ia<sup>d</sup>) macrophages were incubated 2 d with supernatant prepared from C3H/HeN (Ia<sup>k</sup>) spleen cells, and the percentage of Ia<sup>d</sup> expressing macrophages was determined by anti-I-A and complement-mediated cytotoxicity.

\* Con A-stimulated spleen cell supernatant (4).

‡ Con A-supplemented (unstimulated) spleen cell supernatant.

§  $P < 0.05$ .

TABLE II  
*Characterization of the Con A Supernatant: Properties of the Macrophage Ia Antigen Regulatory Activity that Are Shared with the Interferon Antiviral Activity*

Property*	Macrophage Ia antigen regulatory activity	IFN antiviral activity
pH 2-3 sensitive; pH 4-10 resistant	+‡	+
Resistant to 56°C, 1 h	+	+
Trypsin sensitive	+	+
RNase resistant	+	+
40,000 apparent molecular weight on Sephacryl S-200 Superfine	+	+
pI = 5.0-5.5	+	+
Binds phenyl Sepharose; elutes at 35% ethanediol	+	+
Binds poly U Sepharose; elutes in high-salt buffer	+	+
Binds Sepharose	-	-
Neutralized by goat anti-mouse type I IFN antiserum	-	-
Neutralized by rabbit anti-mouse IFN- $\gamma$ antiserum	+	+

\* See Materials and Methods.

‡ Pluses (+) signify that the Con A supernatant, as treated, had significant, dose-dependent activity in the indicated bioassay.

S-200 Superfine columns with an apparent molecular weight of 40,000. Background Ia antigen induction below the sensitivity of the assay (CI <6.0), and therefore insignificant, was found scattered throughout the fractions. The apparent molecular weight of IFN- $\gamma$  obtained in this experiment is consistent with other estimated molecular weights for IFN- $\gamma$ , which vary from 20,000 to 60,000, but are most often in the 40,000-50,000 range (23).

Hydrophobic chromatography of the Con A supernatant macrophage Ia antigen regulatory and IFN- $\gamma$  activities on phenyl Sepharose are shown in Fig. 2. A major peak of macrophage Ia antigen regulatory activity and IFN- $\gamma$  activity bound to the phenyl Sepharose and co-eluted at ~35% ethanediol. Minor levels of macrophage Ia antigen-inducing activity eluted in the initial part of the ethanediol gradient and co-eluted with titers of IFN- $\gamma$  of <5 U/ml (data not shown).

Isoelectric focusing of the Con A supernatant is shown in Fig. 3. Both the macrophage Ia antigen regulatory and IFN- $\gamma$  activities of the Con A supernatant had an isoelectric point (pI) between pH 5.0 and 5.5. The heterogeneity exhibited in this peak is consistent with previous reports for other interferons (24).

Fibroblast IFN (19) and more recently IFN- $\gamma$  (20) have been reported to reversibly bind poly U Sepharose. Fig. 4 demonstrates the elution pattern of the Con A supernatant macrophage Ia antigen regulatory and IFN- $\gamma$  activities on poly U Sepharose. As shown, both activities are retained by the poly U Sepharose, and subsequently co-elute after the application of a high-salt buffer.

The antigenic characteristics of the Con A supernatant macrophage Ia antigen regulatory and IFN- $\gamma$  activities have been determined. Table II indicates that goat anti-mouse type I IFN antiserum, which neutralizes IFN- $\alpha$  and IFN- $\beta$ , does not neutralize either the macrophage Ia antigen regulatory or IFN activities of the Con A supernatant. Table III demonstrates the effect of a rabbit anti-IFN- $\gamma$  antiserum on these bioactivities. Con A supernatants were adsorbed with dilutions of antiserum for

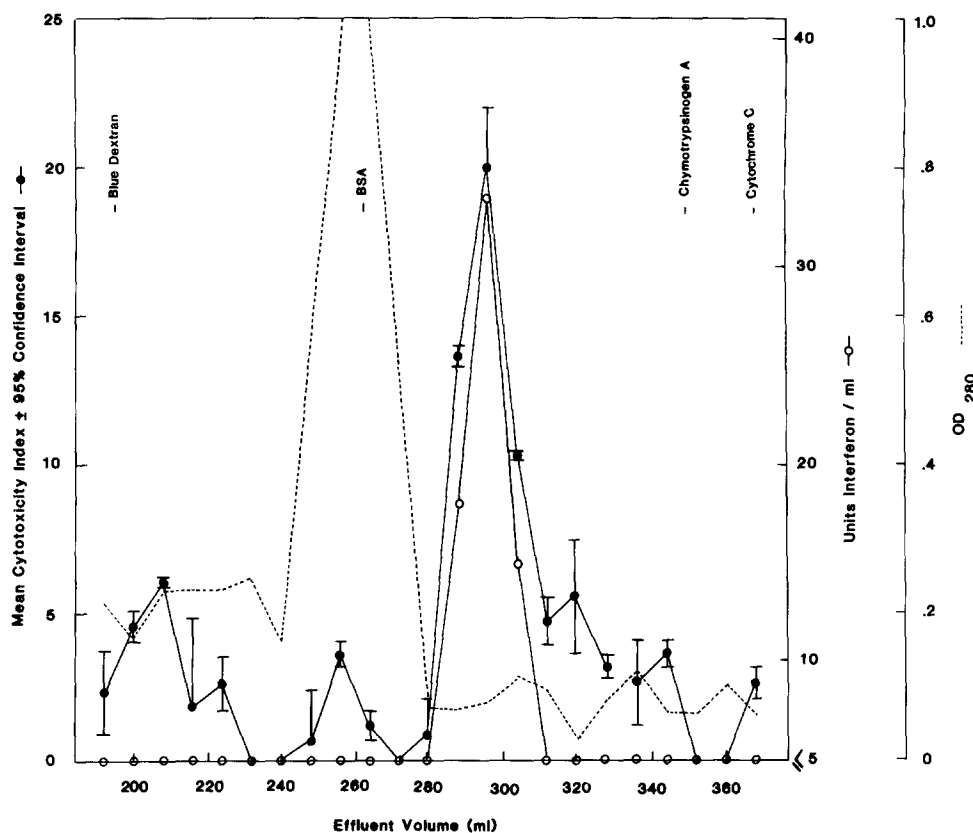


Fig. 1. Gel filtration chromatography of the Con A supernatant. A 2-ml, 20X-concentrated Con A supernatant was applied to a Sephacryl S-200 Superfine column and eluted with 0.05 M Tris-HCl, 0.1 M NaCl buffer, pH 7.5. Alternate fractions were assayed for IFN activity and induction of P388D<sub>1</sub> macrophage Ia antigen expression as determined by antibody- and complement-mediated cytotoxicity (1:100 dilution shown). The peak fractions had a specific activity of 80 U IFN/OD unit, and represented a 41% recovery of the applied IFN sample.

1 h at 4°C and subsequently incubated with P388D<sub>1</sub> cells to induce Ia antigen expression. As shown, the anti-IFN- $\gamma$  antiserum inhibited Con A supernatant induction of P388D<sub>1</sub> macrophage Ia antigen expression to a CI of 5.4, a level below the sensitivity of the assay and therefore indistinguishable from untreated macrophages. The IFN- $\gamma$  content of the Con A supernatant was also abrogated (data not shown). Absorption of the Con A supernatant with normal rabbit serum did not abrogate its macrophage Ia antigen regulatory and IFN- $\gamma$  activities.

*Macrophage Ia Antigen Regulatory Activity of Partially Purified IFN- $\gamma$ .* An independently prepared murine IFN- $\gamma$  purified to 10<sup>7</sup> U/mg protein specific activity was tested for Ia antigen regulatory activity (Fig. 5, panel A). P388D<sub>1</sub> macrophages were incubated with serial dilutions of IFN- $\gamma$  for 3 d, and the percentage of Ia<sup>+</sup> macrophages was determined. As shown, a dose-dependent induction of macrophage Ia antigen expression occurred. Such Ia<sup>+</sup> macrophages, if subsequently reincubated with IFN- $\gamma$ , remained Ia<sup>+</sup> but reverted to the Ia<sup>-</sup> state if reincubated in IFN- $\gamma$ -free medium (data not shown). Thus, IFN- $\gamma$  purified to 10<sup>7</sup> U/mg protein specific activity has macro-

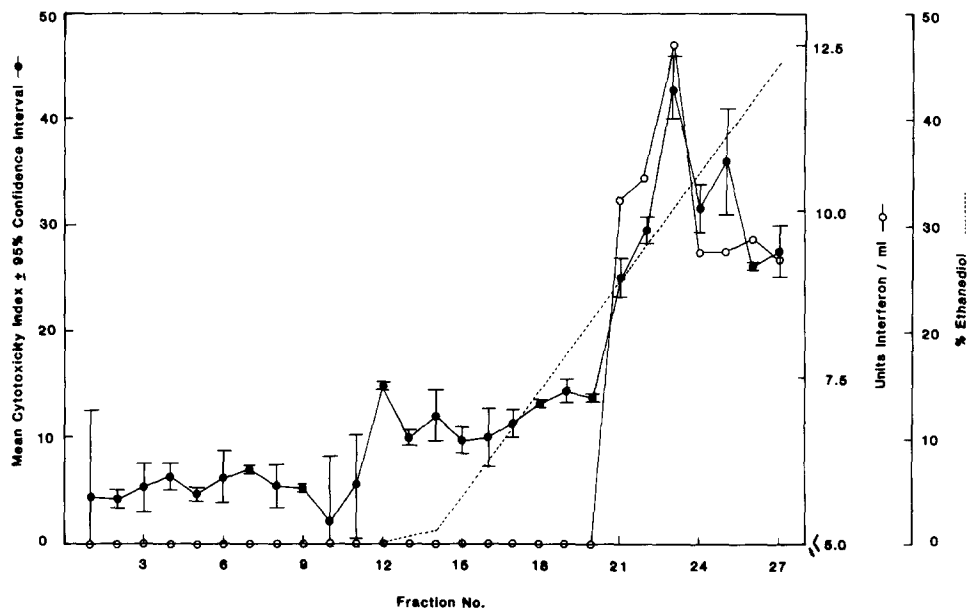


FIG. 2. Hydrophobic chromatography of the Con A supernatant. A 1-ml, 10X-concentrated Con A supernatant was applied to a phenyl Sepharose column, which was then washed with 0.8 M  $(\text{NH}_4)_2\text{SO}_4$  in 0.02 M PBS. Fractions were assayed for IFN content and induction of P388D<sub>1</sub> macrophage Ia antigen expression as determined by antibody- and complement-mediated cytotoxicity (1:100 dilution shown). The peak fractions had a specific activity of 97 U IFN/OD unit and represented a 29% recovery of the IFN in the starting material.

phage Ia antigen regulatory activity.

*Role of Other Lymphokines in the Regulation of Macrophage Ia Antigen Expression.* Another approach to identify the lymphokine(s) that induces macrophage Ia antigen expression was to assay the effect of culture supernatants known to contain a limited number of lymphokine activities. Recently, an IL-2-independent T cell line (BFS) was isolated that produces IFN- $\gamma$  upon stimulation with PMA (11). Culture supernatants of this cell line, however, do not contain detectable CSF, IL-1, IL-2, B cell growth factor, or T cell replacing factor (11). Fig. 5 (panel B) indicates that P388D<sub>1</sub> macrophages, when incubated with crude or partially purified BFS culture supernatants, expressed Ia antigens in a dose-dependent manner. The Ia antigen-inducing activity of the BFS cell line supernatant was sensitive to trypsin treatment and pH 2 dialysis. Thus, an IFN- $\gamma$ -like lymphokine can regulate macrophage Ia antigen expression without the participation of a number of other known lymphokines.

Subsequent experiments investigated whether a non-IFN lymphokine can directly regulate macrophage Ia antigen expression. The following lymphokine preparations, which were devoid of detectable IFN- $\gamma$  activity, had no detectable macrophage Ia antigen regulatory activity (data not shown): (a) EL4 thymoma cell-line-derived, partially-purified CSF (1-10,000 U/ml) or IL-2 (1-100 U/ml); (b) PAM 212 cell-line-derived ETAF, over a 3-log range of dilutions. Data indicating that these cell-line-derived mediators are representative of those found in the Con A supernatant is provided by the inhibition of Con A supernatant macrophage Ia antigen regulatory activity by rabbit anti-mouse IFN- $\gamma$  antiserum, which does not inhibit the IL-1, IL-2,

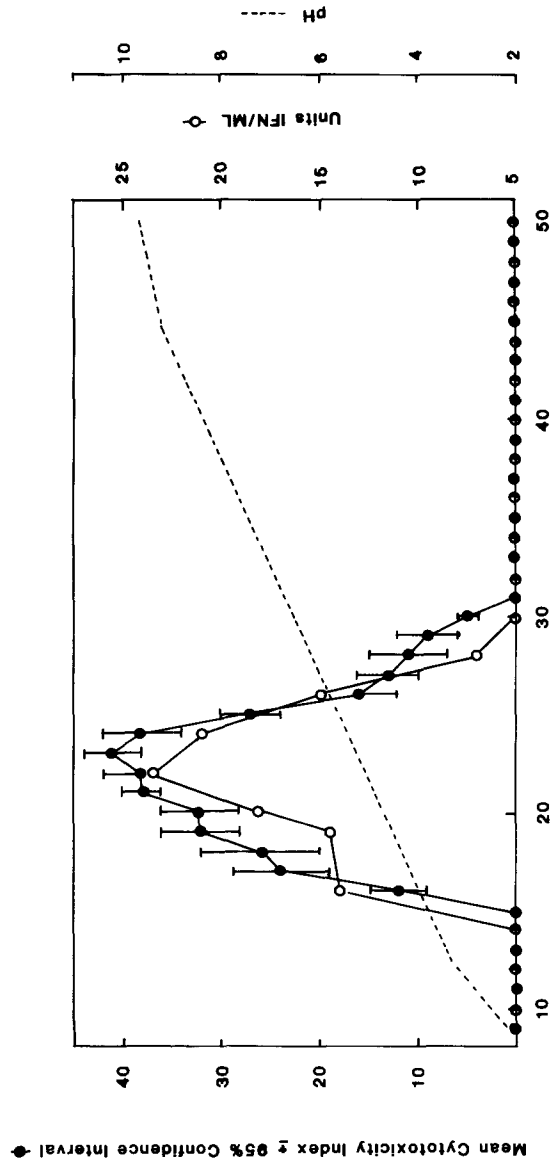


Fig. 3. Isoelectric focusing of the Con A supernatant. A 50-ml Con A supernatant was incorporated into a 5-50% sucrose gradient containing ampholines alternate fractions were tested for IFN content. The peak fractions had a specific expression as determined by antibody- and complement-mediated in a column activity of 19 U IFN/OD unit and represent a 5% recovery of the IFN in the isoelectric focusing unit. After focusing, each fraction was tested for the induction starting material.

Fraction No.



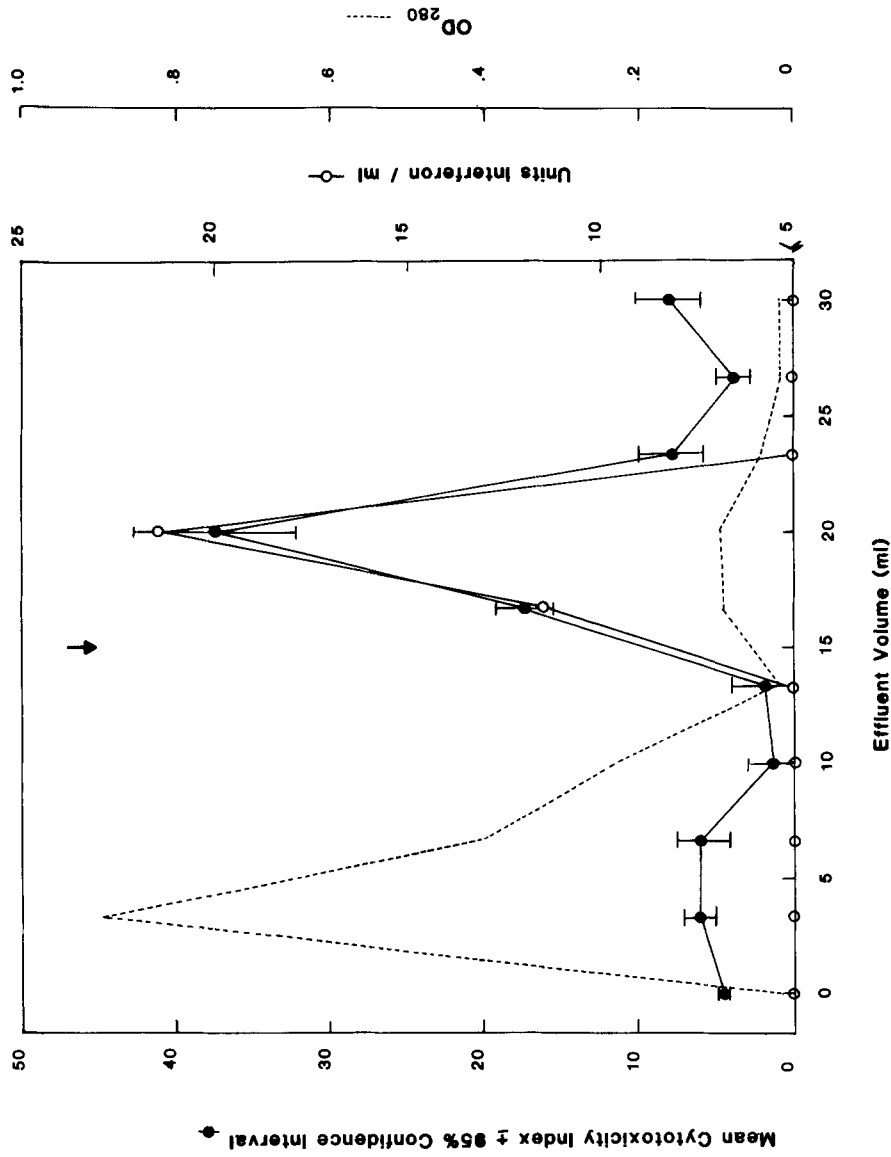


FIG. 4. Affinity chromatography of the Con A supernatant. A 0.5-ml, 20-times concentrated Con A supernatant was applied to a poly U and Sepharose column, which was then washed with 0.01 M Tris-HCl buffer, pH 7.5, and 0.01 M Tris-HCl, 1.0 M NaCl buffer, pH 7.5. Fractions were tested for IFN activity and for the induction of P388D<sub>1</sub> macrophage Ia antigen expression as determined by antibody- and complement-mediated cytotoxicity (1:100 dilution shown). The peak fractions had a specific activity of 400 U IFN/OD unit and represented a 41% recovery of the IFN in the starting material.

TABLE III  
*Abrogation of Con A Supernatant Induction of Macrophage Ia Antigen Expression by Rabbit Anti-IFN- $\gamma$  Antiserum\**

Serum dilution	Mean CI (95% confidence limits)	
	Con A supernatant + rabbit anti- $\gamma$ serum $\ddagger$	Con A supernatant + normal rabbit serum $\ddagger$
0	55.08 (4.9, 66.0)	
1:16	37.94 (19.7, 58.1)	(46.11 (37.2, 55.6)
1:8	17.12 (10.4, 25.1)	46.48 (34.2, 59.8)
1:4	4.42 (0.2, 16.8)	51.36 (40.8, 61.8) $\S$

\* Con A supernatant (1:40 dilution) and either antiserum or control serum were incubated for 1 h at 4°C. The mixtures were then incubated with P388D<sub>1</sub> macrophages for 2 d, and the percentage of Ia<sup>+</sup> macrophages was determined by antibody- and complement-mediated cytotoxicity.

$\ddagger$  Neither serum was directly cytotoxic at the concentrations shown.

$\S$  Differs significantly ( $P < 0.01$ ) from Con A supernatant + normal rabbit serum, not significantly different ( $P < 0.05$ ) from untreated macrophages.

or CSF bioassays (22); (c) a crude macrophage MIF preparation, prepared by absorbing the Con A supernatant with rabbit anti-mouse IFN- $\gamma$  antibody (R. Moulton, unpublished observation) (d) a crude MAF preparation, prepared by absorption of the Con A supernatant with rabbit anti-mouse IFN- $\gamma$  (M. S. Meltzer, personal communication) when tested in serial dilutions ranging from 1:20 to 1:1,000. However, culture supernatants of PMA-stimulated EL4 thymoma cells, upon gel filtration, yield two peaks of MAF activity:<sup>2</sup> (a) a low molecular weight peak that had no detectable IFN activity, nor (at final concentrations of 1:2–1:100) any macrophage Ia antigen regulatory activity; and (b) a second higher molecular weight peak of MAF activity that co-eluted with IFN- $\gamma$  activity-induced P388D<sub>1</sub> macrophage Ia antigen expression. It is not yet known whether both MAF activities are also present in the Con A supernatant. Thus, it appears that IFN- $\gamma$  and the macrophage Ia antigen regulatory activity can be separated from at least one MAF activity.

*Macrophage Ia Antigen Regulatory activity of Other IFN Preparations.* L cell IFN, the culture supernatants of polyinosinic acid:polycytidylic acid-stimulated L929 fibroblasts, were tested for macrophage Ia antigen regulatory activity in both crude and partially purified forms. L cell IFN, in a dose-dependent manner typically induced a maximum of only 10–15% Ia<sup>+</sup> P388D<sub>1</sub> macrophages (Fig. 5, panel C). Optimal macrophage Ia antigen-inducing activity typically occurred between 0.4 and 4.0 U/ml IFN activity and was reduced at higher IFN concentrations.

## Discussion

The data indicate that an IFN- $\gamma$ -like molecule present in a Con A supernatant regulates the in vitro expression of Ia antigens on macrophages. This molecule shares molecular weight, pI, and hydrophobic, affinity, and antigenic characteristics with the antiviral activity of the Con A supernatant. Further, an independently prepared partially purified IFN- $\gamma$  ( $10^7$  U/mg protein sp act) has macrophage Ia antigen regulatory activity.

A number of other lymphokines present in the Con A supernatant had no detectable macrophage Ia antigen regulatory activity. These data are based on: (a) the lack of

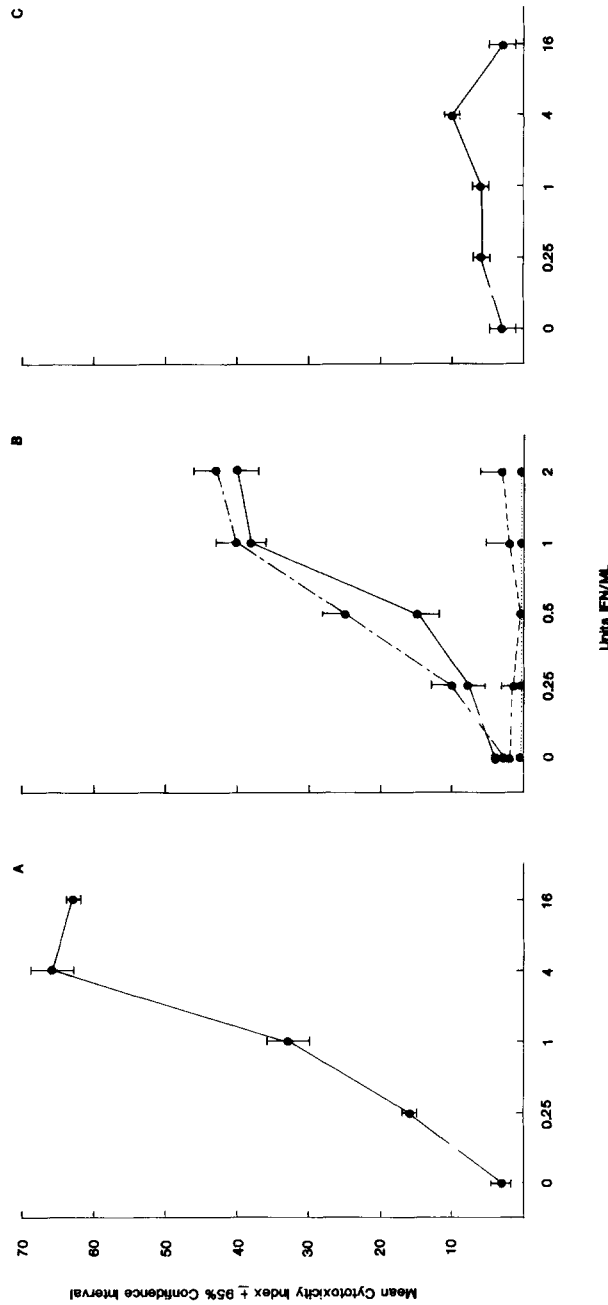


FIG. 5. Induction of macrophage Ia antigen expression by independently prepared IFN- $\gamma$ . P388D<sub>1</sub> macrophages were incubated for 3 d with dilutions of IFN, and the percentage of Ia<sup>+</sup> macrophages was determined by antibody- and complement-mediated cytotoxicity. Panel A: Staphylococcal enterotoxin A-induced IFN- $\gamma$ , purified to 10<sup>7</sup> U/mg protein sp act; panel B: IFN- $\gamma$ -containing BFS cell precipitation and Attigel 202 and poly U Sepharose chromatography. Panel C: L929 fibroblast derived IFN, partially purified by sequential ammonium sulfate precipitation and Attigel 202 and poly U Sepharose chromatography.

macrophage Ia antigen regulatory activity in culture supernatants of cell lines known to contain a limited number of lymphokine activities; and (b) the inhibition of the Con A supernatant macrophage Ia antigen regulatory activity by rabbit anti-IFN- $\gamma$ , without significant reductions in the titers of a number of other lymphokines present in this supernatant. The data, therefore, suggest that IL-1, IL-2, CSF, MIF, and MAF with biochemical and antigenic characteristics distinct from those of IFN- $\gamma$  have no macrophage Ia antigen regulatory activity. However, the data do not preclude the existence of other lymphokines besides IFN- $\gamma$  that are biochemically similar to IFN- $\gamma$  and may have the capacity to directly regulate macrophage Ia antigen expression, such as the high molecular weight MAF activity, or the possibility the IFN- $\gamma$  can act synergistically with a co-factor.

Experiments in progress, which examine the capacity of cloned human IFN to induce DR antigen expression on human monocytes and monocytic cell lines, indicate that cloned IFN can regulate human macrophage DR antigen expression. Using these data, we postulate that an IFN- $\gamma$ -like molecule regulates macrophage Ia antigen expression in vivo. It has been reported that the Ia antigen expression of macrophages from various tissues differs: for example, splenic (25), thymic (26), and liver (27) macrophages are mainly Ia<sup>+</sup>, whereas exudate macrophages are predominantly Ia<sup>-</sup> (25, 28). It can be hypothesized that in those tissues in which a high proportion of Ia<sup>+</sup> macrophages reside, continuous low-level production of an IFN- $\gamma$ -like mediator induces and maintains macrophage Ia antigen expression. The observation that mice reared in a specific-pathogen-free environment have reduced macrophage Ia antigen expression (29) suggests that the background barrage of antigenic stimuli normally encountered may stimulate the continuous low-level lymphokine production. Furthermore, several reports suggest the intriguing possibility that the autoimmune state may, in part, be regulated by IFN modulation of macrophage Ia antigen expression, and consequent enhanced presentation of self-antigens: (a) autoimmune MRL/lpr mice have higher percentages of Ia<sup>+</sup> resident macrophages than normal MRL/+++ mice (30); (b) active systemic lupus erythematosus patients have higher serum IFN titers than patients with an inactive disease state (31); and (c) normal mice that were neonatally thymectomized and injected with the IFN inducers poly I:C plus lipopolysaccharide developed autoimmune disease (32). Experiments in progress are examining in detail the suggested in vivo correlation of serum IFN titers, macrophage Ia antigen expression, and autoimmunity.

### Summary

A culture supernatant of concanavalin A-activated spleen cells (Con A supernatant) induced murine macrophages to express Ia antigens in vitro. Biochemical characterization of the Con A supernatant indicated that the macrophage Ia antigen regulatory activity shares molecular weight, pI, and hydrophobic and affinity characteristics with immune interferon (IFN- $\gamma$ ). Antiserum to mouse IFN- $\gamma$  neutralized both the macrophage Ia antigen regulatory and IFN- $\gamma$  bioactivities of the Con A supernatant. Furthermore, both partially purified murine IFN- $\gamma$  ( $10^7$  U/mg protein sp act) and IFN-containing culture supernatants of the murine BFS T cell line-induced macrophage Ia antigen expression in vitro. Culture supernatants containing colony-stimulating factor, interleukin 1, interleukin 2, macrophage migration inhibitory factor, and a macrophage-activating activity that were distinct from IFN- $\gamma$  did not induce

macrophage Ia antigen expression. Taken together, the data indicate that the in vitro expression of Ia antigens on macrophages is regulated by an activity that has the characteristics of interferon.

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