Immunology 1988 **64** 61–67

Relationship between ineffective antigen presentation by murine alveolar macrophages and their immunosuppressive function

I. N. MBAWUIKE* & H. B. HERSCOWITZ *Department of Microbiology, Georgetown University, Schools of Medicine and Dentistry, Washington, DC, U.S.A.*

Accepted for publication 7 December 1987

SUMMARY

Murine alveolar macrophages (AM) have been shown to be inefficient at providing accessory function for initiation of the *in vitro* plaque-forming cell (PFC) response. In the present study AM, which were obtained either from untreated mice (resident AM) or mice injected i.v. with BCG (activated AM) potently suppressed the PFC response of spleen cells from animals previously primed with sheep erythrocytes (SRBC). Additon of AM at a concentration of 10% with respect to spleen cells resulted in greater than 90% suppression of the PFC response. In order to determine if inefficient antigen presentation was associated with AM-mediated suppression, the role of IL-1 and Ia antigen was studied. Addition of exogenous recombinant IL-1 (rIL-1) stimulated the PFC response in control cultures, but had no effect on AM-mediated suppression. Resident AM could be activated with lipopolysaccharide or antigen to produce significant levels of IL-1. Membrane-bound IL-1, thought to be important in the presentation of particulate antigens, was detected on glutaraldehyde-fixed resident AM and was significantly elevated in BCG-activated macrophages. The frequency of cell surface Ia antigen expression was low in resident AM (4%), but could be increased (35%) after *in vivo* activation with BCG. Recombinant interferon-gamma (IFN-γ), known to enhance expression of Ia antigen and production of IL-1, had no effect on AM-mediated suppression when used either to pretreat AM, when present during the entire period of culture, or when injected into mice before culture initiation. Treatment with IFN-γ, however, resulted in a slight increase in the expression of Ia antigen. These results indicate that the immunosuppressive activity of AM is neither related to a defect in IL-1 production or expression nor to a deficiency in Ia antigen expression and therefore can not be explained by the inefficient antigen-presenting function of alveolar macrophages.

INTRODUCTION

Apart from their non-specific phagocytic and microbicidal functions (Hocking & Golde, 1979), pulmonary alveolar macrophages (AM) have been shown to regulate the induction and expression of specific immune reactions in the lung (Kaltreider, 1982; Herscowitz, 1985). They have been reported to stimulate and inhibit antigen- and mitogen-induced lympho-proliferation (Laughter, Martin & Twomey, 1977; Gorenberg & Daniele, 1978; Holt, 1979; Lipscomb *et al.*, 1981) and suppress antibody response (Pennline *et al.*, 1979; Fournier *et al.*, 1984; Mbawuike, Luhr & Herscowitz, 1986a). As relatively poor antigen-presenting cells, AM have been found to be less efficient in the uptake of particulate antigens (Weinberg & Unanue, 1981), processing of soluble antigens (Ullrich & Herscowitz, 1980) and interacting with T cells (Shellito, Caldwell & Kaltreider, 1983) when compared to peritoneal or splenic macrophages.

It is currently unclear if there is a correlation between the immunosuppressive function of AM and their defective antigen-presenting capabilities. Such a relationship has been suggested by studies which demonstrate decreased ability to suppress and increased ability to present antigen by AM in bronchial asthma (Aubas *et al.*, 1984), sarcoidosis (Venet *et al.*, 1985) and experimental pulmonary granuloma (Kobayashi *et al.*, 1985). In those studies, the increased accessory functions were accompanied by an increased frequency of AM expressing HLA-DR or HLA-DS surface antigens (Venet *et al.*, 1985), augmented levels of IL-1 production and increased responsiveness to migration inhibitory factor (Kobayashi *et al.*, 1985).

IL-1, a polypeptide hormone produced by activated mononuclear phagocytes, modulates the proliferation, maturation and functional activation of T cells (Mizel, 1982) and plays a role in the initiation and amplification of T-cell dependent immune responses. IL-1 induces the synthesis of IL-2 and/or the expression of IL-2 receptors. Recently, antigen-pulsed, parafor-

*Present address: Influenza Research Center, Dept. of Microbiology and Immunology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, U.S.A.

Correspondence: Dr H. B. Herscowitz, Dept. of Microbiology, Schools of Medicine and Dentistry, Georgetown University, 3900 Reservoir Rd, N.W., Washington, DC, 20007, U.S.A.

maldehyde-fixed splenic macrophages were shown to express integral membrane IL-1, which fulfils one of the requirements for T-cell activation in addition to antigen presentation in the context of I-region determinants (Kurt-Jones, Virgin & Unanue, 1985). Only a small percentage of murine AM (5–10%) express Ia antigen when compared to other types of macrophages (Weinberg & Unanue, 1981; Cowing, Schwartz & Dickler, 1978). However, human AM have been shown to secrete spontaneously significant amounts of IL-1 (Koretsky *et al.*, 1983). Further, production of IL-1 was shown to be augmented in pulmonary disease states (Kobayashi *et al.*, 1985; Venet *et al.*, 1985).

In the present study, we asked if murine AM were capable of producing IL-1 and expressing Ia antigen in order to determine if a deficiency in the production of these immunologically relevant molecules played a role in AM-mediated suppression of humoral immunity. In addition, we wanted to determine if treatment of AM with IFN-γ, which is known to stimulate both IL-1 production and Ia antigen expression (Eden & Turino, 1986; Boraschi, Censini & Tagliabue, 1984; Nakamura *et al.*, 1984), could modulate AM-mediated suppression.

MATERIALS AND METHODS

Mice

Certified virus-free BALB/c and C3H/HeJ mice were purchased from Harlan Sprague–Dawley Inc., Walkersville, MD and The Jackson Laboratory, Bar Harbor, ME, respectively, and were maintained at the Georgetown University Research Resources Facility in accordance with NIH guidelines. They were provided with food and water *ad libitum*.

Cell lines

D10.G4.1, a conalbumin-specific AKR T-cell clone, isolated and characterized by Kaye *et al.* (1984), was generously provided by Dr Jonathan Kaye (Yale University Medical School, New Haven, CT). D10.G4.1 cells were maintained by weekly passage with mitomycin *c*-treated C3H/HeJ splenic feeder cells and conalbumin (Sigma Chemical Co., Louis, MO) in medium consisting of Click's EHAA (Irvine Scientific, Santa Ana, CA) supplemented with 10% heat-inactivated fetal bovine serum (FCS), 60 mM 2-mercaptoethanol (2-ME), 100 U/ml penicillin, 100 μg/ml streptomycin and 10% (v/v) of rat spleen cell supernatant containing T-cell growth factor (TCGF). The TCGF-containing supernatant was prepared as follows: rat spleen cells (5×10^6/ml) were cultured for 27 hr in RPMI-1640 medium containing 2·5 μg/ml concanavalin A (Con A; Sigma Chemical Co.). Before use, 20 mg/ml α-methyl-D-mannoside (Sigma) were added to the supernatant followed by filter sterilization.

P388D1, a DBA/2 mouse monocyte-macrophage cell line selected for high IL-1 production (Mizel, 1981) was provided by Dr Robert H. Wiltrout (National Cancer Institute, Frederick, MD) and was maintained by a twice weekly passage in RPMI supplemented with 10% FCS.

Alveolar macrophages (AM)

Murine AM were recovered from untreated animals (resident AM) or from animals given an i.v. injection of heat-killed BCG (activated AM) by bronchoalveolar lavage (Mbawuike, Luhr & Herscowitz, 1986a). For collection of the latter, lyophilized heat-killed *Mycobacterium bovis*, BCG strain was homogenized in phosphate-buffered saline (PBS) at 1 mg/ml by ultrasonic probe sonication (Heat Systems Ultrasonics Inc., Plainview, NY) in an ice bath and 0·5 ml of the homogenate was injected into the tail vein of 7–10-week-old BALB/c mice. Bronchoalveolar lavage was performed 6–8 days later with warm (37°) Ca^{2+}-Mg^{2+}- free PBS containing 17 mM lidocaine-HCl (Xylocaine, Astra Pharmaceutical Products Inc., Framingham, MA). Bronchoalveolar cells (BAC) were immediately placed on ice, washed three times and counted in a haemocytometer. The BAC recovered from untreated or BCG-injected mice consisted of 90–95% macrophages, 4–8% lymphocytes and 1–2% polymorphonuclear leucocytes, as determined by Wright's stain (Diff Quik, Scientific Products Inc., McGaw Park IL) of cytocentrifuge preparations (Mbawuike *et al.*, 1986a).

Peritoneal macrophages

Resident macrophages were obtained from the peritoneal cavity of untreated mice by saline lavage, as described previously (McCarron *et al.*, 1984).

Further purification of macrophage populations, when necessary, was achieved by a one-step adherence on plastic plates for 2–24 hr at 37° followed by removal of non-adherent cells by washing. This procedure resulted in recovery of 95–98% viable macrophages.

Depletion of macrophages from spleen cells

Macrophages were depleted from SRBC-primed spleen cell populations by allowing them to ingest carbonyl iron followed by removal of iron-containing cells by magnets, essentially as described by Wong (1981).

Macrophage-mediated suppression of the in vitro *PFC response*

Spleen cells (1×10^7) obtained from BALB/c mice primed 4 days earlier with sheep erythrocytes (SRBC; Waltz Farm, Smithsburg, MD) by i.p. injection of 0·2 ml 10% (v/v) SRBC in PBS were cultured with 2×10^6 SRBC, with or without 1×10^6 AM, in a total volume of 1 ml RPMI-FCS medium which consisted of RPMI-1640 medium (Hazelton Research Products Inc., Denver, PA) supplemented with 10% fetal bovine serum (Hyclone Laboratories, Logan, UT), 100 U/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine (Hazelton Research Products Inc.). The cell mixture was incubated in 24-well tissue culture plates (no. 3424, Costar, Cambridge, MA) at 37° in 5% CO_2 humidified air. The cultures were harvested after 3 days and the number of cells secreting anti-SRBC antibodies determined in a haemolytic plaque assay, as previously described (Pennline *et al.*, 1979).

Treatment of cell cultures

Recombinant murine IL-1 (Lomedico *et al.*, 1984), a generous gift from Dr Peter T. Lomedico (Hoffman-La Roche Inc., Nutley, NJ), was added to the PFC cultures at various concentrations for the entire period of culture. Recombinant murine IFN-γ (Gray & Goeddel, 1983) was donated by Genentech Inc., S. San Francisco, CA and was added to cultures at various concentrations or used to pretreat macrophages for 24–96 hr before their addition to PFC cultures. *In vivo* treatment with IFN-γ was achieved by i.v. injection of various concentra-

tions of IFN-γ along with 0·5 mg BCG in 0·5 ml PBS in the tail vein of BALB/c mice. AM were harvested 5–9 days later by bronchoalveolar lavage.

Induction of IL-1 in macrophages

Preparation of IL-1-containing supernatants was modified from the method of Fujwara *et al.* (1986). Briefly, AM or the P388D1 cell line were cultured in 24-well tissue culture cluster plates (no. 3424, Costar) at 5×10^5 cells in 1 ml of Click's medium (Irvine Scientific) supplemented with 1% heat-inactivated fetal bovine serum, 100 U/ml penicillin, 2 mM L-glutamine and 5×10^{-5} M 2-ME in the presence or absence of 10 μg/ml of lipopolysaccharide (LPS; *Escherichia* coli, serotype no. 055:B5, Sigma Chemical Co.). After 24–96 hr at 37° in 5% CO_2 in air, the supernatants were harvested and concentrated four-fold with Immersible-CX-10 ultra-filters (Millipore Corp., Bedford, MA). The IL-1-containing culture supernatants were filter-sterilized and stored at −20° until used. The cellular protein content of macrophages that were solubilized with 0·05% Triton X-100 was determined by the Lowry procedure (Lowry *et al.*, 1951).

Assay for IL-1 activity in culture supernatants

Culture supernatants thought to contain IL-1 activity were assayed by a modification of the method of Kurt-Jones *et al.* (1985) as described by J. Kaye, Conrad and C. A. Janeway (personal communication). Briefly, serial dilutions of IL-1-containing supernatants were prepared in 200 μl of Click's EHAA medium (no TCGF) and added to flat-bottomed tissue culture wells (no. 3596, Costar) in triplicate with 2×10^4 D10.G4.1 T cells and 1 mg/ml of Con A. After 48 hr of incubation at 37° each well received 0·5 μCi [methyl-^{3}H]thymidine ([^{3}H]TdR; 50 Ci/mM, ICN Radiochemicals, Irvine, CA). The plates were harvested 18–20 hr later and [^{3}H]TdR incorporation was determined by liquid scintillation counting. Serial dilutions of purified recombinant murine IL-1 (rIL-1) starting at 1 U/ml were included as standard. Regression lines were plotted for c.p.m. of [^{3}H]TdR incorporation against the reciprocal of dilutions of test samples or units of rIL-1 standard. The c.p.m. of test samples at the *y* intercept were compared to the c.p.m. of the rIL-1 standard-treated samples at *y* intercept. The activity in test samples was transformed into units according to the formula:

$$\frac{\text{C.p.m. of test sample at } y \text{ intercept}}{\text{C.p.m. of rIL-1 standard at } y \text{ intercept}} = \text{U/ml sample.}$$

Florescence-activated cell sorter (FACS) for analysis of cell surface Ia expression

Macrophages (1×10^6 cells/ml) were incubated in 5% normal rabbit serum in PBS plus 0·02% NaN_3 for 20 min at 0–4° to block Fc receptors and reduce non-specific interactions. Monoclonal anti-I-A^d antibody (1/100 dilution of MK-D6 hybridoma cell culture supernatant) was added, incubated for 1 hr and the cells were washed four times. A goat anti-mouse IgG-fluorescein-conjugated F(ab)$_2$ fragment was added for 1 hr, and washed four times. Labelled cells resuspended at 5×10^5 cells/ml were analysed by flow microfluorometry using an Ortho Diagnostic System Cytofluorograph (System 50-H).

Enzyme-linked immunosorbent assay (ELISA) for the analysis of membrane Ia, IL-1 and Mac-1 expression

Macrophages (2×10^5) were allowed to adhere overnight in 96-well tissue culture wells (no. 3596, Costar) at 37° in 5% CO_2 in humidified air. Non-adherent cells were aspirated and macrophage monolayers fixed with 0·5% glutaraldehyde (Sigma) in PBS. After incubation with blocking buffer (1% BSA, 100 mM glycine, 10 μg/ml human IgG, 0·02% sodium azide in PBS) at 37° for 30 min, 50 μl of monoclonal anti-I-A^d (1/100 dilution of MK-D6 hybridoma cell culture supernatant), anti-Mac-1 (1/500 dilution of M1/70.15.1.1HL hybridoma supernatant, a generous gift of Dr Timothy A. Springer, Dana-Farber Cancer Institute, Boston, MA) or 1/500 dilution of rabbit anti-human IL-1 serum (RS no. 06993, donated by Dr Richard Chizzonite, Hoffmann-La Roche Inc.) were added to each well. After 30 min of incubation at 22°, the plates were washed six times and rabbit anti-rat IgM + IgG antibodies conjugated to biotin (Zymed Laboratories Inc., South San Francisco, CA) were added to appropriate wells and incubated at 22° for 30 min. Following six washes, avidin alkaline phosphatase (Zymed) or goat anti-rabbit IgG-alkaline phosphatase (Bio-Rad, Richmond, CA) were added to appropriate wells and incubated at 22° for 30 min. After washing six times, the plates were incubated at 37° for 30–60 min, with 100 μl of substrate containing 1 mg/ml of *p*-nitrophenyl phosphate in 1 M dienthanolamine buffer, pH 10·4, containing 6 mg/ml levamisole (to inactivate endogenous alkaline phophatase). The quantity of reaction product was measured at 405 nm by means of a Titertek Multiscan® MC (Flow Laboratories Inc., McLean, VA). Denatured recombinant human IL-1 and recombinant murine IL-1, which cross-react with human IL-1, were always added as controls for membrane IL-1 assays.

Data analysis

Pair-wise *t*-tests of differences between means of various groups were performed using Tukey's studentized range test (HSD), as described in the Statistical Analysis System Computer Software (SAS Institute, Cary, NC).

RESULTS

Suppression and reconstitution of the PFC response of SRBC-primed unseparated spleen cell and non-adherent spleen cell populations by AM

Addition of resident AM, at a concentration of 10% with respect to unseparated spleen cells obtained from animals previously primed with sheep erythrocytes, caused greater than 99% suppression of the *in vitro* plaque-forming cell response (Table 1). Similar results have been reported for activated AM (Mbawuike *et al.*, 1986a). The PFC response could be reduced to background levels when macrophages were depleted from spleen cell populations by treatment with carbonyl iron, an indication of the requirement for macrophages in the generation of this response. Reconstitution of the PFC response by the non-adherent population could not be achieved by addition of up to 1×10^6 (10%) AM, an indication that AM were not only suppressive, but also inefficient in providing accessory function for the generation of the *in vitro* PFC response. Addition of 1×10^6 resident peritoneal macrophages, however, resulted in full reconstitution of this response (data not shown).

Table 1. Suppression and reconstitution of the PFC response of SRBC-primed unseparated and macrophage-depleted splenocytes by resident AM

Culture conditions*	PFC/10^6 cells†	Control response
S	204±79	—
SR	2630±300	—
SRAM	244±27	9·3‡
SN	26±4	1·0‡
SNR	177±38	6·7‡
SNRAM	225±27	8·5‡

*Immunized spleen cells (1×10^7) were cultured alone (S), co-cultured with 2×10^6 SRBC (SR) or with SRBC plus 1×10^6 resident AM (SRAM). SN represents macrophage-depleted spleen cell populations (carbonyl iron plus magnet).

†The PFC response was determined after 3 days and is expressed as PFC/10^6 cells±SEM of six determinations.

‡Values were significantly different from control (SR) cultures ($P < 0.0001$).

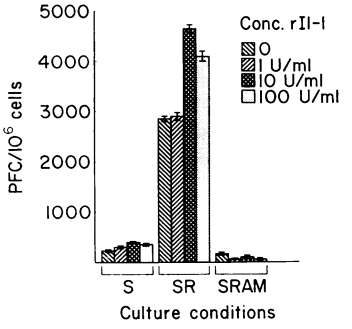

Figure 1. The effect of rIL-1 on the AM-mediated suppression of the PFC response. Conditions were essentially similar to Table 1. IL-1 was added at the beginning of culture. Values represent the mean±SEM of the PFC/10^6 cells in three different experiments. Cultures containing 10% AM were suppressed by greater than 90% ($P < 0.001$).

Effect of rIL-1 on AM-mediated suppression of the PFC response

Since IL-1 has been shown to be required for the initiation and maintenance of T-cell-dependent immune responses (Mizel, 1982), exogenous rIL-1 was added to PFC cultures normally suppressed by the presence of AM. As can be seen in Fig. 1, while addition of 10 and 100 U/ml of rIL-1 significantly stimulated ($P = 0.001$) the control (SR) responses, similar concentrations of rIL-1 could not reverse inhibition of the PFC response in cultures also containing 10% of either resident or activated AM. These results suggest that AM-mediated suppression and possibly inefficient antigen presentation could not be associated with inadequate amounts of IL-1.

Production of IL-1 in resident and activated macrophages stimulated by LPS: kinetics of response

The inability of exogenous IL-1 to reverse AM-mediated suppression, as shown in Fig. 1, did not demonstrate whether AM were capable of producing IL-1 upon immune stimulation. The results presented in Table 2 show that treatment of resident AM or BCG-activated AM with 10 μg/ml of LPS resulted in the production of significant levels of IL-1, expressed as U/mg of cell protein. As can be seen, the amount of IL-1 produced by either or BCG-activated AM was comparable to and, at times, greater than that produced by the macrophage-like cell line

Table 2. Induction of IL-1 in resident and activated macrophages by LPS: kinetics of production

		IL-1 (U/mg cell protein)†		
Cells*	LPS	48 hr	72 hr	96 hr
Resident AM	−	1·67	2·59	NT
Resident AM	+	3·81	4·92	NT
Activated AM	−	0·92	0·92	2·02
Activated AM	+	9·85	8·46	16·16
P388D1	−	0·67	1·28	1·26
P388D1	+	4·11	5·06	3·93

*Activated AM, induced by i.v. injection of BCG and collected by lavage after 5 days, and resident AM were cultured with or without 10 μg/ml LPS for 48, 72 and 96 hr and the cell-free culture supernatant was recovered.

†D10.G4.1 T cells (2×10^4 cells/well) were cultured with macrophage culture supernatants in the presence of 1 μg/ml Con A for 48 hr. [^{3}H]TdR incorporation was assessed during the last 20–24 hr of incubation. IL-1 activity is expressed as units/mg of protein as described in the text.

NT, not tested.

Table 3. Effect of cell concentration on suppression of the PFC response by activated AM and production of IL-1 in PFC cultures

Culture conditions*	PFC/10^6	% suppression	IL-1 U/ml†
S	480±65	—	0·24
SR	3880±118	—	0·51
SRAM (10% AM)	27±11	99‡	0·84
SRAM (5% AM)	307±29	99‡	NT
SRAM (2·5% AM)	1026±39	84‡	0·74
SRAM (1·25 AM)	1893±154	58‡	NT
SRAM (0·6% AM)	3533±256	10	0·65
AM	—	—	0·22
S+AM	—	—	0·29
SRBC+AM	—	—	0·22
AM+LPS (10 μg/ml)	—	—	1·14

*Conditions as described in Table 1.

†Cell-free culture supernatants were assayed for IL-1 activity on D10.G4.1 cells as described in the Materials and Methods. IL-1 activity is expressed as U/ml, as determined by the formula given in the text.

‡Values were significantly different ($P < 0.0001$, $n = 3$) from control response.

NT, not tested.

Table 4. Expression of membrane-bound IL-1 on AM

Antigen	Absorbance*
Resident AM	0·22±0·01
Activated AM	0·38±0·01
Human IL-1 control	0·55±0·04
Murine IL-1 control	1·27±0·08

*Values are mean±SEM of absorbance at 405 nm of triplicate samples in two separate determinations.

Table 5. Frequency of Ia^+ and Mac-1^+ expression in resident and BCG-activated AM populations, as measured by flow cytometry

	% of cells expressing	
Cells	I-A^d	Mac-1
Resident AM	3·9	62·3
Activated AM	34·5	56·4

Resident and BCG-activated (6 days) AM were incubated with monoclonal anti-I-A^d (MK-D6 hybridoma) or anti-Mac-1 (M1/70.15.1.1HL hybridoma) antibodies for 1 hr and washed. Goat anti-rat IgG-FITC-conjugated $F(ab')_2$ fragments were added at 0–4° for 1 hr, washed three times and the frequency of Ia^+ and Mac-1^+ cells analysed by FACS.

Table 6. Effect of pretreatment of AM with IFN-γ on AM-mediated suppression of the PFC response

Culture conditions*	IFN-γ concentration	PFC/10^6 cells	% suppression
S	—	105±38	—
SR	—	3570±261	—
SRAM	—	100±34	97†
SRAM	1×10^2 U/ml	7±7	99†
SRAM	5×10^2 U/ml	53±7	98†
SRAM	1×10^3 U/ml	47±7	98†
SRAM	2×10^3 U/ml	47±7	98†

*Conditions as described in Table 1. AM were pretreated with indicated concentrations of recombinant murine IFN-γ for 24 hr before addition of PFC cultures.

†Values were significantly different ($P<0·0001$; n 3–12) from control (SR) cultures.

P388D1 which was included for reference purposes. IL-1 was detectable in culture supernatants by 48 hr and reached maximum levels after 72–96 hr of incubation. These results indicate that AM were not defective in their ability to produce IL-1.

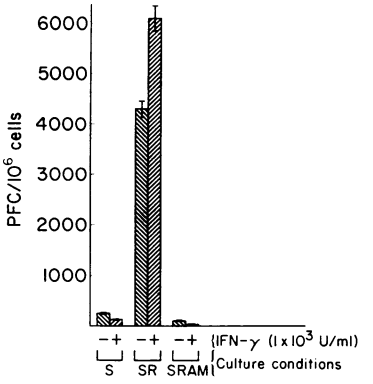

Figure 2. The effect of IFN-γ treatment on AM-mediated suppression of the PFC response. Conditions were as described in Table 1. IFN-γ (1×10^3U/ml) was added at the initiation of culture. IFN-γ stimulated the SR cultures by 47% ($P<0·001$) while 10% AM suppressed responses by 99% ($P<0·001$) in three to four different experiments.

Table 7. Effect of *in vivo* administration of IFN-γ on AM-mediated suppression of the PFC response

Culture conditions*	IFN-γ concentration	PFC/10^6 cells	% suppression
S	—	81±17	—
SR	—	2246±58	—
SRAM	—	34±13	99†
SRAM	5×10^2 U/ml	28±17	99†
SRAM	1×10^3 U/ml	6±6	99†

*Conditions similar to Table 1. IFN-γ was injected i.v. and AM collected after 5–9 days.

†Values were significantly different ($P<0·0001$, $n=3$) from control (SR) cultures.

Production of IL-1 in AM-suppressed PFC cultures

Experiments were carried out to determine if IL-1 production was inhibited in PFC cultures that were also suppressed by AM. As shown in Table 3, addition of varying concentrations of activated AM resulted in a dose-dependent suppression of the PFC response. It should be noted, however, that the AM-suppressed cultures were still able to produce significant levels of IL-1 as measured by [^{3}H]TdR incorporation in D10.G4.1 cells. It is interesting to note that when cultured alone, together with spleen cells or together with SRBC, AM produced only minimal levels of IL-1; however, they produced significant levels of IL-1 when co-cultured with both SRBC and spleen cells or when stimulated by 10 μg/ml LPS. These results further support the notion that the suppressive activity of AM can not be attributed to their inability to synthesize and secrete IL-1.

The recent description of membrane-bound IL-1 (mIL-1) that was involved in the activation of T cells by antigen-pulsed, glutaraldehyde-fixed peritoneal exudate macrophages (Kurt-Jones *et al.*, 1985) prompted us to study the expression of mIL-1

on AM. Using rabbit anti-human IL-1 which cross-reacts with murine IL-1 (Dr Richard Chizzonite, personal communication; March *et al.*, 1985) and glutaraldehyde-fixed macrophages, it was observed that resident AM expressed low levels of mIL-1, while activated AM expressed almost twice as much mIL-1 as that which could be detected on resident AM (Table 4).

I-A^d expression as determined by flow cytometry

In addition to the productin of IL-1, presentation of antigen by macrophages also requires membrane expression of I-region determinants (Kurt-Jones *et al.*, 1985). In order to determine if the defect in antigen-presenting function or the suppressive effect of AM was related to a lack of Ia antigen expression, low frequency in the number of Ia$^+$ AM or the inability of AM to express Ia upon immune stimulation, the number of Ia-bearing AM in resident and activated AM populations was determined by flow cytometry. Table 5 shows the percentage of Ia$^+$ AM labelled with monoclonal anti-I-A^d antibody. As can be seen, 4% of the resident AM were Ia positive; however, after *in vivo* activation by i.v. injection of BCG, 35% of the AM now expressed the Ia antigen. These results were confirmed in an ELISA measuring Ia antigen expression (data not shown). Expression of Mac-1 was determined in the assays as a control. Resident and activated AM were 62% and 56% Mac-1$^+$, respectively.

Effect of IFN-γ on AM-mediated suppression

Since it has been shown that IFN-γ enhances class II (Ia) antigen expression and accessory cell function (Becker, 1985), as well as inhibits the production of non-specific suppressor metabolites and prostaglandins (Boraschi *et al.*, 1984), murine recombinant IFN-γ was used in three types of studies to assess its effect on AM-mediated suppression. In the first study, activated AM were preincubated with various concentrations of IFN-γ for 24–96 hr before their addition to PFC cultures. Data for the 24-hr incubation period are presented in Table 6 (similar results were obtained for 48,72 and 96 hr) and show that IFN-γ had no effect on AM-mediated suppression. In the second study, addition of 1×10^3 U/ml of IFN-γ to the cultures for the entire incubation period of the PFC assay significantly stimulated the control (SR) response, but had no effect on AM-mediated suppression (Fig. 2). Finally, i.v. injection of IFN-γ, at doses which have been shown previously to enhance both expression of class II molecules and antigen presentation by macrophages (Nakamura *et al.*, 1984), had no signficant effect on AM-mediated suppression (Table 7). Similar experiments in which mice were injected with as much as 1×10^4 U of IFN-γ were also without effect (data not shown).

DISCUSSION

The results of this study demonstrate that murine alveolar macrophages can produce IL-1 when stimulated with endotoxin or when co-cultured with SRBC and antigen-specific lymphocytes. They also demonstrate that resident AM express relatively low levels of membrane-bound IL-1 and Ia antigen that can be enhanced by *in vivo* activation with BCG. In spite of possessing these properties which are required for antigen-specific activation of T cells (Mizel, 1982), AM are not only ineffective in presenting antigens to macrophage-depleted spleen cell populations for initiation of an *in vitro* PFC response, but they are also suppressive of this response when added to unfractionated spleen cell cultures. These results suggest the possibility of a causal relationship between the defective antigen-presenting function of AM and their immunosuppressive capabilities.

The objective of the present study was to determine if the immunosuppressive activities of alveolar macrophages could be related to their defective antigen-presenting function. Since increased IL-1 production has been associated with enhanced AM-mediated immune reactivity in sarcoidosis (Eden & Turino, 1986) as well as in experimental pulmonary granuloma in mice (Kobayashi *et al.*, 1985,), we suspected that resident murine AM might be defective in IL-1 production. However, it was observed that addition of exogenous recombinant murine IL-1 did not affect AM-mediated suppression, while at the same time it stimulated PFC responses of control (SR) cultures (Fig. 1). In addition, LPS induced the production of significant levels of IL-1 both in resident and activated AM (Table 2) and significant quantities of IL-1 were detected in AM-suppressed PFC culture supernatants (Table 3). These results show that AM were not defective in IL-1 production and suggest that suppression was not due to lack of IL-1 in PFC culture medium since higher levels of IL-1 were detected in AM-suppressed cultures compared to control (SR) cultures. Expression of mIL-1 was also not defective in AM (Table 4). It has been suggested that membrane-bound IL-1 is as essential for T-cell activation as is Ia expression in the presentation of foreign proteins and alloantigens by macrophages (Kurt-Jones *et al.*, 1985).

The frequency of Ia$^+$ cells, as determined by flow cytometry, was relatively low in the resident AM population, whereas this could be increased after *in vivo* activation with BCG. However, these Ia$^+$ AM were still significantly suppressive of the PFC response, leading to the conclusion that neither the level nor the frequency of Ia$^+$ macrophages play an important role in AM-mediated immunosuppresison.

Treatment of macrophages with IFN-γ, which has previously been shown to stimulate IL-1 production (Newton, 1985), I-A antigen expression (Nakamura *et al.*, 1984) and have an overall augmenting effect on antigen presentation (Becker, 1985), as well as resulting in decreased suppressive activity of macrophages (Boraschi *et al.*, 1984), was without effect on AM-mediated suppression. This was the case whether macrophages were pretreated with IFN-γ, if IFN-γ was added for the entire period of culture or if IFN-γ was administered *in vivo*. These results further support the conclusion that neither IL-1 nor Ia antigen expression play a significant role in AM-mediated suppresison.

Recently, however, we have observed that AM-mediated supression could be attributed to membrane-associated gangliosides, since treatment of AM with anti-ganglioside antibodies reversed their immunosuppressive effect on the PFC response (I. N. Mbawuike and H. B. Herscowitz, manuscript submitted for publication). These results are in agreement with our earlier report which implicated membrane sialoglyco-conjugates in AM-mediated suppression (Mbawuike, Luhr & Herscowitz, 1986b). Since AM exist in the lung microenvironment in a relatively activated state, physiological changes such as increased expression of membrane gangliosides may contribute to the alteration of their potential immunological functions.

The present results indicate that AM are not defective in

IL-1 production, mIL-1 expression or cell surface Ia antigen expression and, therefore, argue against a relationship between the lack of these accessory function molecules and AM-mediated suppression of the immune response.

ACKNOWLEDGMENTS

This work was supported by NIH Grant HL-25478. Appreciation is expressed to Ms Adriana Cabrales for her excellent secretarial assistance.

REFERENCES

AUBAS P., COSSO B., GODARD P., MICHEL F.B. & CLOT J. (1984) Decreased suppressor cell activity of alveolar macrophages in bronchial asthma. *Amer. Rev. Respir. Dis.* **130,** 875.

BECKER S. (1985) Effect of interferon-gamma on class II antigen expression and accessory cell function. *Immunol. Res.* **4,** 135.

BORASCHI D., CENSINI S. & TAGLIABUE A. (1984) Interferon-γ reduces macrophage suppressive activity by inhibiting prostaglandin E_2 release and inducing interleukin-1 production. *J. Immunol.* **133,** 764.

COWING C., SCHWARTZ B.D. & DICKLER H.B. (1978) Macrophage Ia antigens. I. Macrophage populations differ in their expression of Ia antigens. *J. Immunol.* **120,** 378.

EDEN E. & TURINO G.M. (1986) Interleukin-1 secretion by human alveolar macrophages stimulated with endotoxin is augmented by recombinant immune (gamma) interferon. *Amer. Rev. Respir. Dis.* **133,** 455.

FOURNIER M., TOUATY E., GERBER F., MEDRANO G. & PARIENTE R. (1984) Suppression of bronchoalveolar antibody secretion by alveolar macrophages: an *in vitro* study in the rabbit. *Bull. Eur. Physiolpathol. Respir.* **20,** 229.

FUJIWARA H., KLEINHERZ M.E., WALLIS R.S. & ELLNER J.J. (1986) Increased interleukin-1 production and monocyte suppressor cell activity associated with human tuberculosis. *Amer. Rev. Respir. Dis.* **133,** 73.

GORENBERG D.J. & DANIELE R.P. (1978) The alveolar macrophage: its capacity to act as an accessory cell in mitogen-stimulated proliferation of guinea pig lymphocytes, *Cell. Immunol.* **36,** 115.

GRAY W.P. & GOEDDEL D.V. (1983) Cloning and expression of murine interferon cDNA. *Proc. natl. Acad. Sci. U.S.A.* **80,** 5842.

HERSCOWITZ H.B. (1985) In defense of the lung: paradoxical role of the pulmonary alveolar macrophage. *Ann. Allergy*, **55,** 634.

HOCKING W.G. & GOLDE D.W. (1979) The pulmonary alveolar macrophage. *N. Engl. J. Med.* **301,** 580.

HOLT P.G. (1979) Alveolar macrophages. II. Inhibition of lymphocyte proliferation by purified macrophages from rat lung. *Immunology*, **37,** 429.

KALTREIDER H.B. (1982) Alveolar macrophages: enhancers or suppressors of pulmonary immune reactivity. *Chest*, **82,** 261.

KAYE J., GILLIS S., MIZEL S.B., SHEVACH E.M., MALEK T.R., DINARELLO C.A., LACHMAN L.B. & JANEWAY C.A. (1984) Growth of a cloned helper T cell line induced by a monoclonal antibody specific for the antigen receptor: interleukin 1 is required for the expression of receptors for interleukin 2. *J. Immunol.* **133,** 1339.

KOBAYASHI K., ALLRED C., COHEN S. & YOSHIDA T. (1985) Role of interleukin-1 in experimental granulomas in mice. *J. Immunol.* **134,** 358.

KORETZBY G.A., ELIAS J.A., KAY S.L., ROSSMAN M.D., NOWELL P.D. & DANIELE R.P. (1983) Spontaneous production of interleukin-1 by human alveolar macrophages. *Clin. Immunol. Immunopathol.* **29,** 443.

KURT-JONES E.A., VIRGIN H.W. & UNANUE E.R. (1985) Relationship between macrophage Ia and membrane IL-1 expression to antigen presentation. *J. Immunol.* **135,** 3652.

LAUGHTER A.H., MARTIN R.R. & TWOMEY J.J. Lymphoproliferative responses to antigens mediated by human pulmonary alveolar macrophages. *J. Lab. Clin. Med.* **89,** 1326.

LIPSCOMB M.F., TOEWS G.B., LYONS C.R. & UHR J.W. (1981) Antigen presentation by guinea pig macrophages. *J. Immunol.* **126,** 286.

LOMEDICO P.T., GUBLER U., HELLMAN C.P., DUKOVICH M., GIRE J.G., PAN Y.-C.E., COLLIER K., SEMIONOW R., CHUA A.O. & MIZEL S.B. (1984) Cloning and expression of murine interleukin-1 cDNA in *Escherichia coli. Nature (Lond.)*, **312,** 458.

LOWRY O.H., ROSEBROUGH N.J., FARR L.A. & RANDALL R.J. (1951) Protein measurement with Folin phenol reagent. *J. biol. Chem.* **193,** 265.

MCCARRON R.M., GOROFF D.K., LUHR J.E., MURPHY M.A. & HERSCOWITZ H.B. (1984) Methods for collection of peritoneal and alveolar macrophages. *Meth. Enzymology*, **108** (Part G), 274.

MARCH C.J., MOSLEY B., LARSEN A., CERRETI D.P., BRAEDT G., PRICE V., GILLIS S., HENNEY C.S., KRONHEIM S.R., GRABSTEIN K., CONLON P.J., HOPP T.P. & COSMAN D. (1985) Cloning, sequence and expression of two distinct human interleukin-1 complementary DNAs. *Nature (Lond.)*, **315,** 641.

MBAWUIKE I.N., LUHR J.E. & HERSCOWITZ H.B. (1986a) Enhanced recovery of murine alveolar macrophages: morphological and functional characteristics following intravenous injection of heat-killed *Mycobacterium bovis* BCG. *Infect. Immun.* **51,** 483.

MBAWUIKE I.N., LUHR J.E. & HERSCOWITZ H.B. (1986b) Reversal of murine alveolar macrophage-mediated suppression of the plaque-forming cell response by sodium periodate. *Cell Immunol.* **99,** 300.

MIZEL S.B. (1981) Production and quantitation of lymphocyte-activating factor (interleukin-1). In: *Manual of Macrophage Methodology* (eds H.B. Herscowitz, H.T. Holden J.A., Bellanti and A. Ghaffar), p. 407. Marcel Dekker Inc., New York.

MIZEL S.B. (1982) Interleukin-1 and T-cell activation. *Immunol. Rev.* **63,** 51.

NAKAMURA M., MANSER T., PEARSON G.D.N., DALEY M.J. & GEFTER M.L. (1984) Effect of IFN-γ on the immune response *in vivo* and on gene expression *in vitro*. Nature *(Lond.)*, **307,** 381.

NEWTON R.C. (1985) Effect of interferon on the induction of human monocyte secretion of interleukin-1 activity. *Immunology*, **56,** 441.

PENNLINE K.J., CONRAD R.E., GERBER H.R. & HERSCOWITZ H.B. (1979) Suppressive effect of alveolar macrophages in the *in vitro* immune response of rabbit lymphocytes. *J. Reticuloendothel. Soc.* **25,** 495.

SHELLITO J., CALDWELL J.L. & KALTREIDER H.B. (1983) Immune functions of murine alveolar macrophages: binding of lymphocytes and support of lymphoproliferation. *Exp. Lung Res.* **4,** 93.

ULLRICH S.E. & HERSCOWITZ H.B. (1980) Immunological functions of alveolar macrophages: interaction with a soluble protein antigen and the immunogenicity of alveolar macrophage-associated antigen. *J. Reticuloendothel. Soc.* **28,** 11.

VENET A., HANCE A.J., SALTINI C., ROBINSON B.W.S. & CRYSTAL R.G. (1985) Enhanced alveolar macrophage-mediated antigen-induced T-lymphocyte proliferation in sarcoidosis. *J. clin. Invest.* **75,** 293.

WEINBERG D.S. & UNANUE E.R. (1981) Antigen-presenting function of alveolar macrophages: uptake and presentation of *Listeria monocytogenes*. *J. Immunol.* **126,** 794.

WONG D.M. (1981) Ingestion of carbonyl iron. In: *Manual of Macrophage Methodology* (eds H.B. Herscowitz, H.T. Holden, J.A. Bellanti and A. Ghaffar), p. 105. Marcel Dekker, Inc., New York.