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Potentiation of Interferon-Mediated Inhibition of *Chlamydia* Infection by Interleukin-1 in Human Macrophage Cultures

JOSEPH M. CARLIN* AND JENNIFER B. WELLER

Department of Microbiology, Miami University, Oxford, Ohio 45056

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One mechanism by which interferons (IFNs) can inhibit chlamydial infection is by the induction of the enzyme indoleamine 2,3-dioxygenase (IDO), which restricts the availability of tryptophan, which is required for chlamydial growth. Other immunomodulating agents, including interleukin-1 (IL-1), can interact synergistically with IFNs, resulting in increased IDO activity in macrophages. The objectives of this study were to establish that IL-1 can enhance IFN-mediated inhibition of chlamydial growth by increasing the amount of IDO activity induced by IFNs and to identify immunomodulatory agents in culture supernatants from chlamydia-infected macrophages that interact synergistically with IFNs in restricting chlamydial growth. Monocyte-derived macrophages were treated with IL-1 combined with gamma IFN (IFN-γ) or IFN-β. The ability of treated cells to support the growth of *Chlamydia psittaci* was directly related to the amount of IDO activity induced; as IDO activity increased, so did inhibition of chlamydial growth. Furthermore, concentrations of IFNs were identified at which little IDO activity was induced and chlamydial growth was permitted yet which in the presence of IL-1 resulted in increased IDO activity and restriction of chlamydial growth. The addition of exogenous tryptophan reversed the effect of combined IFN and IL-1 treatment, indicating that IDO activity induced by combined cytokine treatment was responsible for chlamydial inhibition. Supernatants from chlamydia-infected macrophages were capable of potentiating IDO induction by IFN-γ and of restricting the growth of *C. psittaci*. Antibody to IL-1β neutralized the potentiating effects of supernatants from chlamydia-infected cells on both IDO induction and chlamydial inhibition. Thus, IL-1 produced in response to chlamydial infection may contribute to the elimination of the infection.

Chlamydiae are obligate intracellular pathogens of major public health concern; among the diseases they cause are trachoma, pneumonia, and sexually transmitted diseases. Because of their ability to invade and replicate within host cells, the chlamydiae are relatively isolated from humoral immune responses; however, they, as well as other intracellular pathogens, remain susceptible to the effects of cytokines produced during infection. Interferons (IFNs) have been implicated in the restriction of a variety of facultative and obligate intracellular nonviral pathogens, including *Rickettsia prowazekii* (33), *Leishmania* spp. (13, 20), *Brucella abortus* (17), *Toxoplasma gondii* (22, 25), and *Chlamydia* spp. (26, 30). Several antimicrobial mechanisms in which host cell physiology is modulated by IFNs have been demonstrated. Among the mechanisms shown to inhibit growth of intracellular pathogens are triggering of the oxidative burst, resulting in the release of reactive oxygen metabolites (22); generation of reactive nitrogen metabolites by induction of nitric oxide synthase (1, 13); and induction of indoleamine 2,3-dioxygenase (IDO). IDO catalyzes oxidative cleavage of the indole ring of the essential amino acid L-tryptophan to *N*-formylkynurenine (31), limiting the availability of tryptophan for pathogen protein synthesis. The induction of IDO is not restricted to gamma IFN (IFN-γ); type I IFNs (IFN-α and IFN-β) also induce IDO activity in mononuclear phagocytes (10, 11). By inducing IDO activity, both type I and type II IFNs can restrict the growth of *T. gondii* (25, 29) and *Chlamydia psittaci* (9, 19) in vitro.

IFN-induced mechanisms of resistance to intracellular pathogens can be influenced by the combination of IFNs with other cytokines and immunomodulatory agents. For example, IFN-γ in combination with lipopolysaccharide (LPS), interleukin-1 (IL-1), or tumor necrosis factor alpha works synergistically to induce nitric oxide synthase (14). Furthermore, IL-1, LPS, and muramyl tripeptide independently potentiate IDO induction by both type I and type II IFNs in human macrophages (16). Infection with chlamydiae can result in the production of cytokines that can induce IDO activity synergistically. Human macrophages infected with *C. psittaci* produce type I IFNs as well as IL-1β (24). Inasmuch as both IDO-inducing and -potentiating agents are produced in response to infection with chlamydiae and since chlamydiae are susceptible to IDO-mediated tryptophan depletion, the present study was undertaken. The objectives were twofold: to determine if IL-1 can enhance the antichlamydial effect of IFN treatment by increasing the amount of IDO activity induced by IFNs and to determine the effect of cytokines present in culture supernatants from chlamydia-infected macrophages on IDO induction and chlamydial growth.

MATERIALS AND METHODS

Cytokines and reagents. Human recombinant IFN-β (specific activity = 10^8 U/mg of protein; <0.5 ng of LPS per mg) was obtained from Triton Biosciences (Berlex Laboratories, Alameda, Calif.). Human recombinant IFN-γ (specific activity = 1.8×10^7 U/mg of protein; <0.4 ng of LPS per mg) was a gift from Biogen Corp. (Cambridge, Mass.). Recombinant human IL-1α (specific activity = 3×10^8 U/mg of protein; <0.5 ng of LPS per mg) was generously provided by Peter Lomedico of Hoffman-LaRoche (Nutley, N.J.). Recombinant human IL-1β (specific activity ≅ 3×10^8 U/mg of protein; <0.1 ng of LPS per μg) was purchased from R & D Systems (Minneapolis, Minn.). Normal goat immunoglobulin G (IgG) and goat anti-human IL-1β-neutralizing IgG (0.05 to 0.1 μg of the antibody neutralizes 50% of the biological activity of 50 pg of IL-1β per ml) were purchased from R & D Systems. Fetal bovine serum (FBS) was obtained from HyClone (Logan, Utah). Streptomycin sulfate, gentamicin sulfate, RPMI 1640, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), Hanks' balanced salt solution (HBSS), and Histopaque 1077 were purchased from Sigma Chemical Co. (St. Louis, Mo.). Radiolabeled [5-^{3}H]tryptophan (specific activity = 20 Ci/mmol) was purchased from New England Nuclear (Boston, Mass.).

* Corresponding author. Electronic mail address: CarlinJM@MUOhio.edu.

Cell and chlamydia cultivation. Monocyte-derived macrophages were cultivated as described previously (11). Briefly, peripheral blood mononuclear cells obtained from adult donors were isolated from Histopaque 1077 density gradients and resuspended in RPMI 1640 supplemented with 10% FBS, 25 μM HEPES buffer, 10 μg of gentamicin sulfate per ml, and 100 μg of streptomycin sulfate per ml (complete medium). Cells were plated on 12-mm-diameter acid-washed glass coverslips at 10^6 cells per coverslip and incubated for 1 h to allow monocytes to attach. Each coverslip was rinsed three times with warm HBSS to remove nonadherent cells. Monocytes (approximately 2×10^5 per coverslip) were cultured in 24-well plates at 37°C in 5% CO_2 in air. The culture medium was replaced on day 7, and cultivation was continued for a total of 10 to 14 days to allow maturation of the monocytes to monocyte-derived macrophages. The recovery of viable monocyte-derived macrophages was routinely 1.4×10^5 cells per coverslip.

Stocks of *C. psittaci* 6BC were propagated in confluent L929 cell monolayers in M199 medium supplemented with 10% FBS, 10 μg of gentamicin sulfate per ml, 100 μg of streptomycin sulfate per ml, and 2 μg of cycloheximide per ml at 37°C in 5% CO_2 in air (4, 7). The cells were infected with 10 times the 50% infectious dose of 6BC, incubated for 2 days, and removed from the tissue culture plate by trypsin. After infected cells were disrupted by sonication, 6BC elementary bodies were partially purified by differential centrifugation, resuspended in phosphate-buffered saline (PBS) containing 0.2 M sucrose and 2% FBS, distributed into cryovials, and frozen at −70°C until needed. Infectivity titers were quantified by the 50% infectious dose method in L cells (15).

IDO induction and potentiation. Duplicate sets of 24-well plates containing monocyte-derived macrophages were treated with combinations of IFNs and IL-1α. One set of plates was used to determine the effect of cytokine combinations on chlamydial growth; the other set was used to determine the amount of IDO activity at the time of infection. In some experiments, the IFN concentration was varied, and a constant amount of IL-1α was added. Medium was removed from triplicate samples per treatment group per set and replaced with fresh complete medium containing increasing concentrations of either IFN-β or IFN-γ or with complete medium alone. For each treatment, some wells also received either IL-1α or complete medium alone. In other experiments, cells were treated with varied amounts of IL-1α, while the IFN concentration was held constant. The medium was replaced with complete medium containing increasing amounts of IL-1α or complete medium alone. For each treatment, some wells also received either IFN-β, IFN-γ, or medium alone. The final medium volume was 0.5 ml. Each set of plates then was incubated for 48 h at 37°C in 5% CO_2, after which time either IDO activity was determined or the plates were infected with *C. psittaci.*

For determination of IDO activity, the medium in each well was replaced with 0.4 ml of HBSS containing 25 μM unlabeled tryptophan carrier and 1 μCi of [5-^{3}H]tryptophan per ml. Some wells received radiolabeled medium in the absence of cells to determine the amount of nonspecific tryptophan decyclization that occurred. Plates were incubated for an additional 4 h (6), and the supernatants were collected and frozen at −20°C until analyzed for tryptophan metabolites.

In plates in which growth of *C. psittaci* was to be determined, sufficient *C. psittaci* was added to infect approximately 30% of the cells. The plates were incubated for 28 h at 37°C in an atmosphere of 5% CO_2 in air, at which time coverslips from the plates were washed with PBS, fixed with methanol, stained with 5% Giemsa stain, and mounted on slides. The percentage of *C. psittaci*-infected macrophages was determined by light microscopy (5).

Effect of exogenous tryptophan on *C. psittaci* replication. Monocyte-derived macrophages were treated with combined IFN-γ and IL-1α, IFN-β and IL-1α, or IL-1α alone and infected with *C. psittaci* as described above. Two hours after the addition of infectious chlamydiae, either systematically varied concentrations of tryptophan in HBSS or HBSS alone was added to the culture medium. After an additional 26 h, coverslips were fixed and stained, and the amount of chlamydial growth was determined.

Assay of IDO activity. IDO activity was determined by assaying the conversion of [5-^{3}H]tryptophan to its decyclized metabolites, L-*N*-formylkynurenine and kynurenine, by a previously described reversed-phase high-pressure liquid chromatography (HPLC) technique (34). Fifty-microliter aliquots of culture supernatants were injected into a μBondapak C_{18} column (30 cm by 3.9 mm [inner diameter]; Millipore Waters, Milford, Mass.) and eluted with 10% methanol in 1 mM KH_2PO_4 buffer (pH 4.0) at a flow rate of 1.6 ml/min. Tryptophan and tryptophan metabolites were quantified by flowthrough scintillation spectroscopy with a fully automated HPLC (Isco, Lincoln, Nebr.) equipped with a radioisotope detector (Radiomatic Instruments, Tampa, Fla.). Enzymatic activity was expressed as a percentage of the specific tryptophan metabolism that was calculated with the equation % specific catabolism = $[(cpm_{test} - cpm_{spontaneous})/(cpm_{total} - cpm_{spontaneous})] \times 100$, where cpm_{test} is the counts per minute present in the L-*N*-formylkynurenine and kynurenine fractions, $cpm_{spontaneous}$ is the counts per minute resulting from nonspecific breakdown of tryptophan to L-*N*-formylkynurenine and kynurenine, and cpm_{total} is the total counts per minute from all fractions. Statistical significance was determined by the *t* test for independent samples.

Antibody neutralization of IDO potentiation. Supernatants from chlamydia-infected macrophage cultures (conditioned medium) were prepared as described previously (24). Briefly, monocyte-derived macrophages on coverslips were infected with 6BC (2.5 50% infectious doses) in a total well volume of 500 μl. After 24 h, supernatants were collected from infected cells, filter sterilized (0.2-μm-pore-size filters), and supplemented with tryptophan to compensate for tryptophan depletion (final concentration of 25 mM tryptophan). Conditioned medium was previously shown by enzyme-linked immunosorbent assay (ELISA) to contain IL-1β at a concentration of 20 ± 5 ng/ml (24).

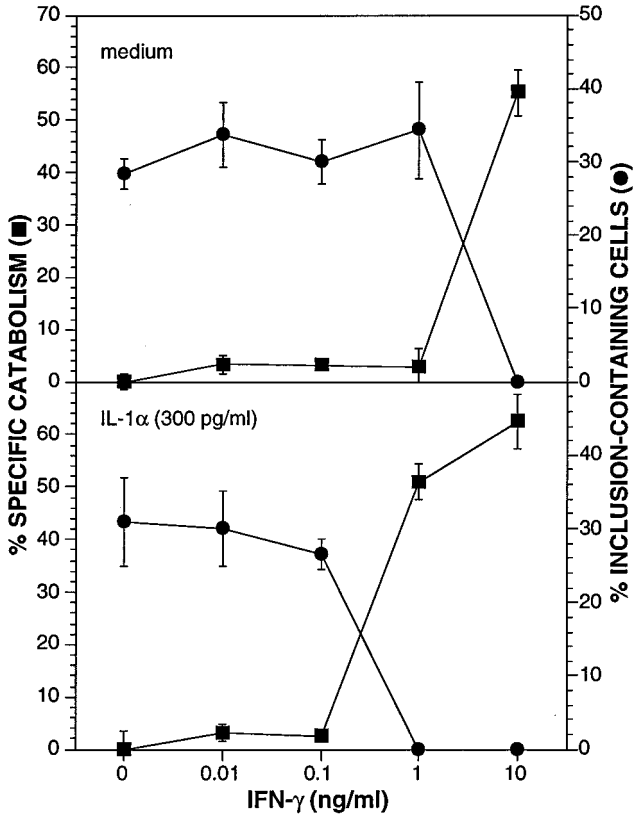

FIG. 1. Effect of IFN-γ concentration on IDO activity and inhibition of chlamydial growth. Monocyte-derived macrophages were treated with IFN-γ (top) or IFN-γ combined with IL-1α (bottom) for 48 h before assessment of IDO activity and infection with *C. psittaci*. The growth of chlamydiae is expressed as the percentage of inclusion-containing cells ± standard deviation, and IDO activity is represented as the percent specific catabolism ± standard deviation. The results from one of three similar experiments are presented.

Triplicate coverslips of macrophages were treated with either medium alone or IFN-γ (1 ng/ml) in duplicate sets. Coverslips also received a 1:100 dilution of conditioned medium, IL-1β (100 pg/ml), IL-1α (100 pg/ml), or medium alone. To determine whether potentiation of IDO activity and of chlamydial inhibition by conditioned medium was due to the presence of IL-1β produced by macrophages in response to infection, potentiators (conditioned medium, IL-1β, or IL-1α) were combined with goat anti-human IL-1β-neutralizing IgG at 2 μg/ml (10 50% neutralizing doses for IL-1β at 100 pg/ml) or normal goat IgG (2 μg/ml) for 1 h at room temperature before addition to macrophage cultures. Cells were cultivated for 48 h, and both IDO activity and chlamydial growth were assessed as described above.

RESULTS

Potentiation of IDO activity and inhibition of *C. psittaci*. To establish the baseline effect of IFN concentration on IDO activity and chlamydial inhibition, 10-fold dilutions of IFN were used to treat cells before assessment of IDO activity and infection with *C. psittaci*. Concentrations of IFN-γ of ≤1 ng/ml were ineffective in inducing IDO activity and had no effect on chlamydial growth (Fig. 1, top). However, IFN-γ at 10 ng/ml induced significant IDO activity ($P \leq 0.001$) and completely inhibited chlamydial replication ($P \leq 0.001$) compared with cells receiving no IFN-γ. When IL-1α was added to IFN-γ-

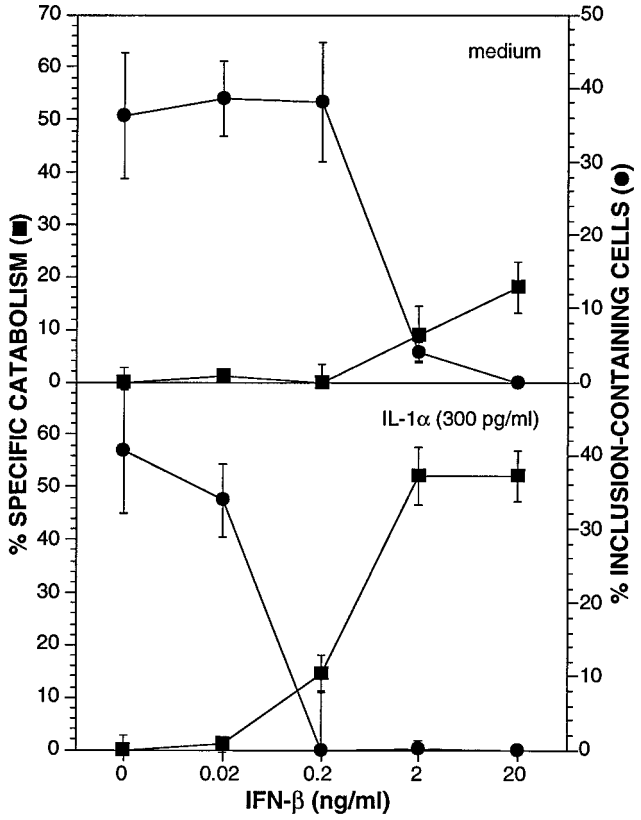

FIG. 2. Effect of IFN-β concentration on IDO activity and inhibition of chlamydial growth. Monocyte-derived macrophages were treated with IFN-β (top) or IFN-β combined with IL-1α (bottom) for 48 h before assessment of IDO activity and infection with *C. psittaci*. The growth of chlamydiae is expressed as the percentage of inclusion-containing cells ± standard deviation, and IDO activity is represented as the percent specific catabolism ± standard deviation. The results from one of three similar experiments are presented.

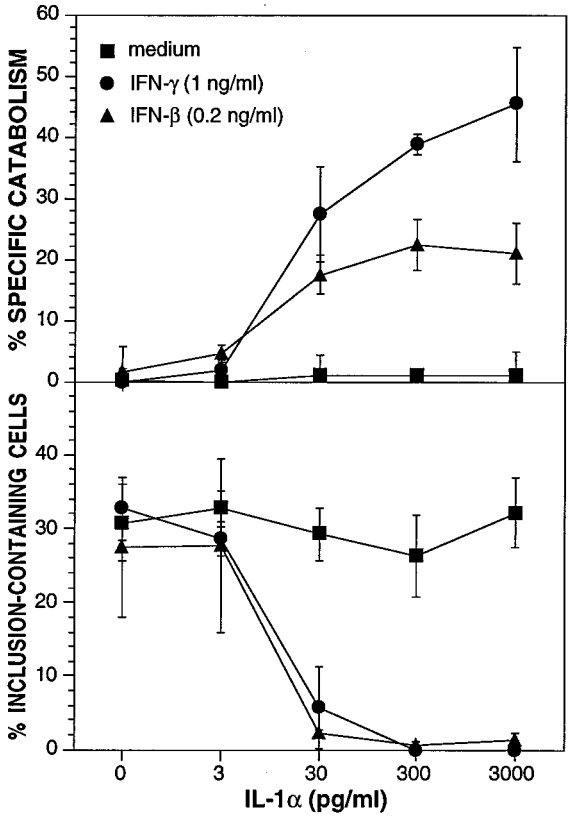

FIG. 3. Effect of IL-1α concentration on IFN-mediated induction of IDO activity and inhibition of chlamydial growth. Monocyte-derived macrophages were treated with IFN-γ, IFN-β, or medium alone for 48 h before assessment of IDO activity and infection with *C. psittaci*. IDO activity is expressed as the percent specific catabolism ± standard deviation (top), and the growth of chlamydiae is represented as the percentage of inclusion-containing cells ± standard deviation (bottom). The results from one of three similar experiments are presented.

treated macrophage cultures (Fig. 1, bottom), significant IDO activity and inhibition of *C. psittaci* growth were induced at a 10-fold-lower IFN-γ concentration ($P \leq 0.001$). Furthermore, *C. psittaci* growth was inhibited at an IFN-γ concentration 10 times lower than that observed in the absence of IL-1α ($P \leq 0.001$). Experiments with IFN-β yielded similar results. Concentrations of IFN-β of ≤0.2 ng/ml did not induce IDO activity, and chlamydial growth was no different from that observed in cells cultivated in medium alone (Fig. 2, top). At an IFN-β concentration of 2 ng/ml, significant inhibition of chlamydial growth ($P \leq 0.001$) occurred; at 20 ng/ml, complete inhibition of growth was observed. When IL-1α was combined with IFN-β and used to treat macrophages, IDO activity was induced and chlamydial growth was inhibited at lower IFN-β concentrations (Fig. 2, bottom). In the presence of IL-1α, IFN-β at 0.2 ng/ml induced significant IDO activity ($P \leq 0.03$) and completely blocked inclusion development. Furthermore, only in the presence of IL-1α did IFN-β at higher concentrations induce IDO activity that was similar in amount to the maximum activities induced by IFN-γ treatment (Fig. 1).

Additional experiments were performed in which the IFN concentration was held constant while the concentration of IL-1α was varied. IFN concentrations of 0.2 ng/ml for IFN-β and 1 ng/ml for IFN-γ were used in these studies, since these concentrations were observed to stimulate almost no IDO activity in the absence of IL-1α and because in the presence of IL-1α they resulted in IDO activity sufficient to inhibit chlamydial growth. Although IL-1α alone at concentrations of up to 3 ng/ml had no effect on either IDO activity or chlamydial inhibition (Fig. 3), IL-1α at 30 pg/ml potentiated IDO induction by IFN-γ and IFN-β ($P \leq 0.001$) and enhanced the antichlamydial activity of IFN-β ($P \leq 0.02$) and IFN-γ ($P \leq 0.001$). Raising the IL-1α concentration to 300 pg/ml resulted in maximal chlamydial inhibition and potentiation of IDO induction by either IFN-γ or IFN-β. When the experiments were repeated with IL-1β to enhance the effect of IFN treatment, nearly identical results were obtained (data not shown).

Effect of tryptophan on reversal of potentiated inhibition of chlamydial growth. In previous studies, the addition of supraphysiologic concentrations of L-tryptophan to the culture medium reversed the effect of induced IDO activity on inhibition of intracellular pathogens (6, 9). To confirm that the mechanism by which combined IFN and IL-1 treatment inhibited *C. psittaci* also was mediated by IDO, various amounts of tryptophan were added 2 h after infection to macrophages treated with IFN-γ combined with IL-1α, with IFN-β combined with IL-1α, or with IL-1α alone. The percentage of inclusion-containing cells was determined 26 h later (Fig. 4). In IFN-treated cells that received no exogenous tryptophan, inclusion development was completely inhibited. However, the addition of tryptophan reversed the inhibitory effect of combined IFN and IL-1α treatment in a concentration-dependent manner. Al-

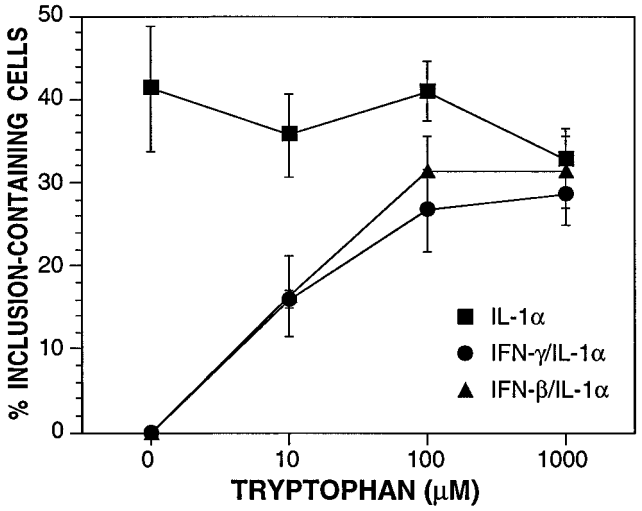

FIG. 4. Effect of tryptophan concentration on inhibition of chlamydial growth. Monocyte-derived macrophages were treated with IL-1α (300 pg/ml) combined with IFN-γ (1 ng/ml), IFN-β (2 ng/ml), or medium alone for 48 h before infection with *C. psittaci* and assessment of chlamydial growth. The growth of chlamydiae is expressed as the percentage of inclusion-containing cells ± standard deviation. The results from one of three similar experiments are presented.

though tryptophan concentrations of 10 and 100 μM partially reversed the effect, chlamydial growth was still significantly inhibited in IFN-γ and IFN-β-treated cells ($P \leq 0.02$ and $P \leq 0.04$, respectively); only at 1 mM tryptophan was chlamydial inhibition completely reversed.

Potentiation of chlamydial growth inhibition by supernatants from chlamydia-infected cultures. Since prior studies have shown that mononuclear phagocytes produce IL-1β in response to chlamydial infection (24, 27), experiments were performed to determine if IL-1β-containing supernatants from chlamydia-infected macrophages (conditioned medium) were capable of potentiating IDO induction and enhancing the antichlamydial effect of IFN-γ treatment. Parallel sets of macrophage cultures were treated with IFN-γ combined with conditioned medium, with IL-1α, with IL-1β, or with medium alone; both IDO activity and chlamydial growth were assessed in treated cells (Fig. 5). Significant potentiation of IDO activity was observed in IFN-induced cells treated with conditioned medium, IL-1β, or IL-1α ($P \leq 0.001$). In addition, inhibition of chlamydial growth was significantly enhanced in the presence of potentiators ($P \leq 0.002$). To determine if the potentiating activity in conditioned medium was mediated by IL-1β, potentiators were pretreated with neutralizing antibody to IL-1β or isotype-matched normal immunoglobulin. Anti-IL-1β significantly blocked IDO potentiation by conditioned medium ($P \leq 0.001$) and by IL-1β ($P \leq 0.001$) and significantly reversed the effect of conditioned medium ($P \leq 0.002$) and IL-1β ($P \leq 0.001$) on enhancement of chlamydial inhibition. The lack of effects of anti-IL-1β antibody on IL-1α-mediated enhancement of both chlamydial inhibition and IDO potentiation confirmed the specificity of the antibody against IL-1β.

DISCUSSION

The characterization of the roles of specific cytokines generated during an immune response has been impeded by the complexity of the cytokine system, not only because of the pleiotropic effects of individual cytokines but also because of

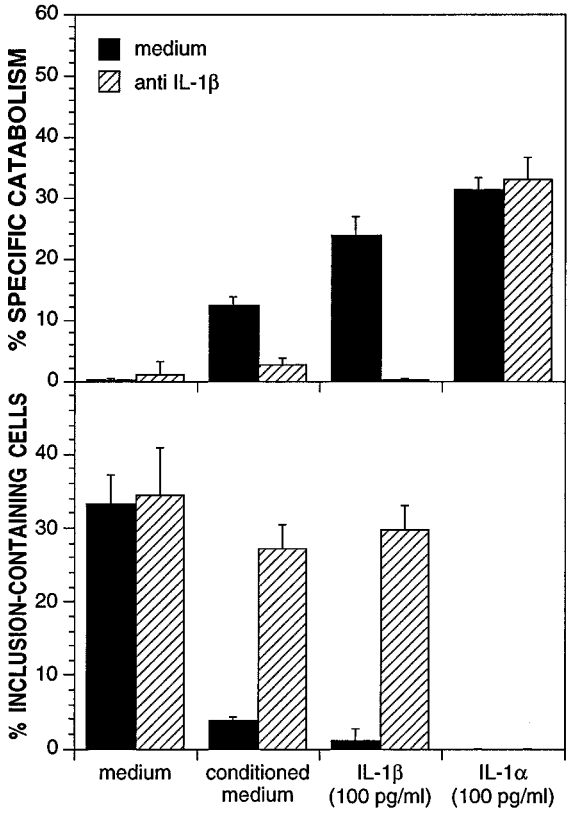

FIG. 5. Role of IL-1β in potentiation of IDO activity and enhancement of chlamydial inhibition by culture supernatants from chlamydia-infected macrophages (conditioned medium). Monocyte-derived macrophages were treated with IFN-γ (1 ng/ml) combined with conditioned medium (1:100 dilution), IL-1β (100 pg/ml), or IL-1α (100 pg/ml) for 48 h before assessment of IDO activity and infection with *C. psittaci*. Cultures also received 10 50% neutralizing doses of antibody to IL-1β (anti IL-1β) or isotype-matched immunoglobulin (medium). IDO activity is expressed as the percent specific catabolism ± standard deviation (top), and the growth of chlamydiae is represented as the percentage of inclusion-containing cells ± standard deviation (bottom). The results from one of three similar experiments are presented.

antagonistic and synergistic effects of cytokine combinations. Examples of cytokine antagonism include the capacities of transforming growth factor β, IL-4, and IL-10 to suppress the release of nitric oxide by IFN-γ-activated macrophages (23) and of IL-4 to inhibit the induction of IDO activity by IFN-γ in human monocytes (21). Synergistic interactions between cytokines have been shown to contribute to the inhibition of pathogens; the combination of IFN-γ with tumor necrosis factor alpha or IL-1 leads to the elimination of *Francisella tularensis* in mice (14), and IFN-β in combination with LPS restricts the growth of *C. psittaci* and *T. gondii* in human macrophages (9, 29). In this study, it has been demonstrated that a synergistic interaction between IL-1 and IFNs enhances IDO induction, resulting in inhibition of *C. psittaci* growth. While treatment of macrophages with relatively low concentrations of either IFN-γ or IFN-β induces no IDO activity and permits replication of chlamydiae, the addition of IL-1 to these IFN-treated macrophages induces significant IDO activity and renders these cells capable of suppressing chlamydial growth. That the mechanism of chlamydial inhibition is due to IDO activity was confirmed by experiments in which tryptophan was shown to reverse the inhibitory effect of combined IFN and IL-1 treatment in a dose-dependent manner.

Previous studies have shown that infection of human macrophages with *C. psittaci* results in production of type I IFNs and induction of IDO activity (24). Furthermore, IL-1β also is secreted by mononuclear phagocytes in response to infection with *C. psittaci* (24) and *Chlamydia trachomatis* (27). In this study, we have shown that IL-1β produced by *C. psittaci*-infected macrophages is capable of contributing to IDO induction. Although chlamydia-conditioned medium diluted 1:100 alone was incapable of inducing IDO activity in uninfected cells, conditioned medium potentiated IDO induction in macrophages treated with IFN-γ and enhanced the antichlamydial effect of IFN treatment. Neutralizing antibody to IL-1β blocked the synergy between IFN-γ and conditioned medium, suggesting that IL-1β, and not chlamydial LPS (8) present in the conditioned medium, was the agent responsible for enhancing the effect of IFN treatment. These experiments revealed not only that IDO is induced by cytokines generated by chlamydia-infected macrophages but also that IDO induction is potentiated by the cytokines produced. Although IL-1α was also detected by ELISA in conditioned medium (data not shown), the IL-1β concentration was greater than 20 times that of IL-1α. At a 1:100 dilution, the concentration of IL-1α remaining in conditioned medium was insufficient to affect either IDO potentiation or chlamydial inhibition, yet sufficient IL-1β remained to enhance both activities. Since the dependencies of IDO potentiation and antichlamydial effects on IL-1α and IL-1β concentrations are similar, it appears that IL-1β is the major contributor to the effects of conditioned medium and that the contribution of IL-1α is minor in comparison.

The effect of IL-1 production in response to chlamydial infection in vivo may be that of a double-edged sword. The results presented here demonstrate that IL-1 may contribute to the elimination of chlamydial infection by potentiating the effect of IFNs. On the other hand, it has been suggested that IL-1 may contribute to the inflammation and fibrosis associated with trachoma and chlamydial tubal infertility (18, 27) because of its effects on the production of collagen (32) and collagenase (12), as well as on stimulation of fibroblast proliferation (28). It is possible that IL-1 also contributes to the establishment of persistent infection. A previous study has demonstrated that at low concentrations of IFN-γ, persistent infection is established rather than complete inhibition of growth occurring (3). Since analyses of chlamydial antigen profiles from cells cultivated in tryptophan-deficient medium were consistent with profiles from persistently infected cells, it has been suggested that nutrient depletion mediated by IFN-induced IDO leads to the persistent state (2). If IDO induction contributes to the establishment of persistent infection, the presence of IL-1 and its ability to potentiate IDO induction by IFNs may permit persistence to be established under conditions of lower IFN concentration. However, one must interpret the potential relationship between IDO enhancement by IL-1 and development of persistent infection with caution. With the exception of the LGV biovar, *C. trachomatis* grows poorly within macrophages, and enhancement of IDO activity by IL-1 has thus far been demonstrated only in macrophages.

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