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Antiparasitic and Antiproliferative Effects of Indoleamine 2,3-Dioxygenase Enzyme Expression in Human Fibroblasts

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Studies were carried out to evaluate the proposed role of indoleamine 2,3-dioxygenase (INDO) induction in the antimicrobial and antiproliferative effects of gamma interferon (IFN-γ) in human fibroblasts. The INDO cDNA coding region was cloned in the pMEP4 expression vector, containing the metallothionein (MTII) promoter in the sense (+ve) or the antisense (−ve) orientation. Human fibroblasts (GM637) stably transfected with the sense construct expressed INDO activity after treatment with $CdCl_2$ or $ZnSO_4$, but cells transfected with the antisense construct did not. The growth of *Chlamydia psittaci* was strongly inhibited in INDO +ve cells but not in INDO −ve cells after treatment with Cd^{2+} or Zn^{2+}. The inhibition correlated with the level of INDO activity induced and could be reversed by the addition of excess tryptophan to the medium. The growth of *Toxoplasma gondii* was also strongly inhibited in INDO +ve cells but not in INDO −ve cells after treatment with Cd^{2+}. Expression of Cd^{2+}-induced INDO activity also inhibited thymidine incorporation and led to cytotoxicity in INDO +ve cells but not in INDO −ve cells. Thus, the induction of INDO activity by IFN-γ may be an important factor in the antimicrobial and antiproliferative effects of IFN-γ in human fibroblasts.

Gamma interferon (IFN-γ), a cytokine produced by activated T lymphocytes and natural killer cells, has pleiotropic effects; it plays a vital role in host self-defense against a variety of nonviral microbial pathogens, regulates immune responses, and inhibits cell growth (10, 37). Administration of neutralizing antibodies against IFN-γ abrogates the resistance of mice to various microbial pathogens (3, 4, 34, 40), indicating a primary role of IFN-γ in host defense against such pathogens. IFN-γ induces the synthesis of a number of gene products in cells (38). Several of the induced gene products have been implicated in the IFN-γ-mediated resistance to microbial infection. This includes the production of nitric oxide resulting from the induction of nitric oxide synthase (1, 14, 21, 24), activation of macrophages (22, 23), and starvation for tryptophan due to induction of the indoleamine 2,3-dioxygenase (INDO) enzyme (6, 7a, 26, 27, 33), which catalyzes the first step in tryptophan degradation. (The office of Human Gene Mapping nomenclature [Department of Human Genetics, University of Manitoba, Canada] brought to our attention that the term IDO had been assigned to indole 2,3-dioxygenase [EC 1.13.11.17]. Indoleamine 2,3-dioxygenase [EC 1.13.11.42] has therefore been abbreviated INDO.)

Since a variety of gene products are induced by IFN-γ, their relative importance in the biological effects of IFN-γ is difficult to determine. To assess the significance of INDO as a part of this host resistance mechanism, we cloned cDNA for human INDO (8) in an expression vector containing the metallothionein (MTII) promoter. Cells transfected with this construct provide a way to induce INDO activity independent of other IFN-γ-induced proteins. We have examined whether expression of INDO activity in cells stably transfected with the INDO cDNA expression plasmid would inhibit the growth of *Chlamydia psittaci* and *Toxoplasma gondii* in the absence of IFN-γ, which would demonstrate whether the expression of INDO alone can block the replication of these pathogens. In addition, we have tested whether the expression of INDO in transfected cells would also result in cytostatic and cytotoxic effects, as observed after treatment with IFN-γ (9, 25, 30, 35, 36).

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MATERIALS AND METHODS

Cloning of INDO cDNA in an inducible expression vector. A nearly full-length, ca. ~2-kb INDO cDNA was cloned originally in plasmid Bluescript SK− and named C42 (8). In order to express this cDNA in mammalian cells, it was first recloned in an expression vector. We chose vector pMEP4 (Invitrogen, San Diego, Calif.), based on the Epstein-Barr virus, because of the presence of the inducible metallothionein (MTII) promoter and its ability to replicate to a high copy number. The INDO cDNA contained more than 600 nucleotides of 5′ untranslated sequence. Therefore, in order to obtain optimal expression of INDO enzyme, we decided to clone (i) the full-length cDNA, including >600 bp of untranslated 5′ sequence, and (ii) the coding region after deletion of most of this untranslated sequence. In both cases, the cDNA was cloned in the sense orientation as well as the antisense orientation with respect to the MTII promoter. The 2-kb insert was obtained by digestion of plasmid C42 with *Kpn*I plus *Not*I enzymes and cloned into the pMEP4 vector digested with the same enzymes to insert the INDO cDNA in the sense orientation (see Fig. 1). For cloning in the antisense orientation, the 2-kb cDNA was obtained by digestion of plasmid C42 with *Xho*I plus *Sma*I enzymes and ligated to pMEP4 digested with *Xho*I plus *Pvu*II enzymes. After transformation of *Escherichia coli* DH1 cells with each of the two constructs, several colonies were isolated, and plasmid minipreps were prepared and analyzed for the

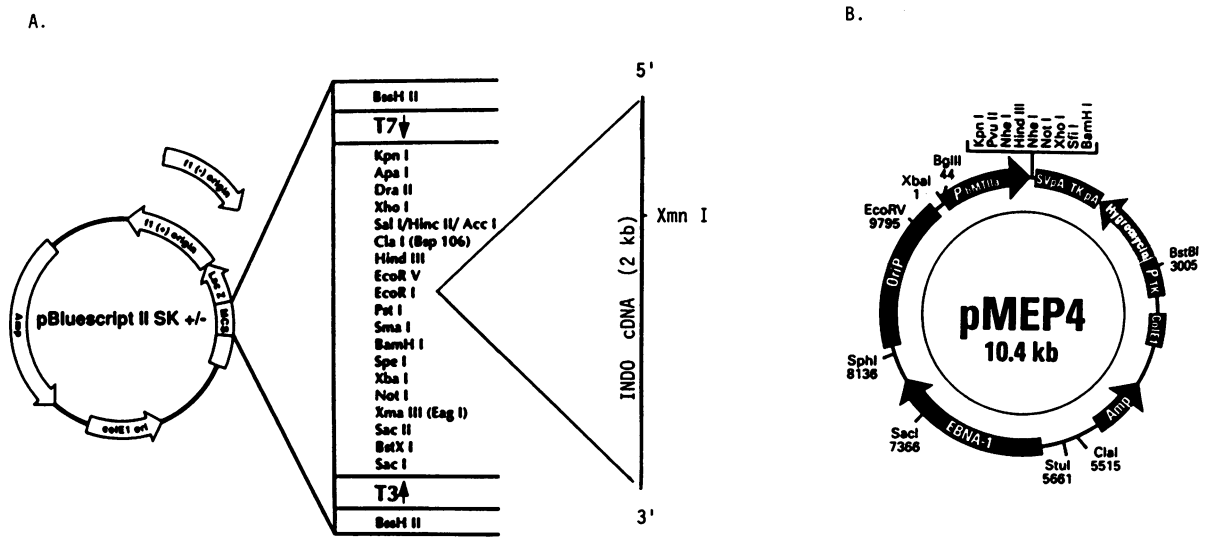

FIG. 1. INDO cDNA (ca. ~2 kb) cloned into Bluescript SK− at the *Eco*RI site (A) was isolated either in its entirety or as a 1.4-kb subfragment after deletion of 592 bp of 5′ untranslated sequence as described in Materials and Methods and cloned into the pMEP4 vector (B) containing the metallothionein (MT II) promoter in both the sense and antisense orientations. (The Bluescript vector map has been reproduced with the permission of Stratagene, and the pMEP4 map has been reproduced with the permission of Invitrogen.) MCS, multiple cloning site.

presence of the insert and its orientation with respect to the MTII promoter by restriction analysis. Separately, a ca. ~1.4-kb INDO cDNA fragment containing the entire coding sequence but depleted of much of the untranslated 5′ sequence was isolated by making use of an *Xmn*I site at position 592 from the 5′ end of the 2-kb INDO cDNA insert. Plasmid C42 was digested with *Xmn*I and then with *Xho*I and *Sma*I enzymes. This allowed the isolation of a 1.4-kb fragment with both ends blunt (*Xmn*I and *Sma*I). (The *Xho*I digestion was included because the vector also has an *Xmn*I site which yielded another fragment of approximately the same size that could be cleaved by *Xho*I to facilitate the isolation of the INDO 1.4-kb fragment.) The 1.4-kb fragment isolated after agarose gel electrophoresis was ligated to the pMEP4 vector digested with *Pvu*II enzyme. After transformation of DH1 cells, plasmid minipreps were obtained from several colonies and analyzed to determine the presence of the 1.4-kb insert and its orientation with respect to the MTII promoter by restriction analysis. Clones containing the 2-kb cDNA as well as the 1.4-kb cDNA insert in both the sense and antisense orientations with respect to the MTII promoter were obtained and grown at a preparative scale, and the plasmids were purified by previously described procedures (31).

Stable transfection of human fibroblasts with INDO cDNA cloned in pMEP4 expression vector. Transformed human fibroblast cell line GM637 was obtained from the Coriell Institute for Medical Research, Camden, N.J., and maintained in Eagle's minimal essential medium (MEM) supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), and 10% fetal bovine serum (FBS). Individual cultures of GM637 cells were transfected with plasmid preparations containing either the 2-kb INDO cDNA or the 1.4-kb fragment containing the INDO coding region in either the sense or the antisense orientation. The calcium phosphate coprecipitation method was used for transfection of cells (2), and the transformants were selected with hygromycin (250 U/ml), as the vector contains the hygromycin resistance gene. Cells stably transfected with each of the four plasmid constructs were obtained and maintained in the presence of hygromycin (250 U/ml).

Stocks of *C. psittaci* and *T. gondii*. Stocks of *C. psittaci* 6BC were propagated in confluent L929 mouse 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. The cells were infected with 10 times the 50% infectious dose (ID_{50}) of 6BC, incubated for 2 days, and removed from the tissue culture plate with trypsin. Infected cells were then disrupted by sonication, and 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 ID_{50} method in L cells (16). A cloned line of the RH strain of *T. gondii* was maintained in normal human fibroblast cultures as described previously (29).

Replication of *C. psittaci* and *T. gondii* in transfected cells. For experiments on *C. psittaci* replication, cells transfected with the sense or antisense INDO construct were cultivated in 24-well plates on cover slips in triplicate wells (2×10^5 cells per well) in Eagle's MEM supplemented with 10% FBS, 100 μg of gentamicin sulfate per ml, and 10 μg of streptomycin sulfate per ml. Some wells received $ZnSO_4$ or $CdCl_2$ at final concentrations of 75 and 5 μM, respectively. In experiments measuring the effect of metal ion concentration on INDO induction and inhibition of chlamydiae, half-log dilutions of the metal ion inducers were used. After 24 h of incubation, cells were infected with 0.3 ID_{50}s of *C. psittaci* in 50 μl of Hanks' balanced salt solution (HBSS), which resulted in infection of between 30 and 50% of the cells. Incubation was continued for an additional 24 h to permit sufficient development of chlamydial inclusions. After incubation, cover slips from these wells were washed with PBS, fixed with methanol, stained with 5% Giemsa, and mounted on slides. The percentage of *C. psittaci* inclusion-containing cells was determined by light microscopy (5).

For experiments on *T. gondii* replication, transfected cells were grown in Eagle's MEM supplemented with 10% FBS, antibiotics, and 250 U of hygromycin per ml. Cells were washed

three times with PBS, trypsinized, and pelleted by centrifugation. The cells were suspended in medium containing 5% FBS and seeded in 0.5-cm^2 wells at a density calculated to yield a confluent monolayer without further cell division. The next day, the cultures were incubated in medium either without or with $CdCl_2$ added. After 2 days, both treated and control cultures were supplemented with EDTA (45 μM) to chelate Cd^{2+} ions. The addition of EDTA was essential to block the inhibitory effect of Cd^{2+} on the growth of the parasite. The cultures were then infected with 1.5×10^4 *T. gondii*, and the growth of the parasite was measured 2 days later by adding 0.6 μCi of [5,6-^{3}H]uracil (specific activity, 20 Ci/mmol; Moravek Biochemicals) and measuring the acid-precipitable radioactivity 4 h later, as described previously (28).

Effect of exogenous tryptophan on INDO-mediated inhibition of *C. psittaci* replication. Transfected cell cultures were treated with inducers or medium alone and infected with *C. psittaci* as described above. After a 2-h incubation, serial log dilutions of tryptophan in HBSS or HBSS alone was added to the culture medium. After an additional 24 h., cover slips were fixed and stained, and chlamydial growth was determined.

Determination of INDO activity in transfected cells. INDO activity was determined in transfected cell cultures set up in parallel to cultures infected with *C. psittaci*. After 44 h of incubation, the culture medium was aspirated and replaced with 400 μl of 50 μM tryptophan in HBSS containing 1 μCi of [5-^{3}H]tryptophan (specific activity, 20 to 30 Ci/mmol; New England Nuclear) per ml. Some wells received radiolabeled medium without cells to determine the amount of nonspecific tryptophan decyclization that occurred. After 4 h of incubation, supernatants were collected and frozen at −20°C until analyzed for accumulated tryptophan metabolites. INDO activity was assessed by a modification of a reversed-phase high-performance liquid chromatographic (HPLC) technique (39). Briefly, 50-μl 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. Radioactivity was measured by integration of peak areas with a fully automated HPLC (Isco, Lincoln, Neb.) equipped with a radioisotope detector (Radiomatic Instruments, Tampa, Fla.). The percentage of specific tryptophan catabolism was calculated by the following equation:

$$\% \text{ specific catabolism} = \frac{CPM_{test} - CPM_{spontaneous}}{CPM_{total} - CPM_{spontaneous}} \times 100 \quad (1)$$

where CPM_{test} is the counts per minute (CPM) present in *N*-formylkynurenine and kynurenine-containing fractions, $CPM_{spontaneous}$ represents the CPM of nonspecific tryptophan degradation products, and CPM_{total} is the sum of CPM from all fractions (7a).

Effect of INDO expression on cell growth. Cells transfected with the 1.4-kb INDO cDNA coding region in either the sense or antisense orientation were seeded in 24-well tissue culture plates (4×10^4 cells per well). The next day, duplicate wells were treated with either IFN-γ (250 U/ml) or Cd^{2+} (5 μM) or left untreated (controls). To measure the effect on cell growth, cells were labeled with [^{3}H]thymidine (3 μCi per well) for 2 h on day 3 or day 5 from the start of treatment. The medium was then removed, and the cells were washed with cold PBS and solubilized in 0.2 ml of 1 N NaOH. Aliquots of each sample were used for determination of trichloroacetic acid-precipitable counts in duplicate.

RESULTS

Stable transfection of human fibroblasts with INDO cDNA cloned in pMEP4 expression vector. Cultures of GM637 cells were transfected with plasmid preparations containing either the 2-kb INDO cDNA or the 1.4-kb fragment containing the INDO coding region cloned in the pMEP4 vector in either the sense or antisense orientation with respect to the MTII promoter, as described in Materials and Methods. In parallel, cells were transfected with pMEP4 vector alone, and the transformants were selected with hygromycin (250 U/ml), as the vector contains the hygromycin resistance gene. Cells stably transfected with each of the four plasmid constructs were obtained and maintained in the presence of hygromycin (250 U/ml).

Cultures of stably transfected cells were tested for the expression of INDO activity after treatment with $CdCl_2$ (0 to 10 μM) or $ZnSO_4$ (0 to 75 μM) or with IFN-γ (250 U/ml) for 24 h. Cells were then washed, and the medium was replaced with Earle's basal salt solution containing tryptophan (100 μM) and 10% FBS. After incubation for a further 24 h, the medium was collected and assayed for kynurenine by a colorimetric assay as described before (11). We found that INDO activity was strongly induced in cells transfected with the plasmid containing the 1.4-kb INDO coding region in the sense orientation after treatment with Cd^{2+} or Zn^{2+} in a concentration-dependent manner. Stronger induction was obtained with Cd^{2+}, reaching a maximum at 5 to 10 μM, which was comparable to the level of INDO activity induced (from the cellular INDO gene) after treatment with 250 U of IFN-γ per ml (Fig. 2A). Cells transfected with the plasmid containing the 1.4-kb INDO coding region in the antisense orientation showed no detectable INDO activity after treatment with Cd^{2+}, as expected for the antisense construct, but responded normally to IFN-γ (Fig. 2A).

By Northern (RNA blot) analysis, INDO-specific RNA transcripts were found in cells transfected with either the sense or antisense construct after treatment with $CdCl_2$ (Fig. 2B). The Cd^{2+}-induced INDO transcripts from the transfected plasmid constructs (Fig. 2B, lanes 3 and 6) were slightly longer than the IFN-γ-induced INDO transcripts from the cellular gene (Fig. 2B, lanes 2 and 5), apparently because of the contribution from the vector. The level of Cd^{2+}-induced INDO transcripts was lower in cells transfected with the antisense construct (Fig. 2B, lane 6) than in cells transfected with the sense construct (lane 3). This could be due to a shorter half-life of the antisense transcripts. The cells transfected with the plasmid containing the entire 2-kb INDO cDNA did not express any measurable INDO activity when treated with Cd^{2+}, although RNA transcripts were found in Northern blots (data not shown). We believe that this is due to the rather long untranslated 5′ sequence in the 2-kb clone, which contains termination codons in each reading frame (8). Therefore, the studies reported here were carried out with cells transfected with the 1.4-kb INDO cDNA constructs. The cells transfected with the vector alone showed no Cd^{2+}-induced INDO activity but responded normally to IFN-γ (not shown), as expected.

Inhibition of *C. psittaci* replication upon expression of INDO activity. To determine whether the expression of INDO activity in transfected cells would have an effect on *C. psittaci* infection, cells transfected with either the sense or antisense INDO construct were treated with $CdCl_2$ (5 μM) or $ZnSO_4$ (75 μM) for 24 h and then infected with 0.3 ID_{50} of *C. psittaci* as described in Materials and Methods. After incubation for a further 24 h, cells were fixed and scored for percentage of inclusion-containing cells by light microscopy (5). The results

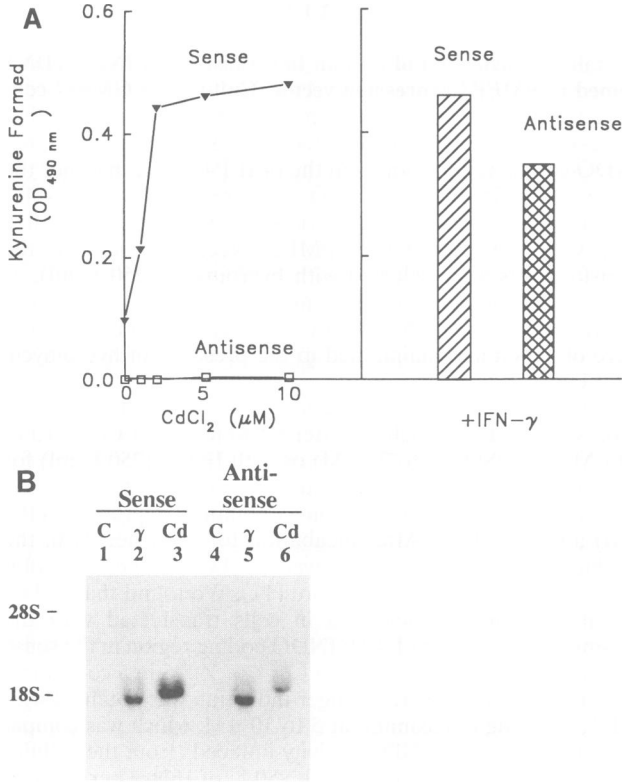

FIG. 2. Expression of INDO cDNA in cells transfected with the sense and antisense constructs. (A) Transfected cells were grown to confluence in 24-well plates and then incubated either with IFN-γ (250 U/ml; right panel) or with increasing concentrations of $CdCl_2$ (0 to 10 μM; left panel) for 24 h. The cells were then washed with PBS and incubated with 100 μM L-tryptophan in Earle's basal salt solution containing 10% FBS for 24 h at 37°C. The medium was collected, treated with 5% trichloroacetic acid at 50°C for 30 min, and centrifuged. The supernatant was assayed for L-kynurenine by adding an equal volume of 0.4% Ehrlich's reagent in glacial acetic acid and measuring the color developed by the A_{490} (11, 35). (B) Cells transfected with the sense or antisense construct were grown in 100-mm plates and treated with either IFN-γ (250 U/ml) or $CdCl_2$ (5 μM) for 24 h. Cells were washed with PBS, and total RNA was isolated by the acid-phenol-chloroform extraction procedure (7b) and precipitated with an equal volume of isopropanol. The precipitate was extracted with 4 M $LiCl_2$–1.5 M guanidine isothiocyanate–25 mM sodium citrate (pH 7.0)–0.1 M 2-mercaptoethanol at 0 to 4°C for 2 h, washed with 75% ethanol, and then dissolved in 0.1% sodium dodecyl sulfate. A 15-μg amount of each sample was fractionated by electrophoresis in 1% agarose and transferred to a nitrocellulose membrane. The membrane was baked and prehybridized and then hybridized with the ^{32}P-labeled INDO cDNA probe. The membrane was washed and autoradiographed as described before (7). The positions of the 28S and 18S rRNA markers are indicated.

showed that treatment with Cd^{2+} or Zn^{2+} strongly inhibited the growth of *C. psittaci* in cells transfected with the sense construct but not in cells transfected with the antisense construct (Fig. 3). In the absence of heavy metal ions, both cell types supported *C. psittaci* growth to a comparable extent. In parallel cultures, tryptophan catabolism was assayed by HPLC (39) as described in Materials and Methods. After treatment with Cd^{2+} or Zn^{2+}, a large part of the tryptophan in the culture medium was catabolized in cells transfected with the INDO sense construct but not in cells transfected with the antisense construct (Fig. 3, bottom).

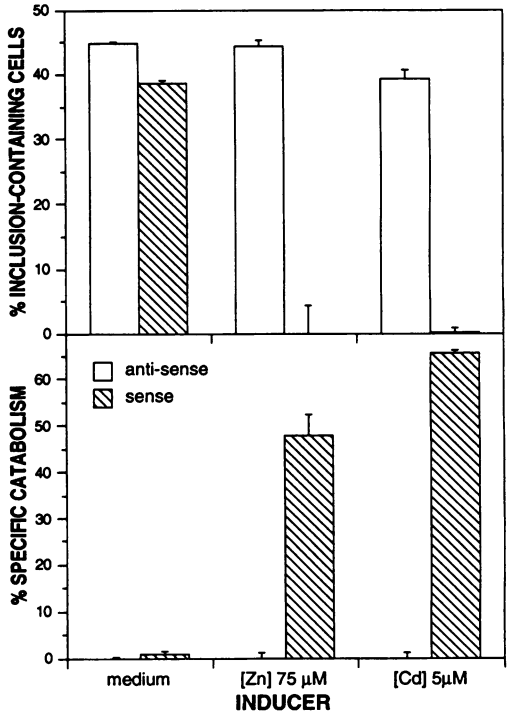

FIG. 3. Effect of Zn^{2+} and Cd^{2+} on the induction of INDO activity (lower panel) and the inhibition of *C. psittaci* (upper panel) in human GM637 fibroblasts transfected with INDO cDNA cloned in the pMEP4 expression vector in either the sense orientation (hatched bars) or the antisense orientation (open bars). Infection with chlamydiae is expressed as percentage of inclusion-containing cells ± standard deviation (SD), and INDO activity is represented as percent specific catabolism ± SD. See Materials and Methods for details.

The effects of the Cd^{2+} and Zn^{2+} concentration on the induction of INDO activity and the growth of chlamydiae in cells transfected with the INDO sense construct are shown in Fig. 4. The results showed that the level of INDO activity induced (as measured by tryptophan catabolism) was dependent on the Cd^{2+} or Zn^{2+} concentration and that the inhibition of chlamydial inclusions upon treatment with increasing concentrations of $CdCl_2$ or $ZnSO_4$ correlated closely with tryptophan degradation. Complete inhibition was obtained at 5 μM Cd^{2+} and 75 μM Zn^{2+}. In order to determine whether this inhibition of chlamydial growth was indeed due to the depletion of tryptophan, we tested whether it would be reversed by the addition of excess tryptophan. The inhibitory effect observed after treatment of INDO sense-transfected cells with Cd^{2+} (5 μM) or Zn^{2+} (75 μM) was completely overcome by the addition of 100 μM L-tryptophan (Fig. 5), indicating that induction of the transfected INDO gene construct caused depletion of tryptophan and thus inhibited chlamydial growth in these cells after treatment with Cd^{2+} or Zn^{2+}.

Inhibition of *T. gondii* replication in INDO-transfected cells. The replication of *T. gondii* in human fibroblasts is strongly inhibited by treatment with IFN-γ, which has been attributed to tryptophan depletion due to induction of the INDO enzyme (26, 27). Since IFN-γ induces a number of gene products, we tested whether the expression of INDO enzyme alone in transfected cells would block the replication of *T. gondii* in the absence of IFN-γ. Cells transfected with the sense or antisense INDO construct were incubated with or without $CdCl_2$ (10 μM) for 2 days. Then, EDTA was added to 45 μM (final

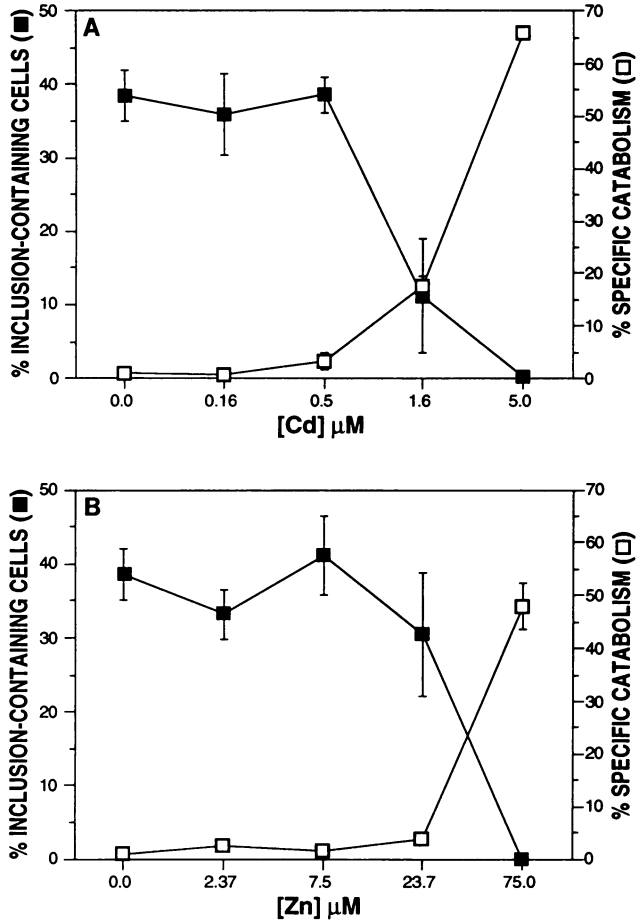

FIG. 4. Effect of increasing concentrations of Cd^{2+} (A) and Zn^{2+} (B) on the induction of INDO activity (□) and the inhibition of chlamydial growth (■) in cells transfected with the sense construct of INDO cDNA (1.4 kb) in the pMEP4 vector. Chlamydial growth and INDO activity are expressed as described in the legend to Fig. 2. Error bars represent SD.

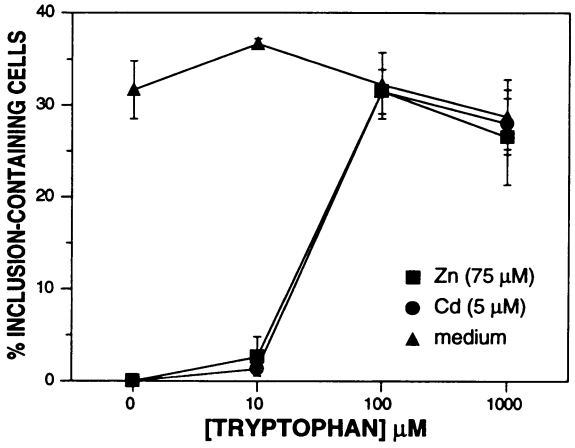

FIG. 5. Inhibition of chlamydial growth by Cd^{2+} and Zn^{2+} in cells transfected with the INDO cDNA sense construct and its reversal by the addition of excess tryptophan. Error bars represent SD.

concentration) to chelate Cd^{2+}, which was inhibitory to *T. gondii* replication, and the cultures were infected with *T. gondii*. The growth of the parasite was measured by the incorporation of [^{3}H]uracil 2 days later. The results showed >90% inhibition of *T. gondii* growth in cells transfected with the sense construct after treatment with Cd^{2+}. No inhibition of *T. gondii* growth was observed in cells transfected with the antisense construct after treatment with Cd^{2+} (data not shown).

While our studies were in progress, Habara-Ohkubo et al. (15) reported that expression of mouse INDO cDNA in murine cells blocked the growth of *T. gondii*. Our experiments showed a similar result with human INDO in human cells. In view of this published report (15), we did not carry out any further experiments to study the effect of INDO expression on *T. gondii* growth.

INDO expression and cell growth. IFN-γ has been shown to have an antiproliferative effect on tumor cells in culture (9, 25, 30, 35, 36), which has been correlated with the induction of INDO enzyme. We examined whether the expression of INDO activity alone in transfected cells would have a cytostatic and/or cytotoxic effect in the absence of IFN-γ. Cells transfected with the sense or antisense INDO construct were seeded in 24-well plates, and duplicate wells were treated with either IFN-γ (250 U/ml) or $CdCl_2$ (5 μM) or left untreated (control). The effect on cell growth was determined by measuring the incorporation of [^{3}H]thymidine after 3 or 5 days. The results showed that thymidine incorporation was strongly inhibited in cells transfected with the INDO sense construct after treatment with Cd^{2+} (Fig. 6A) but not in cells transfected with the antisense construct (Fig. 6B). Both cell populations showed comparable inhibition after treatment with IFN-γ (Fig. 6). Continued treatment with Cd^{2+} resulted in profound cytotoxicity in cells transfected with the sense INDO construct (Fig. 7, right), whereas the cells transfected with the antisense construct continued to grow normally (Fig. 7, left), indicating that the cytotoxicity observed was not due to the presence of Cd^{2+}. Both cell populations showed similar cytotoxicity in response to IFN-γ (Fig. 7). These results indicate that the growth-

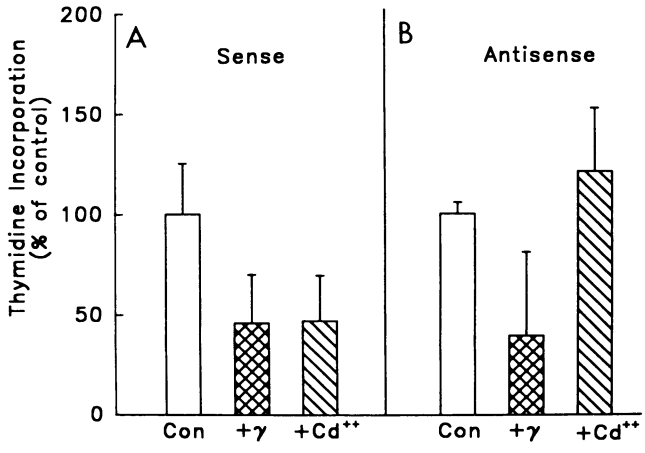

FIG. 6. Incorporation of [^{3}H]thymidine into cells transfected with the sense construct (A) or antisense construct (B) of INDO cDNA after treatment with IFN-γ (250 U/ml) or $CdCl_2$ (5 μM) for 3 days compared with the corresponding controls (Con). The average counts in control samples were normalized to 100%, and the relative values in different samples were calculated. Error bars represent SD.

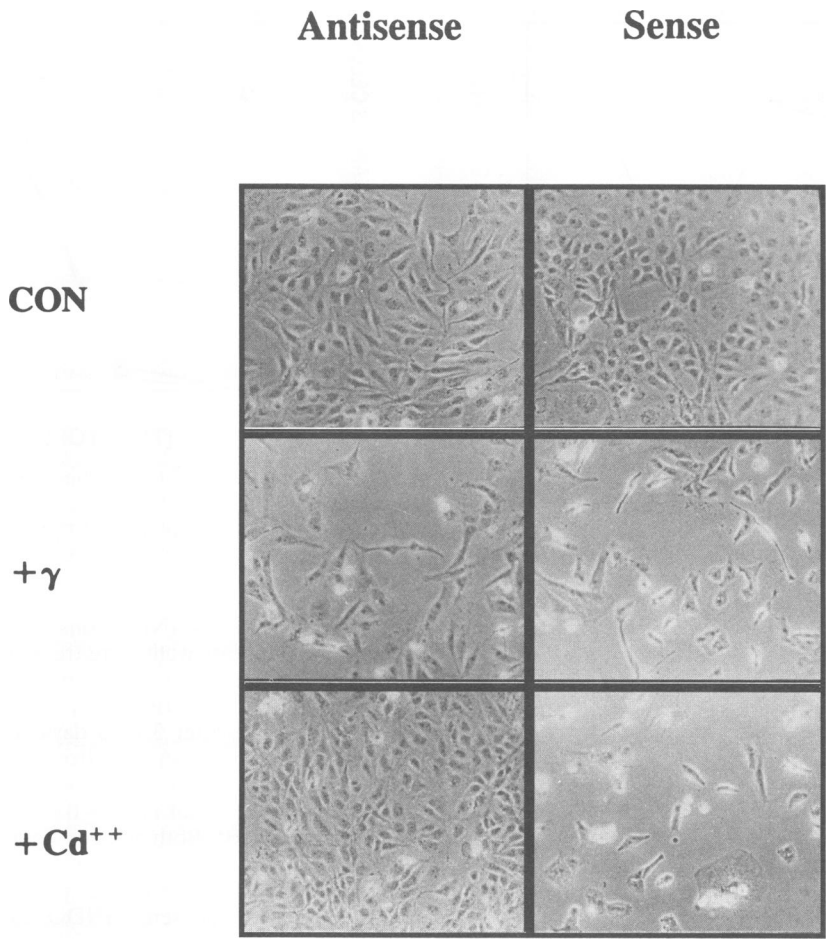

FIG. 7. Effect of IFN-γ and $CdCl_2$ on the growth and survival of cells transfected with the INDO sense construct (right panels) or antisense construct (left panels). See Materials and Methods for details. Cells were treated for 6 days before pictures were taken. CON, control.

inhibitory and cytotoxic effects of IFN-γ could be reproduced by the expression of INDO enzyme alone (Fig. 6 and 7).

DISCUSSION

The antimicrobial activities of IFN-γ have been correlated with the activation of various different mechanisms. In order to assess the relative importance of these effector mechanisms, it is important to determine the role of each pathway independent of other IFN-induced mechanisms. We have tested whether expression of INDO enzyme in cells transfected with INDO cDNA in an expression vector would block the growth of two pathogens, *C. psittaci* and *T. gondii*, in the absence of IFN-γ. The results presented here demonstrate that the growth of the two microbes was strongly inhibited by the expression of INDO activity. In experiments with *C. psittaci*, the inhibition of the microbe was closely correlated with the induction of INDO activity, as reflected by tryptophan degradation, and could be reversed by the addition of excess tryptophan to the medium (Fig. 4 and 5). These results provide strong support to the view that induction of INDO enzyme is an important factor in the antimicrobial activity of IFN-γ. Earlier studies showed that purified recombinant murine IFN-γ did not induce measurable levels of INDO activity in murine fibroblasts and failed to inhibit the growth of *T. gondii* (32). Expression of INDO activity in mouse cells by transfection conferred inhibition of *T. gondii* growth (15), indicating an important role for INDO expression in the inhibition of *T. gondii*. Our results with INDO-transfected human fibroblasts support this conclusion and are in agreement with the recent report that mutant human cells deficient in IFN-γ-induced expression of the INDO gene showed reduced capacity to suppress *Chlamydia* and *Toxoplasma* growth (35a).

The results presented here also showed that expression of INDO activity alone from transfected cDNA resulted in the inhibition of cell growth and led to cytotoxicity, as shown earlier with IFN-γ (9, 25, 30, 35, 36). Thus, the antiproliferative and cytotoxic effects of IFN-γ may be due in part to the induction of INDO and the resulting starvation for tryptophan, an essential amino acid.

IFN-γ, produced by activated T cells and natural killer cells, is a pleiotropic cytokine with multiple biological activities. The biological effects of IFN-γ are apparently mediated through the induction of multiple cellular genes. The fact that the expression of INDO alone could reproduce some of the biological effects of IFN-γ suggests that it may be an important factor in the biological activities of IFN-γ. It is not implied, however, that INDO induction is the sole mechanism for the antimicrobial activity of IFN-γ. Other mechanisms activated by IFN-γ may be equally important in host defense against

microbial pathogens in different cell types and different host species. The induction of INDO by IFN-γ may also play a role in the pathogenesis of certain diseases. For example, increased levels of IFN-γ have been found in the serum of AIDS patients who are seropositive for human immunodeficiency virus type 1 antibodies (13), which has been correlated with reduced levels of tryptophan and serotonin (a product derived from tryptophan) in these patients, apparently due to the induction of INDO by circulating IFN-γ (12, 20). Furthermore, greatly increased levels of quinolinic acid, a neurotoxic metabolite of tryptophan, have been found in the serum and cerebrospinal fluid of AIDS patients, especially those with neurological dysfunction, i.e., AIDS dementia complex (17, 18), suggesting a correlation. Increased INDO activity and toxic levels of quinolinic acid were also found in the spinal cord and the cerebrospinal fluid of macaques infected with poliovirus, suggesting a possible role in neurological disease (19). Therefore, the induction of INDO activity by IFN-γ may be a significant factor in the physiological resistance to pathogens as well as the pathogenesis of various diseases involving IFN-γ.

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