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Role of Tryptophan Degradation in Respiratory Burst-Independent Antimicrobial Activity of Gamma Interferon-Stimulated Human Macrophages

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To determine whether extracellular tryptophan degradation represents an oxygen-independent antimicrobial mechanism, we examined the effect of exogenous tryptophan on the intracellular antimicrobial activity of gamma interferon (IFN-γ)-stimulated human macrophages. IFN-γ readily induced normal monocyte-derived macrophages (MDM) to express indoleamine 2,3-dioxygenase (IDO) activity and stimulated MDM, alveolar macrophages, and oxidatively deficient chronic granulomatous disease MDM to degrade tryptophan. All IFN-γ-activated, tryptophan-degrading macrophages killed or inhibited *Toxoplasma gondii*, *Chlamydia psittaci*, and *Leishmania donovani*. Although exogenous tryptophan partially reversed this activity, the increases in intracellular replication were variable for normal MDM (*T. gondii* [5-fold], *C. psittaci* [3-fold], *L. donovani* [2-fold]), chronic granulomatous disease MDM (*T. gondii* [2.5-fold], *C. psittaci* [5-fold]), and alveolar macrophages (*T. gondii* [1.5-fold], *C. psittaci* [1.5-fold]). In addition, IFN-α and IFN-β also stimulated normal MDM to express IDO and degrade tryptophan but failed to induce antimicrobial activity, and IFN-γ-treated mouse macrophages showed neither IDO activity nor tryptophan degradation but killed *T. gondii* and *L. donovani*. These results suggest that while tryptophan depletion contributes to the oxygen-independent antimicrobial effects of the activated human macrophage, in certain cytokine-stimulated cells, tryptophan degradation may be neither sufficient nor required for antimicrobial activity.

Gamma interferon (IFN-γ-), a soluble T-cell product, directly stimulates both the oxygen-dependent and oxygen-independent antimicrobial mechanisms of the human macrophage, enabling this host defense cell to act against a diverse group of intracellular and extracellular pathogens (reviewed in reference 7). Although the oxygen (respiratory burst)-dependent mechanisms of the activated mononuclear phagocyte have been well characterized (15), relatively little is known about its oxygen-independent antimicrobial pathways. Recent reports (1–3, 17–19, 26, 27), however, have indicated that IFN-γ can stimulate a variety of human cells, including blood monocytes (3, 17), to display increased activity of indoleamine 2,3-dioxygenase (IDO), resulting in the degradation of extracellular tryptophan. Moreover, for human fibroblasts parasitized by *Toxoplasma gondii* (19, 20) and human bladder carcinoma cells infected with *Chlamydia psittaci* (2), the microbistatic activity induced by IFN-γ can be entirely reversed by exogenous tryptophan.

To test the hypothesis that tryptophan depletion may represent one of the respiratory burst-independent antimicrobial mechanisms of the activated human macrophage, we first surveyed the capacity of blood- and tissue-derived cells to respond to IFN-γ with tryptophan degradation, then tested oxidatively deficient macrophages, and finally determined the effect of tryptophan supplementation on intracellular antimicrobial activity.

MATERIALS AND METHODS

Cells. Human peripheral blood monocytes, human alveolar macrophages, and mouse peritoneal macrophages (BALB/c) were obtained and prepared as previously described (8–12). The T24 human bladder carcinoma cell line (2) was generously provided by G. Byrne (Madison, Wis.). All human cells were cultivated in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) containing 10% heat-inactivated heterologous human serum plus antibiotics; mouse macrophages were cultivated in Eagle minimal essential medium (GIBCO) containing 15% heat-inactivated fetal bovine serum plus antibiotics (8–12). A total of 2×10^5 cells of each type were seeded onto 12-mm round glass cover slips and washed after 1 h, and adherent cells were maintained at 37°C in 5% CO_2–95% air. After ≥5 days in culture, adherent monocytes were designated as monocyte-derived macrophages (MDM) (9).

IFN treatment. Human recombinant IFN-γ (rIFN-γ) was obtained from Amgen, Inc. (Thousand Oaks, Calif.), human rIFN-β was from Triton Biosciences (Alameda, Calif.), and murine rIFN-γ was provided by Genentech, Inc. (South San Francisco, Calif.). Natural human IFN-α (10^8 U/mg of protein) was purified with an NK-2 antibody column (Celltech, Slough, United Kingdom) (24). Cells were treated with rIFN-γ under in vitro conditions reported to induce antimicrobial activity: (i) fresh monocytes, MDM, and alveolar macrophages, 100 or 500 U of human rIFN-γ per ml for 72 h (10–12); (ii) T24 cells, 300 U of human rIFN-γ per ml for 24 h (2); and (iii) mouse peritoneal macrophages, 100 U of murine rIFN-γ per ml for 24 h (13). Human IFN-α and IFN-β were used at 500 U/ml (16) for 72 h for MDM and alveolar macrophages.

In vitro infection. After pretreatment with the IFN preparations, all cells were washed and cells on three cover slips in a 35-mm plastic dish were challenged with *T. gondii* (11), *C. psittaci* (8), or *Leishmania donovani* amastigotes (12). After 30 to 60 min, uningested *T. gondii* and *L. donovani*

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were removed by washing (time zero) and cells were reincubated in standard medium for 20 or 72 h, respectively (11, 12). At time zero, there were 26 to 43 *T. gondii* per 100 cells and 78 to 112 *L. donovani* per 100 cells. The *C. psittaci* inoculum, suspended in standard medium without penicillin, was not removed during the 24 h of challenge (22). The course of intracellular infection was determined by microscopic enumeration of (i) the number of *T. gondii* per 100 cells at time zero and 20 h (11), (ii) the number of *L. donovani* per 100 cells at time zero and 72 h (12), and (c) the percentage of cells infected with *C. psittaci* (inclusion bearing) 24 h after infection (8, 22).

Tryptophan supplementation. To test the effect of tryptophan supplementation, we added 100 μg of L-tryptophan (Sigma Chemical Co., St. Louis, Mo.) per ml to the culture medium at the start of the IFN-γ pretreatment period, and a similar concentration was included in the IFN-γ-free standard medium added after challenge with *T. gondii* and *L. donovani*. For *C. psittaci* infection, the challenge inoculum was suspended in medium containing 100 μg of tryptophan per ml (2). The addition of 100 μg/ml increased the tryptophan concentration in all culture media to >400 μM, and this level remained constant during up to 72 h of incubation (data not shown; see Table 4, footnote *a*). For all control cells pretreated with medium alone, the presence of 100 μg of tryptophan per ml before and after infection did not appreciably influence the intracellular replication of any of the three pathogens (data not shown).

Assays for tryptophan degradation and IDO activity. Single cover slips containing cells were placed in wells of a 24-well plastic tissue culture tray and incubated in triplicate in 0.5 ml of medium or medium plus IFN. After 24 to 72 h (depending on the cell population), the culture supernatants were removed and stored at −20°C until assayed for tryptophan levels. In some experiments, the medium was removed from cultures 20 to 24 h after infection and assayed for tryptophan. Tryptophan was measured by amino acid analysis in a Durrum D-500 amino acid analyzer in an assay with a lower limit of detection of 5 μM. For the purposes of calculation, <5 μM was assumed to equal zero. In the absence of cells, tryptophan levels in the medium remained constant for up to 72 h of incubation at 37°C and were not altered by the presence of IFN-γ alone (data not shown). To measure IDO activity, three cover slips containing cells were placed in a 35-mm plastic dish, cultivated alone or with IFN, and washed, and then the cells were lysed in a buffer containing 10 mM Tris hydrochloride (pH 7.4), 50 mM KCl, 5 mM $MgCl_2$, and 0.2% Triton X-100 (B. Y. Rubin, S. L. Anderson, G. R. Hellermann, N. K. Richardson, R. M. Lunn, and J. E. Valinsky, submitted for publication). The IDO reaction was performed as described elsewhere (21), and radiolabeled tryptophan and kynurenine were resolved by ascending chromatography on Whatman 3MM paper with 0.1 N HCl as a solvent. The tryptophan and kynurenine spots were located by UV fluorescence and cut out, and the amount of radioactivity present in each was measured in a scintillation counter. Protein determinations were done with the Bio-Rad dye reagent (Bio-Rad Laboratories, Rockville Center, N.Y.) with bovine serum albumin as a standard. IDO activity is expressed as nanomoles of tryptophan converted per hour per mg of protein (Rubin et al., submitted).

RESULTS

Tryptophan degradation and induction of IDO activity. Table 1 summarizes the capacity of human bladder carcinoma (T24) cells (2) and human macrophages to degrade extracellular tryptophan. Unstimulated cells did not alter the concentration of tryptophan in the culture medium during up to 72 h of incubation. In contrast, after exposure to rIFN-γ under in vitro conditions known to induce intracellular antimicrobial activity (2, 8, 10–13, 22), all activated cells including both tissue-derived (alveolar) and blood-derived (MDM) macrophages readily degraded tryptophan. In most experiments, cells treated with 300 or 500 U of IFN-γ per ml depleted the culture medium of detectable tryptophan (<5 μM). IDO activity, as judged by the capacity of cell lysates to metabolize labeled tryptophan (21; Rubin et al., submitted), was measured in T24 cells and MDM. For both cell populations, induction of enzymatic activity correlated closely with the ability to degrade tryptophan (Table 1).

TABLE 1. Degradation of extracellular tryptophan and expression of IDO activity[a]

Cells and treatment (U/ml)	Tryptophan (μM)	IDO activity (nmol/h per mg)
T24		
Medium[b]	34 ± 1 (3)	
Cells + medium	34 ± 1 (3)	0 (3)
IFN-γ (300)	<5 (3)	5.3 ± 0.8 (3)
Normal MDM		
Medium[b]	37 ± 1 (13)	
Cells + medium	36 ± 2 (13)	0 (4)
IFN-γ (100)	<5 (13)	9.7 ± 0.9 (4)
IFN-γ (500)	<5 (13)	10.7 ± 1.4 (4)
IFN-α (500)	6 ± 3 (3)	5.4 (2)
IFN-β (500)	<5 (3)	5.3 (2)
Alveolar macrophages		
Medium[b]	36 ± 1 (7)	
Cells + medium	37 ± 1 (7)	NT[c]
IFN-γ (100)	12 ± 4 (3)	NT
IFN-γ (500)	6 ± 1 (7)	NT
Mouse macrophages		
Medium[b]	46 ± 2 (3)	
Cells + medium	45 ± 1 (3)	0 (2)
IFN-γ (100)	42 ± 2 (3)	0 (2)
IFN-γ (500)	41 ± 3 (3)	0 (2)

[a] Adherent cells were treated with the indicated agents for 24 h (T24 cells, mouse peritoneal macrophages) or 72 h (MDM, alveolar macrophages). Culture supernatants were then removed for tryptophan assay or lysates were prepared for IDO assay. Results indicate the means± standard errors of the means of *n* experiments (number in parentheses) each performed in triplicate (tryptophan level) or duplicate (IDO activity), except for IDO activity of MDM treated with IFN-α and IFN-β and IFN-γ-treated mouse macrophages (means of two similar experiments for each).

[b] Culture medium without cells incubated for 24 to 72 h at 37°C.

[c] NT, Not tested.

Activity of oxidatively deficient mononuclear phagocytes. We next examined monocytes and MDM from a previously studied patient with X-linked chronic granulomatous disease (CGD). His cells fail to reduce Nitro Blue Tetrazolium following either stimulation with phorbol myristate acetate or ingestion of zymosan particles, *L. donovani*, or *T. gondii* and release essentially no H_2O_2 after phorbol myristate acetate triggering (8, 9, 11, 12). While 72 h of treatment with rIFN-γ did not alter the respiratory burst activity of these cells (8, 9, 11, 12), it readily induced both his monocytes and MDM to degrade extracellular tryptophan (Table 2). Thus, the induction of this effect for human mononuclear phagocytes appeared to be independent of respiratory burst activity.

Persistence of tryptophan-degrading capacity. Once fully stimulated by rIFN-γ, normal MDM maintain their respective activities toward *T. gondii* and *C. psittaci* for 24 to 48 h (11, 22, 23). These effects persist despite placing MDM in

TABLE 2. Tryptophan degradation by oxidatively deficient CGD cells[a]

Pretreatment of cells (U/ml)	Extracellular tryptophan (μM)			
	Monocytes[b]		MDM[b]	
	Normal	CGD	Normal	CGD
Medium alone	36 ± 1	37 ± 1	34 ± 2	32 ± 2
IFN-γ (100)	21 ± 2	18 ± 1	2 ± 2	0
IFN-γ (500)	0 ± 3	6 ± 2	0	0

[a] Cells were pretreated with medium or rIFN-γ for 72 h before culture supernatants were removed. Results are the means ± standard errors for three monocyte cover slips (one experiment) and nine MDM cover slips (two experiments). In the absence of either monocytes or MDM, the level of tryptophan in the culture medium ranged from 32 ± 2 to 35 ± 3 μM.

[b] Fresh monocytes were stimulated with rIFN-γ after an initial hour of adherence; MDM were first cultivated for 5 or 7 days prior to IFN-γ stimulation.

standard IFN-γ-free RPMI medium which contains 32 to 46 μM tryptophan. Therefore, if tryptophan depletion contributes to the antimicrobial activity of the MDM, the capacity to degrade tryptophan should still be evident for at least 24 h after removal of IFN-γ. Activated MDM continued to degrade tryptophan after transfer to standard medium (Table 3), and this activity was not disturbed by the presence of intracellular *T. gondii*, *C. psittaci*, or *L. donovani*.

Tryptophan supplementation and antimicrobial activity. (i) Tryptophan levels. The standard RPMI medium used contained 37 ± 1 μM tryptophan (n = 14). To test the effect of exogenous tryptophan, 100 μg/ml was added along with rIFN-γ during the pretreatment period and was readded by itself after infection (see Materials and Methods). For IFN-γ-activated macrophages cultivated with supplemented medium, the extracellular tryptophan concentration was 471 ± 15 μM (n = 16) at the end of the 72-h pretreatment period (just prior to infection) and remained at this level (430 ± 9 μM [n = 6]) 20 to 24 h after infection.

(ii) Effect on T24 cells and macrophages. For IFN-γ-activated T24 cells, the addition of exogenous tryptophan entirely reversed the capacity to inhibit the replication of both *C. psittaci* (2) and *T. gondii* (Table 4). In contrast, for IFN-γ-treated human macrophages, tryptophan supplementation achieved variable effects depending on both the cell and pathogen examined. Tryptophan repletion (i) permitted an increase in the survival and replication of *T. gondii*, *C. psittaci*, and *L. donovani* within activated normal MDM, (ii) induced comparatively little or no change in the activity of alveolar macrophages, and (iii) enhanced the replication of *T. gondii* and *C. psittaci* by 2.5- and 5-fold, respectively, in activated CGD MDM. Despite the ability of exogenous tryptophan to partially block antimicrobial effects, all IFN-γ-treated macrophages continued to show clear activity against each of the test pathogens (Table 4). Given the overriding role of oxygen-dependent mechanisms in the activity of macrophages against at least *T. gondii* (11) and *L. donovani* (9, 12), this latter observation was not unexpected.

In the experiments reported in Table 4 in which exogenous tryptophan proved to have an inhibitory effect on antimicrobial activity, a similar result could also be achieved by adding tryptophan (100 μg/ml) after infection only (data not shown). Supplementing cultures of activated MDM and alveolar and CGD macrophages before and/or after infection with 100 μg of either isoleucine or lysine per ml (2),

TABLE 3. Tryptophan degradation by macrophages after removal of IFN-γ[a]

Macrophage pretreatment	Extracellular tryptophan (μM)		
	Time zero	24 h after removal of IFN-γ	
		Uninfected	Infected
Normal MDM			
Medium alone	39 ± 5	32 ± 4	33 ± 3
IFN-γ	0	2 ± 1	1 ± 1
CGD MDM			
Medium alone	37 ± 1	39 ± 4	NT[b]
IFN-γ	0	8 ± 2	NT
Alveolar macrophages			
Medium alone	39 ± 2	41 ± 2	NT
IFN-γ	6 ± 3	10 ± 5	NT

[a] Macrophages were treated with medium or 500 U of rIFN-γ per ml. After 72 h, the medium was removed (time zero) and cells were washed and either reincubated uninfected in standard medium or infected in standard medium with *T. gondii*, *C. psittaci*, or *L. donovani*. Results for infected cultures (one experiment for each pathogen) were similar and were pooled. Results indicate the means ± standard errors of triplicate values for the following number of experiments: MDM, three; CGD MDM, two; and alveolar macrophages, three. IDO activity was measured in one experiment for uninfected normal MDM activated for 72 h by 500 U of rIFN-γ per ml: time zero, 7.4 nmol/h per mg; and 24 h after IFN-γ removal, 5.6 nmol/h per mg (mean of duplicate cultures).

[b] NT, Not tested.

TABLE 4. Effect of tryptophan supplementation on intracellular antimicrobial activity[a]

Cells and treatment	*T. gondii* (fold increase)[b]	*C. psittaci* (% cells infected)	*L. donovani* (fold increase)[b]
T24 cells			
Medium alone	5.5 ± 0.8	38 ± 2	NT[c]
IFN-γ	1.1 ± 0.2	3 ± 1	NT
IFN-γ + T	5.2 ± 0.7	40 ± 3	NT
Normal MDM			
Medium alone	7.5 ± 1.0	31 ± 2	2.2 ± 0.2
IFN-γ	0.4 ± 0.1	3 ± 2	0.7 ± 0.1
IFN-γ + T	2.0 ± 0.2	10 ± 4	1.4 ± 0.2
IFN-α	6.8 ± 1.2	33 ± 4	2.5 ± 0.3
IFN-β	6.5 ± 0.8	28 ± 3	2.3 ± 0.3
Alveolar macrophages			
Medium alone	6.0 ± 0.8	32 ± 5	NT
IFN-γ	1.3 ± 0.3	6 ± 2	NT
IFN-γ + T	2.0 ± 0.3	9 ± 3	NT
CGD MDM			
Medium alone	5.7 ± 0.4	35 ± 2	NT
IFN-γ	1.5 ± 0.5	3 ± 1	NT
IFN-γ + T	3.7 ± 1.0	16 ± 7	NT
Mouse peritoneal macrophages			
Medium	6.7 ± 0.5	NT	1.5 ± 0.2
IFN-γ	1.2 ± 0.1	NT	0.4 ± 0.1
IFN-γ + T	1.1 ± 0.2	NT	0.5 ± 0.2

[a] Prior to infection, cells were treated with rIFN-γ as follows: T24 cells, 300 U/ml for 24 h; human macrophages, 500 U/ml for 72 h; and mouse macrophages, 100 U/ml for 24 h. Normal MDM treated with IFN-α or IFN-β received 500 U/ml for 72 h. Cultures designated by + T received 100 μg of tryptophan per ml both during the IFN-γ pretreatment period and after infection (see Materials and Methods). Results for *T. gondii*, *C. psittaci*, and *L. donovani* are for 20, 24, and 72 h after infection, respectively, and indicate the means ± standard errors of three to eight experiments for each pathogen and cell.

[b] Fold increase in intracellular replication was calculated by dividing the number of parasites per 100 cells at the end of the infection period by the number originally ingested at time zero (see Materials and Methods) (14, 29). A fold increase of <1 indicates parasite killing. There were insufficient CGD cells to examine *L. donovani*.

[c] NT, Not tested.

however, did not impair antimicrobial activity toward any of the three pathogens (data not shown).

(iii) Effect on fresh monocytes. Although monocytes from normal donors and the CGD patient did not degrade tryptophan (Table 2), these cells exert well-defined intrinsic activity against both *T. gondii* (6, 11, 28) and *C. psittaci* (22). Therefore, to complete this analysis of the effects of tryptophan supplementation, fresh unstimulated monocytes were cultivated overnight with exogenous tryptophan (100 μg/ml), challenged with *T. gondii* or *C. psittaci*, and then reincubated with tryptophan for 20 to 24 h. This treatment did not influence the activity of either normal or CGD monocytes toward either pathogen (data not shown). These results also served to indicate that exogenous tryptophan was not acting as a potential oxygen intermediate scavenger (5); exogenous catalase, for example, inhibits the antitoxoplasma activity of both normal monocytes and IFN-γ-stimulated MDM (11).

Dissociation of tryptophan depletion and intracellular antimicrobial activity. The preceding results suggested that tryptophan depletion was involved to some degree in the capacity of IFN-γ to induce MDM to display antimicrobial activity. The results of the following experiments, however, indicated that under different conditions, tryptophan depletion alone was neither sufficient nor necessarily required to achieve intracellular antimicrobial effects. First, normal MDM were stimulated for 72 h with IFN-α and IFN-β, which also induce the capacity to degrade tryptophan (3, 17). While MDM treated with 500 U of these IFNs per ml readily depleted the culture medium of tryptophan (Table 1), these cells displayed no antimicrobial activity (Table 4). Second, resident mouse peritoneal macrophages were activated overnight by murine rIFN-γ. These cells killed or inhibited *T. gondii* and *L. donovani* (Table 4) but showed no IDO activity nor any capacity to degrade tryptophan (Table 1). Further, their antimicrobial effects were not diminished by tryptophan supplementation (Table 4).

DISCUSSION

The results of this study suggest the following conclusions. IFN-γ stimulates both blood- and tissue-derived human macrophages to degrade extracellular tryptophan. This effect, presumably mediated by the induction of IDO, is not unique to IFN-γ and can also be achieved by exposure to either IFN-α or IFN-β (3, 17). While other cytokines such as tumor necrosis factor and interleukin-2 do not directly stimulate human monocytes to degrade tryptophan (3), interleukin-2 can activate this mechanism by inducing the secretion of IFN-γ (3, 14).

Although superoxide anion is thought to play a substrate or cofactor role in some cells for the activity of IDO (25), our results with CGD cells indicated that the capacity to undergo a respiratory burst is not required for IFN-γ to induce tryptophan degradation by human mononuclear phagocytes. Thus, this putative antimicrobial mechanism can probably be categorized as oxygen (respiratory burst) independent.

Our results also suggest that tryptophan degradation contributes, albeit to varying degrees, to the antimicrobial activity of the IFN-γ-stimulated MDM toward *T. gondii*, *C. psittaci*, and *L. donovani*. However, the minor effects of tryptophan supplementation on activated alveolar macrophages and the absence of any effect on fresh monocytes further suggest that this mechanism is not necessarily relevant to all populations of human mononuclear phagocytes.

Since oxygen-dependent mechanisms predominate in the activity of normal macrophages toward both *T. gondii* (11) and *L. donovani* (9, 12), these two pathogens and oxidatively intact macrophages may not represent optimal targets or effector cells with which to analyze the role of tryptophan depletion. Although successful inhibition of *C. psittaci* does not require respiratory burst activity (8, 22), we were still unable to assign a major antimicrobial effect to tryptophan degradation using this pathogen and normal MDM (Table 4). In contrast, the results obtained in the absence of respiratory burst activity (e.g., with CGD cells) suggested that tryptophan depletion contributes to as much as 50% of the oxygen-independent antitoxoplasma and antichlamydial effects of the IFN-γ-activated MDM. For *C. psittaci*, the differences in the inhibitory effect of tryptophan supplementation for normal MDM versus CGD MDM also raise the possibility that tryptophan degradation has a more prominent antimicrobial role in oxidatively deficient cells. The capacity of exogenous tryptophan to entirely reverse the microbistatic activity of IFN-γ-stimulated human fibroblasts (19) and T24 cells (2), cells likely to be devoid of effective respiratory burst mechanisms (8, 22; unpublished data), supports this speculation.

Despite the preceding results, our data and those of other investigators also indicate that tryptophan depletion by itself is not necessarily sufficient to achieve effective antimicrobial activity. Thus, *T. gondii*, *C. psittaci*, and *L. donovani* all flourished intracellularly within IFN-α- and IFN-β-treated MDM despite being surrounded by an extracellular milieu largely free of tryptophan. Turco and Winkler (27) have also reported that although capable of degrading tryptophan, IFN-γ-activated human fibroblasts and HeLa cells continue to fully inhibit *Rickettsia prowazekii* in the presence of exogenous tryptophan. Both of these observations contrast directly with prior work with human fibroblasts (19) and T24 cells (2) (Table 4) which originally suggested that IFN-γ-induced inhibition of intracellular microbial replication resulted from simply having deprived *T. gondii* and *C. psittaci* of an amino acid essential for their growth (2, 19, 20). However, in view of our results, we believe that this mechanism is more complex and that the potential antimicrobial role of tryptophan degradation in human cells depends on at least three variables: the cell targeted for cytokine treatment, other mechanisms stimulated by the cytokine, and the test pathogen used.

Our observations can be interpreted as suggesting that some but not all IFN-γ-activated human cells possess a separate oxygen-independent antimicrobial mechanism which requires tryptophan depletion. If the inhibition of *T. gondii* or *C. psittaci* by IFN-γ-activated fibroblasts and tumor cells results from deprivation of an essential amino acid (2, 19, 20) or the production of a potentially toxic tryptophan metabolite (19), why then do the same two pathogens replicate freely within tryptophan-degrading IFN-α- and IFN-β-stimulated MDM? A possibility worth considering is that IFN-γ (but not IFN-α or IFN-β) simultaneously induces a distinct mechanism which acts in concert with tryptophan degradation and is reversible by tryptophan supplementation. In IFN-γ-stimulated oxidatively deficient cells (CGD MDM, fibroblasts, T24 cells), such a mechanism would appear to play a clear and primary role; in oxidatively active cells (normal MDM, alveolar macrophages), this mechanism also appears to be utilized but is overshadowed by the antimicrobial effects of the respiratory burst. Further study to examine this hypothesis is currently under way.

Finally, although human cells readily respond to IFN-γ with the expression of IDO and tryptophan degradation, it is now apparent that cells of murine origin do not. Human cells

which degrade tryptophan in response to IFN-γ include fibroblasts (1, 19, 20), HeLa cells (27), a variety of tumor cell lines (2, 18, 26), monocytes (3, 17), and as shown here, MDM and alveolar macrophages. Murine cells which do not appear to respond to IFN-γ with tryptophan degradation include a variety of fibroblasts (McCoy cells [4], embryo fibroblasts [27], L929 cells [27]) and peritoneal macrophages (Table 1). However, as judged by the in vitro and in vivo results of Yoshida and colleagues (29), who utilized mouse lung slices stimulated by lipopolysaccharide or L cell-derived IFN, IDO activity can be induced in certain murine cells. Nevertheless, the inability of IFN-γ-treated murine cells to degrade tryptophan provides a ready explanation for the failure of exogenous tryptophan to affect antimicrobial activity toward *C. trachomatis* (4), *R. prowazekii* (27), and *T. gondii* and *L. donovani* (Table 4). The results of these latter studies not only indicate that IFN-γ-induced antimicrobial activity can be entirely dissociated from tryptophan metabolism for certain cells (e.g., of murine origin), but also reemphasize the presence of innate species differences among the antimicrobial mechanisms of both phagocytic and nonphagocytic host defense cells.

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