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Intracellular Growth Inhibition of *Histoplasma capsulatum* Induced in Murine Macrophages by Recombinant Gamma Interferon Is Not Due to a Limitation of the Supply of Methionine or Cysteine to the Fungus

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Recombinant murine gamma interferon (rMuIFN-γ) stimulates mouse peritoneal macrophages to inhibit the intracellular growth of the zoopathogenic fungus *Histoplasma capsulatum*. In some systems, the inhibition of growth of an intracellular parasite by rIFN-γ has been related to nutritional constraints induced in the host cells by the lymphokine. Such an explanation might apply to *H. capsulatum* because the fungus is a functional methionine-cysteine (Met-Cys) auxotroph at 37°C; its sulfite reductase is repressed at that temperature. For this reason, we set about to examine whether or not the antihistoplasma state induced in rMuIFN-γ is due to a restriction in the availability of Met-Cys. Omission of Met-Cys from the medium in which macrophages were cultivated prevented *H. capsulatum* from growing within them. Addition of Met or Cys to the macrophage cultures did not antagonize the inhibitory effect induced in the cells by rMuIFN-γ. Thus, there was no evidence from our work that rMuIFN-γ evokes the antihistoplasma effect in mouse peritoneal macrophages by limiting the supply of Met-Cys to the fungus.

The growth of *Histoplasma capsulatum* within mouse peritoneal macrophages is inhibited by recombinant murine gamma interferon (rMuIFN-γ) (23–25). The intracellular yeasts are not killed by the interaction (28), but their growth is often completely suppressed (26, 28).

Pfefferkorn and his colleagues have shown that the growth of *Toxoplasma gondii* in human fibroblasts is suppressed by recombinant human IFN-γ (17). Such growth inhibition is believed to be due to tryptophan (Trp) starvation of the parasite caused by the degradation of that amino acid induced in the fibroblasts by recombinant human IFN-γ (17–19, 29). Trp deprivation does not affect the intracellular growth of *H. capsulatum* within mouse peritoneal macrophages (see Table 1) (28). Nevertheless, a nutritional basis for intracellular growth inhibition of *H. capsulatum* within IFN-γ-treated mouse macrophages is plausible because the fungus is a functional methionine-cysteine (Met-Cys) auxotroph at 37°C (1, 2, 13, 20) and limitation in the supply of Met-Cys would inhibit its growth (13). On the basis of these considerations, we set about to study the effect of Met-Cys on the intracellular inhibition of *H. capsulatum* induced by rMuIFN-γ.

H. capsulatum 505 (13, 26, 28) was used in these studies. Resident peritoneal macrophages harvested from C57BL/6 mice were washed and suspended in complete or deficient Earle's minimal essential medium (MEM) devoid of the amino acid(s) in question. The macrophages were set up in cell culture as described previously (24, 25). The media devoid of certain amino acids were prepared from Earle's MEM Select-Amine Kit (GIBCO Laboratories, Grand Island, N.Y.). The media were supplemented with 10% fetal bovine serum (FBS) (Hyclone Laboratories, Inc., Logan, Utah) that had been dialyzed extensively against H_2O in tubing with a molecular weight cutoff of 3,000. The rMuIFN-γ was supplied by Genentech, Inc., South San Francisco, Calif. The assay for intracellular growth was similar to that previously reported on a number of occasions (24, 25, 28).

The effect of amino acid depletion on the intracellular growth of *H. capsulatum* within normal mouse peritoneal macrophages is illustrated in Table 1. Removal of Trp from the medium had little effect on the intracellular growth of *H. capsulatum*. Cys deprivation alone did not markedly affect the intracellular growth because the medium still contained Met, which mammalian cells convert to Cys. Depletion of both Met and Cys from the medium halted the intracellular growth of *H. capsulatum*. Thus, it is possible to show that Met-Cys starvation does interrupt intracellular multiplication within mouse peritoneal macrophages. The macrophages appeared morphologically normal over the 20-h incubation period in a medium depleted of Met-Cys.

In a series of reconstitution experiments, it could be shown that adding Cys or Met to a culture medium devoid of these amino acids did not alter the antihistoplasma state induced in mouse peritoneal macrophages by rMuIFN-γ. Results from a representative experiment are recorded in Table 2. Complete medium supported the inhibition-inducing activity of rMuIFN-γ (Table 2, rows 1, 2, and 3). Macrophages suspended in a medium devoid of Met-Cys did not support the intracellular growth of *H. capsulatum* (Table 2, rows 4, 7, and 9). Adding back 0.1 mM Cys (the level in MEM) restored intracellular growth of the fungus (Table 2, rows 5 and 6). However, adding back Cys did not antagonize the effect of rMuIFN-γ (Table 2, rows 8, 10, and 11). Similarly, adding back Met restored growth of *H. capsulatum* within macrophages cultured in a medium depleted of that amino acid but did not abrogate the action of IFN (data not shown).

Thus, withdrawal of Met-Cys from the medium in which murine macrophages were cultured completely inhibited the

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TABLE 1. Effect of amino acid deprivation on the intracellular growth of *H. capsulatum* within normal mouse peritoneal macrophages

Amino acid omitted[a]	No. of yeasts/ infected macrophage[b]	Growth[c]	Reduction of growth (%)[d]
None	6.1 ± 1.4	3.7	
Trp	5.6 ± 0.9	3.2	14
Cys	5.3 ± 0.4	2.9	22
Met-Cys	2.5 ± 0.1	0.1	97

[a] The complete medium was Earle's MEM with 10% FBS. The depleted media were prepared from Earle's MEM Select-Amine Kit (GIBCO). The media depleted of certain amino acids were supplemented with dialyzed 10% FBS.

[b] The results are shown as the mean ± standard error for two experiments. The mean ± standard error at time zero was 2.4 ± 0.5 yeasts per infected macrophage.

[c] Growth is recorded as the net growth, calculated by subtracting the time zero mean number of yeasts per infected macrophage from the mean number of yeasts per infected macrophage after 20 h of incubation at 37°C.

[d] Percent inhibition is calculated with the following formula: [1 − (growth in macrophages in depleted media)/(growth in macrophages in complete medium)] × 100.

growth of *H. capsulatum* within them (Table 1). But adding Met or Cys to IFN-γ-treated macrophages did not abrogate the inhibitory effect. From the results of other work, it is known that Met is taken up by IFN-γ-treated cells (22) and that amino acids are taken into the phagosomes of macrophages activated by sublethal infection with *H. capsulatum*

TABLE 2. Effect of cysteine on the inhibition of intracellular growth of *H. capsulatum* induced in mouse peritoneal macrophages by rMuIFN-γ

Row	Medium[a]	rMuIFN-γ (U/ml)	Cys (mM)	Growth[b]	Reduction of growth (%)[c]
1	MEM	0	0	3.7[d]	
2	MEM	100	0	2.0[d]	46
3	MEM	200	0	1.0[d]	73
4	MEM(−Met/Cys)	0	0	0.5	86
5	MEM(−Met/Cys)	0	0.1	4.0	0
6	MEM(−Met/Cys)	0	0.3	4.5	0
7	MEM(−Met/Cys)	100	0	0	100
8	MEM(−Met/Cys)	100	0.1	1.9	52
9	MEM(−Met/Cys)	200	0	0	100
10	MEM(−Met/Cys)	200	0.1	1.3	67
11	MEM(−Met/Cys)	200	0.3[e]	1.7	62

[a] MEM is Earle's MEM with 10% FBS. MEM(−Met/Cys) is synthetic Earle's MEM constructed without Met or Cys and supplemented with dialyzed 10% FBS.

[b] Growth is recorded as net growth, which was calculated by subtracting the time zero mean number of yeast cells per infected macrophage from the mean number of yeast cells per infected macrophage after 17 h of incubation at 37°C (see Table 1 for an example of the calculations). The data recorded are averages from the results of two experiments. Components of the experiments depicted have been repeated on a number of other occasions with comparable results. The results are the averages from two experiments.

[c] Percent reduction in growth is the reduction in growth of yeast cells within infected macrophages incubated in the various test situations compared with growth of yeast cells within macrophages incubated in control medium. The percent reductions recorded in rows 2, 3, 4, 7, and 9 were made with results from MEM. The percent reductions recorded in rows 8, 9, 10, and 11 were made with results from MEM(−Met/Cys) supplemented with Cys as indicated.

[d] This portion of the protocol (rows 1, 2, and 3) has been repeated a very large number of times with very similar results in the context of other experiments (23–26, 28).

[e] The experiment recorded in this row was repeated one additional time with 0.4 mM cysteine. The percent reduction in growth was 50%.

(14). rMuIFN-γ does not inhibit the uptake of Met-Cys nor the conversion of Met to Cys and does not induce a degradation of Cys (data not shown). Thus, there was no evidence from our work that rMuIFN-γ evokes the antihistoplasma effect in mouse peritoneal macrophages by limiting the supply of Met-Cys to the fungus. However, the antihistoplasma state of IFN-treated macrophages does appear to have a different nutritional basis. We have recently found (27) that the antihistoplasma effect requires L-arginine and is inhibited by the arginine analog N-^{G}-monomethyl-L-arginine. The arginine-dependent effect appears to be the same as that directed toward *Cryptococcus neoformans* (9–12). The ultimate effect of the products of IFN-augmented arginine metabolism is the interaction of nitric oxide with non-heme iron proteins (16). This is interesting because we now have evidence that down-regulation of transferrin receptors by IFN-γ (7) affects the intracellular growth of *H. capsulatum* within IFN-treated macrophages (15). Serum transferrin binds enough iron to inhibit *H. capsulatum* in media containing serum (8, 21). *H. capsulatum* acquires iron in vitro by a hydroxamic type of siderophore (4–6) but, unlike many bacteria, cannot use the siderophore to remove iron in vivo from transferrin or lactoferrin (3, 7). In recent work, we have shown that (i) *H. capsulatum* requires iron for intracellular growth and (ii) iron restriction may be one of the bases for the IFN-γ-induced antihistoplasma effect of mouse macrophages (15).

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