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Gamma Interferon-Activated Human Macrophages and *Toxoplasma gondii*, *Chlamydia psittaci*, and *Leishmania donovani*: Antimicrobial Role of Limiting Intracellular Iron

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Iron-saturated transferrin did not reverse the intracellular killing or inhibition of *Toxoplasma gondii*, *Chlamydia psittaci*, or *Leishmania donovani* by gamma interferon-activated human macrophages. Deferoxamine, an iron chelator, also did not impair replication within unstimulated macrophages. Limiting the availability of intracellular iron is an unlikely mechanism in human macrophage activity against these three diverse pathogens.

In previous studies, we utilized *Toxoplasma gondii*, *Leishmania donovani*, and *Chlamydia psittaci* as intracellular microbial targets to characterize the antimicrobial mechanisms of the human monocyte and the activated macrophage (12–14, 16). These results indicated that human mononuclear phagocytes utilize both respiratory burst-dependent and -independent antimicrobial mechanisms and that the activating lymphokine gamma interferon (IFN-γ) can enhance the effect of both pathways (12–14, 16). In contrast to the human mononuclear phagocyte's respiratory burst-dependent mechanisms, those apparently independent of the oxidative burst remain less well characterized (15).

Recent data from an in vitro model of human monocytes infected with *Legionella pneumophila* have suggested that limiting the availability of iron may represent a broadly effective intracellular antimicrobial mechanism (1). This conclusion was reached after demonstrating that (i) deferoxamine, an iron chelator, inhibited *L. pneumophila* replication within unstimulated monocytes, and (ii) the capacity of IFN-γ-activated monocytes to inhibit *L. pneumophila* correlated with downregulation of transferrin receptor expression and was entirely reversed by iron-saturated transferrin (1).

To determine whether this mechanism is relevant when the activated human mononuclear phagocyte encounters other diverse intracellular pathogens, we treated resting macrophages with deferoxamine, and we treated IFN-γ-activated macrophages with excess iron during infection with *T. gondii*, *L. donovani*, and *C. psittaci*.

Adherent peripheral blood monocytes from healthy donors were cultivated on glass coverslips at 37°C in 5% CO_2–95% air in RPMI 1640 medium (GIBCO, Life Technologies, Inc., Grand Island, N.Y.) containing 15% heat-inactivated heterologous human serum and antibiotics (12, 13, 16). After 5 to 7 days in culture, unstimulated cells (monocyte-derived macrophages [12, 13, 16]) were pretreated for 24 h with 5, 10, 15, 20, or 30 μM deferoxamine mesylate (CIBA-GEIGY, Suffern, N.Y.) (1) and, in the presence of fresh deferoxamine, were then challenged with *T. gondii* trophozoites (10^6/ml) (13), *L. donovani* amastigotes (5×10^6/ml) (12), or *C. psittaci* (one 50% infectious dose) (16). *C. psittaci*-infected cultures were left undisturbed for 20 h, at which time the percentage of Giemsa-stained cells bearing inclusions was counted microscopically (16). The percent inhibition of *C. psittaci* replication was determined as follows: [(% control cells infected − % treated cells infected)/% control cells infected] × 100 (16). Thirty to sixty minutes after initial challenge with *T. gondii* or *L. donovani*, uningested parasites were removed by washing (time zero), and cultures were reincubated with deferoxamine for 20 h (*T. gondii*) or 72 h (*L. donovani*) (12, 13). By using Giemsa-stained coverslips, the number of parasites per 100 macrophages present at time zero was counted microscopically and compared with the number present at 20 or 72 h to determine the fold increase in intracellular replication (12, 13).

Although deferoxamine was present before, during, and after challenge, none of the concentrations used, including 30 μM (Table 1), affected the intracellular replication of the three test pathogens. In the *L. pneumophila* model, intracellular replication is completely inhibited by ≥15 μM deferoxamine (1).

Five- to seven-day-old human macrophages were activated by 3 days of pretreatment with 1,000 U of human recombinant IFN-γ per ml (2×10^7 U/mg; Amgen, Thousand Oaks, Calif.), challenged with the three test pathogens (12, 13, 16), and then cultured in IFN-γ-free medium for 20 to 72 h, depending upon the pathogen (12–14, 16). Iron-saturated transferrin (Sigma Chemical Co., St. Louis, Mo.), 6 mg/ml (1), was added to the culture medium during the 3 days of IFN-γ pretreatment, at the time of challenge, and for up to 72 h after infection. Iron transferrin had no effect on microbial replication within unstimulated control cells and did not alter the capacity of IFN-γ-activated cells to inhibit or kill the three test pathogens (Table 1). In a single experiment, 10 mg of iron transferrin per ml also had no appreciable effect (data not shown). In contrast, the activity of the IFN-γ-stimulated human monocyte towards *L. pneumophila* is >50% reversed by 0.7 mg of iron transferrin per ml and is completely inhibited by treatment with 6 mg/ml (1).

These results suggest that limiting the availability of iron is not likely to represent a broadly applicable antimicrobial mechanism through which the activated human macrophage exerts its control over intracellular pathogens. Exogenous iron did not diminish IFN-γ-induced activity against *T. gondii*, *C. psittaci*, or *L. donovani*, and as judged by the absence of a deferoxamine effect, the replication of these

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TABLE 1. Effect of treatment with Deferoxamine and iron transferrin

Macrophage treatment[a]	Replication[b]		
	Fold increase		% Inhibition of *C. psittaci*
	T. gondii	*L. donovani*	
None (control)	7.1 ± 0.9	2.5 ± 0.4	0
Deferoxamine	6.8 ± 0.9	2.4 ± 0.6	8 ± 3
IFN-γ	1.0 ± 0.1	0.7 ± 0.1	70 ± 5
Iron transferrin	7.0 ± 0.6	2.3 ± 0.4	3 ± 1
IFN-γ + iron transferrin	0.9 ± 0.2	0.8 ± 0.1	68 ± 3

[a] Unstimulated macrophages were treated with medium alone (control) or medium containing 30 μM deferoxamine 24 h before and throughout the infection period. Unstimulated and IFN-γ-activated macrophages were treated with 6 mg of iron transferrin per ml starting 72 h before and throughout the infection period.

[b] Results (means ± standard error of the mean for three to eight experiments per pathogen) are from observations made 20 h (*T. gondii* and *C. psittaci*) or 72 h (*L. donovani*) after infection. At time zero, there were 32 to 50 *T. gondii* and 48 to 77 *L. donovani* organisms per 100 macrophages; parasite ingestion by cells treated with medium or any of the three reagents was comparable. A fold increase of >1.0 indicates parasite replication, an increase of 1.0 indicates inhibition, and an increase of <1.0 indicates parasite killing (12, 13). Twenty hours after *C. psittaci* challenge, 37 to 73% of control macrophages were infected.

organisms did not appear to be dependent upon the intracellular labile iron pool (1). It is possible that our results reflect differential iron availability within the various intracellular compartments of the macrophage where microbial replication takes place (1). However, *T. gondii* and *C. psittaci* reside, as does *L. pneumophila*, in phagosomes which do not fuse with lysosomes (1, 13, 16), and *L. donovani* multiplies in fused phagolysosomes (12, 14). Thus, the particular compartment in which intracellular replication occurs did not appear to influence our findings.

At the same time, however, it is clear that limiting the availability of iron represents a key antimicrobial effect of the human macrophage towards *L. pneumophila*, a bacterium whose intracellular replication is iron dependent (1). It is also possible that this mechanism may be relevant to macrophage activity against other intracellular bacteria which have high iron requirements. In addition, in mouse peritoneal macrophages, deferoxamine (30 to 35 μM) inhibits the intracellular multiplication of *Trypanosoma cruzi* (9) and, at much higher concentrations (≥500 μM), suppresses the replication of *Histoplasma capsulatum* (8). (Our attempts to use deferoxamine at >30 μM, however, resulted in toxicity with loss of the cell monolayer similar to the previous experience of Byrd and Horwitz with human monocytes [1].) In addition, IFN-γ-activated macrophages show reduced transferrin receptor expression (1, 5) and/or decreased cellular iron stores (2, 18), a state which appears to account for the capacity of iron-saturated transferrin to reverse the inhibition of *L. pneumophila* (1) and *H. capsulatum* (8) by IFN-activated human and mouse macrophages, respectively. However, recent data from an in vivo animal model of legionellosis (17) have suggested that restriction of iron transport (e.g., transferrin receptor downregulation) is not necessarily required for activated macrophages to inhibit *L. pneumophila* (17). Excess ferrous sulfate also partially or completely reverses the antimicrobial capacity of activated mouse macrophages towards *Schistosoma mansoni* (7) and *Leishmania enrietti* (10), respectively. In both of these models, the effect of iron was ascribed to diminishing the toxicity of L-arginine-dependent reactive nitrogen intermediates (7, 10). These products, however, do not appear to be involved in the in vitro antimicrobial activity of human macrophages (3, 6, 11) or at best may play a pathogen-restricted role (4).

Therefore, as judged by our results with *T. gondii*, *C. psittaci*, and *L. donovani*, limiting the availability of intracellular iron is not necessarily required for effective macrophage antimicrobial activity. Similar observations have been reported for tryptophan deprivation (15), another recently identified respiratory burst-independent pathway, which also seems to represent a narrow-spectrum antimicrobial mechanism of the activated human macrophage.

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