Role of iron in Trypanosoma cruzi infection of mice.

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Research Article

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Role of Iron in *Trypanosoma cruzi* Infection of Mice

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Abstract. The role of iron in experimental infection of mice with *Trypanosoma cruzi* was investigated. B_6 mice had a transient parasitemia and a transient anemia, both of maximal intensity 28 d after the inoculation of *T. cruzi*. There was a biphasic hypoferremic host response to infection with *T. cruzi* with the peak hypoferremia also occurring 28 d after inoculation of the parasite. The mortality rate from infection was increased from 23% in phosphate-buffered saline-treated B_6 mice to 50% in a group of B_6 mice receiving iron-dextran ($P \leq 0.025$), whereas depletion of iron stores with the iron chelator desferrioxamine B and an iron-deficient diet provided complete protection of B_6 mice ($P \leq 0.05$). The mortality rate in the highly susceptible C_3H strain was reduced from 100% in the control group to 45% ($P \leq 0.025$) in the iron-depleted group. The tissue iron stores were altered in mice receiving either iron-dextran or desferrioxamine B and an iron-deficient diet. In vitro, *T. cruzi* was shown to require both a heme and a nonheme iron source for an optimal growth rate. The effects of iron excess or depletion on the outcome of infection with *T. cruzi* correlated both with the growth requirements of the parasite for iron and with the availability of intracellular iron. Thus, it was suggested that the hypoferremic response, by sequestering iron within intracellular stores, potentially enhanced the pathogenicity of the intracellular parasites. Furthermore, the in vivo effects of iron excess and depletion correlated with an effect of iron on the growth rate and pathogenicity of the parasite.

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Introduction

Many systemic infections provoke a host hypoferremic response that reduces the level of iron in the plasma transferrin iron pool and thus limits the availability of extracellular iron (1, 2). This host protective response deprives invading extracellular pathogens of essential iron and thus restricts their growth and pathogenicity (3). The hypoferremia appears to arise from the sequestration of iron within the reticuloendothelial system (RES)[1] (4, 5). This host response is thereby of questionable benefit in defence against intracellular parasites replicating within the RES and using intracellular host iron pools.

Clinical investigation of iron-deficient subjects in Africa has shown that, compared with an untreated group, a group treated with iron had significantly more symptomatic infections with facultative and obligate intracellular pathogens (6). These observations have suggested that, in the absence of normal tissue iron stores, intracellular organisms were restricted in their growth and pathogenicity, whereas, restitution of iron stores promoted growth and pathogenicity of intracellular parasites.

Trypanosoma cruzi is a protozoan parasite causing widespread human disease in Latin America. Although found transiently in extracellular compartments, its growth is strictly intracellular, and it preferentially parasitises cells of the RES and muscle cells (7, 8). Laboratory mice inoculated with *T. cruzi* have served as an important animal model of acute Chagas' disease. During the course of infection, parasites replicate within the murine RES and muscle cells (8). Thus, murine Chagas' disease is a convenient and pertinent model to investigate the role of iron metabolism during systemic infection with intracellular pathogens. Here, we report on the influence of mouse iron status on the pathogenicity of *T. cruzi*.

Methods

Animals. A substrain of C57BL/6 (B6) mice was obtained from the University of Calgary Medical Vivarium (Calgary, Alberta, Canada) and C3H/HeJ (C_3H) females were obtained from the Jackson Laboratory (Bar Harbor, ME). Unless otherwise specified, animals were fed rat chow

1. *Abbreviations used in this paper:* FCS, fetal calf serum; LIT, liver infusion tryptose; RES, reticuloendothelial system; TIBC, total iron binding capacity; UIBC, unsaturated iron binding capacity.

5012 (Ralston Purina Co., St. Louis, MO) and given tap water ad lib. These mouse strains were chosen because of their resistance (B_6) or susceptibility (C_3H) to the Brazil strain of *T. cruzi* (9).

Parasite maintenance and culture. The Brazil strain of *T. cruzi* was used throughout these experiments. It was a gift of Dr. Wittner and Dr. Tanowitz, Albert Einstein College of Medicine, Bronx, NY. The blood form of the parasite was maintained in C_3H mice by serial passage of infected blood. Axenic *T. cruzi* epimastigotes were cultured from infected mouse blood and maintained in liver infusion tryptose (LIT) broth (Difco Laboratories, Detroit, MI) supplemented with hemin (Eastman Kodak Co., Rochester, NY), and 10% fetal calf serum (FCS) (Gibco Laboratories, Grand Island, NY) (10) at 27°C on a shaker platform rotating at 70 rpm.

Infection studies. Groups of sex- and age-matched mice were infected intraperitoneally with either 1×10^3 or 1×10^4 parasites. Parasitemia was determined at intervals during the infection, by removing a 10-μl sample of blood from the retroorbital venous plexous, with dilution in 0.84% (wt/vol) NH_4Cl and direct microscopic counting in an improved Neubauer chamber (American Optical Corp., Buffalo, NY). Hematocrits were also determined at various times during the course of infection. Additional mice were removed from the various experimental groups at various stages of infection and serum from these mice was used to assay for total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC), transferrin iron, and percent saturation of the transferrin iron pool by ^{59}Fe radioassay as described before (2). The cumulative mortality in the various groups was also scored.

Influence of iron addition or depletion on infection. The effects of exogenous iron on *T. cruzi* infection were examined by the administration of a colloidal iron dextran preparation (Dextran Products Ltd., Scarborough, Canada), which had been shown previously to be nontoxic for these mice to dosages >2,000 mg/kg (11). Infected mice were injected intraperitoneally with either 5 mg of Fe as iron dextran (~250 mg/kg) or phosphate-buffered saline (PBS) (control) 5 d after the inoculation with *T. cruzi*. One group of mice received five additional 1-mg injections of Fe every other day after the first 5-mg dose.

Iron depletion was instituted by the intraperitoneal injection of 10 mg of desferrioxamine B (Desferal, Ciba-Geigy, Dorval, Canada) on both the 5th and 6th d after the initiation of infection. Mice were placed on an iron-deficient diet (ICN Nutritional Biochemicals, Cleveland, OH) and iron-free glass-distilled water immediately after the first desferrioxamine B injection and maintained on this dietary regime until the completion of the experiments.

The effects of the various treatments (above) on iron status of control noninfected mice were also determined. PBS (control)- , iron dextran- , and desferrioxamine B-treated mice were examined 53 d after the treatments for weight, hematocrit, TIBC, transferrin iron saturation, and iron contents of both liver and spleen. Liver and spleen iron levels were determined in samples obtained from exsanguinated mice. The tissues were homogenized in iron-free glass-distilled water using a Ten Broeck tissue grinder (Corning Glass Works, Corning, NY), and iron concentrations were determined by atomic absorption spectrophotometry (Perkin-Elmer Corp., Norwalk, CT).

Iron requirements of T. cruzi. To evaluate the iron requirements of *T. cruzi*, axenic epimastigotes maintained in LIT containing 20 μg/ml of hemin and 10% FCS (complete medium) were transferred into and subsequently passaged in a hemin-deficient medium. After the second passage in the absence of hemin, the generation time of epimastigotes was increased. Epimastigotes depleted of hemin by three passages in hemin-deficient medium were inoculated either into the complete medium or into various test media containing 10% FCS. The growth rate of hemin-deficient epimastigotes in the complete medium was compared with the growth rate in LIT without hemin, LIT without hemin containing 21 μg/ml of the iron chelator desferrioxiamine B, and LIT without hemin containing desferrioxiamine B previously referrated with an equimolar concentration of ferric ammonium sulphate (Fisher Scientific Ltd., Montreal, Canada). The concentration of desferrioxamine B was sufficient to chelate all the iron in LIT containing 10% FCS. The iron content of the medium was determined by flame photometry (Perkin-Elmer Corp.).

Statistical analysis. Data were analyzed by a chi-square test with Yates correction, or a two-tailed *t* test. Statistical analyses were performed with a preprogrammed Hewlett-Packard Co. (Palo Alto) HP-67 Computer. The values of chi-square and *t* were determined. The corresponding value of *P* was obtained from tables published by Glantz (12).

Results

T. cruzi infection in B_6 mice. Female B_6 mice infected with *T. cruzi* had a transient detectable parasitemia beginning 12 d after, and persisting until 53 d after the intraperitoneal inoculation of blood forms of the parasite (Fig. 1 *A*). The intensity

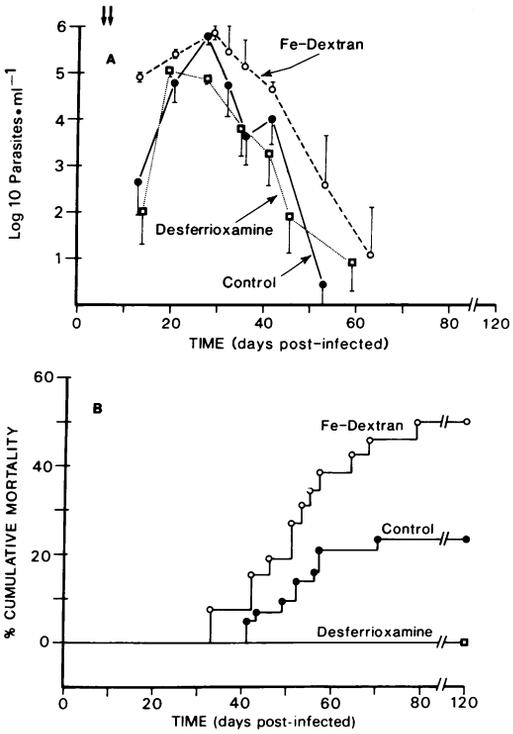

Figure 1. Effects of iron on *T. cruzi* infection in B_6 mice. Mice were infected with 10^4 parasites. The blood parasitemia (*A*) and the cumulative mortality rates (*B*) were monitored throughout the course of infection. On day 5, groups of infected mice received either PBS (●), iron-dextran (○), or 10 mg desferrioxamine given on both day 5 and day 6 (□). PBS- and iron-dextran-treated groups were maintained on normal rat chow while the desferrioxamine-treated group received an iron-deficient diet. The arrows indicate the days of treatment of infected mice.

of the parasitemia peaked 28 d after the inoculation of parasites, and 23% of infected mice died, the first deaths being recorded 41 d after the inoculation (Fig. 1 *B*).

Alterations in iron metabolism and hematocrit levels during infection with T. cruzi. The effects of *T. cruzi* infection on iron metabolism in B_6 mice were investigated. There was a biphasic hypoferremic response. Initially, the serum iron gradually decreased over the first 5 d of infection and there was a corresponding desaturation of transferrin (Fig. 2). The serum iron levels subsequently returned to normal, but a second more pronounced hypoferremia was detected with serum iron levels reaching 52% of control values 28 d after the inoculation of *T. cruzi* (Fig. 2). The iron saturation of transferrin was proportional to the level of serum iron, whereas the TIBC rose to a level 52% above control values on the 28th d of infection. The intensity of the second phase hypoferremia correlated with the intensity of the parasitemia (Figs. 1 and 2).

T. cruzi infection in B_6 mice also provoked a transient anemia. On the 25th d of infection, the hematocrit was reduced to 38% whereas, on the 52nd d, the hematocrit had returned to 46% (Fig. 3). Interestingly, the intensity of the anemia correlated with the degree of parasitemia.

Thus, *T. cruzi* infection of B_6 mice provoked a biphasic reduction in serum iron levels, an increase in serum transferrin, and a transient anemia. The intensity of these events correlated with the intensity of the parasitemia.

Influence of exogenous iron on infection with T. cruzi. The influence of exogenous iron on the course of infection was then investigated. 5 d after the inoculation of *T. cruzi,* groups of female B_6 mice either received PBS or iron-dextran containing 5 mg of iron. One group of iron-treated mice received five supplementary 1-mg doses of iron-dextran over the next 10 d. The mortality rate in iron-dextran treated mice was 50%, a significant increase when compared with the control group of mice ($P \leq 0.025$) (Fig. 1 *B* and Table I). Furthermore, the level and duration of parasitemia were increased slightly, although the differences between treated and control groups were not

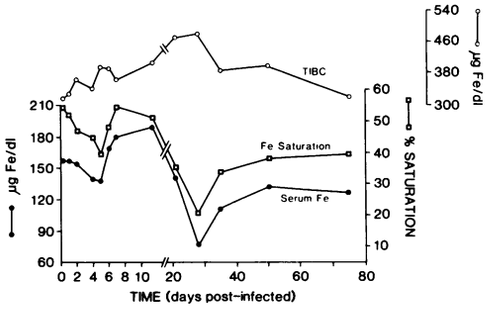

Figure 2. Influence of *T. cruzi* infection on the level of serum iron, TIBC, and saturation of transferrin. Male B_6 mice were infected with 10^4 parasites. Assays were performed on serum samples obtained at various intervals during the infection. The values obtained represent the mean of three to eight samples.

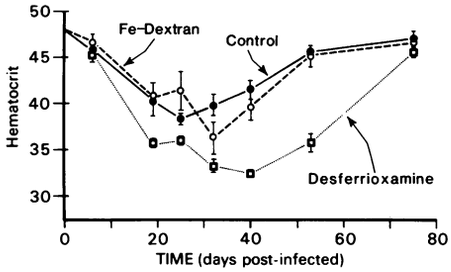

Figure 3. Influences of iron on the hematocrit in *T. cruzi*-infected mice. B_6 mice were infected with 10^4 parasites and the mean hematocrit ±SEM was determined at various times throughout the course of infection. After the inoculation, groups of mice received PBS (●), iron-dextran (○), or desferrioxamine B and iron-deficient diets (□). The differences between the control and desferrioxamine B group had the following *P* values: day 25, ≤0.025; day 32, ≤0.005; day 40 and 53, ≤0.001. All other comparisons resulted in a *P* value >0.05.

significant at the $P \leq 0.05$ level (Fig. 1 *A*). The mortality rate in the group of mice receiving repeated doses of iron-dextran was enhanced further to 61% (Table I).

Iron-dextran-treated mice also developed a transient anemia that did not differ from the anemia of control mice in either intensity or duration (Fig. 3). Thus, iron excess enhanced the pathogenicity of *T. cruzi* infection in B_6 mice without affecting the infection-induced anemia.

Groups of mice also received one injection of iron dextran either before or 53 d after the inoculation of *T. cruzi.* The course of infection in these groups of mice did not differ from control groups. The mortality rate in male B_6 mice was also enhanced by treatment with iron-dextran 5 d after the inoculation of *T. cruzi.* However, the number of mice in each group was not sufficient to reach a statistically significant difference.

Effects of partial iron depletion on the course of infection with T. cruzi. It had thus been determined that *T. cruzi* provoked a hypoferremic response, presumably by intracellular sequestration of iron within the RES (4, 5). Furthermore, it was observed that iron-dextran, which preferentially delivers iron to the intracellular RES stores (13), enhanced the pathogenicity of *T. cruzi* infection. Hence, it was of interest to determine the effects of reduction of intracellular iron stores on the course of infection with *T. cruzi.* Desferrioxamine B preferentially chelates intracellular iron and thus promotes its depletion by enhanced excretion in urine (14). Groups of B_6 mice were given desferrioxamine B 5 d after the inoculation of *T. cruzi,* while control mice received PBS. The desferrioxamine B groups of mice were fed an iron-deficient diet and the next day they received a second dose of desferrioxamine B. Mice iron-depleted in this fashion had a reduced mortality rate (0%) ($P \leq 0.05$) after inoculation of *T. cruzi,* whereas, the level of parasitemia was not significantly altered when compared with control-infected mice (Figs. 1 *A* and *B,* Table I).

Compared with infected control mice, iron-depleted infected mice had a significantly greater reduction in hematocrit (*P*

*Table I. Effect of Iron on T. cruzi Infection of B_6 Mice**

Mouse groups	Peak parasitemia	Mortality rate	Mean survival time	*P* value by chi-square
	log 10±SD/ml	*dead/infected (%)*	*d±SD*	
Control; female	5.6±0.5	10/43 (23%)	51.7±9	—
Control; male	6.4±0.2	1/14 (7%)	33	—
Iron-dextran 5 mg; female	5.8±0.7	13/26 (50%)	51.8±13	≤0.025
Iron-dextran 5 mg; male	6.4±0.4	5/14 (36%)	48.3±5	≤0.10
Iron-dextran-repeated doses; female	6.0±0.6	11/18 (61%)	49.1±7	≤0.05
Desferrioxamine B; female	5.0±0.4	0/14 (0%)	—	≤0.05
Desferrioxamine B; male	6.5±0.4	0/14 (0%)	—	≥0.1

* All mice were infected with 10^4 parasites. After 5 d, mice received either PBS, iron-dextran, or desferrioxamine B (see Methods).

≤ 0.05) from the 25th to the 53rd d of infection. However, after resolution of the acute stage of infection, the hematocrit returned to a normal level in the control and iron-depleted groups (Fig. 3).

Subsequently, the influence of iron depletion on the course of infection with *T. cruzi* was investigated in the highly susceptible C_3H mouse strain. The mortality rate in C_3H mice receiving 10^3 parasites was 100%, with a mean survival time of 36.5 d (Fig. 4, Table II). The mortality rate was significantly reduced to 45% in the iron-depleted groups ($P \leq 0.001$). Furthermore, in the iron-depleted C_3H mice that died, survival time was significantly prolonged to 43.7 d (Fig. 4, Table II).

Thus, reduction of intracellular iron stores reduced the pathogenicity of infection with *T. cruzi,* both in the moderately resistant B_6 mouse strain and in the highly susceptible C_3H strain. Furthermore, iron-depletion provoked a greater level of anemia during the acute stage of infection.

Effects of administration and chelation of iron on the status of uninfected mice. Uninfected B_6 female mice received treatments with iron-dextran or desferrioxamine B in a fashion similar to infected mice. The influence of these modalities of treatment on the body weight, hematocrit, serum iron, TIBC, percent saturation of transferrin, and tissue levels of iron in the liver and spleen were evaluated. Untreated, iron-treated, and iron-depleted uninfected mice all gained weight normally (Table III). 53 d after institution of treatment, the hematocrit level was not altered in either group of treated mice (Table III). However, mice treated with iron-dextran containing 5 mg of iron had 5.9 and 2.8 times more hepatic and splenic iron, respectively, than control mice, while their serum iron level was not significantly different (Table III). In mice treated with desferrioxamine and an iron-deficient diet, the storage of iron was also altered, with a reduction of 48 and 15% of hepatic and splenic iron and with modest changes in the serum iron level (Table III). Furthermore, the alteration in storage iron did not provoke any changes in the hematocrit level, thus indicating that the iron stores were sufficient to meet the normal requirements for erythropoiesis.

Iron requirements of T. cruzi epimastigotes. Axenic cultures of epimastigotes require a source of heme for optimal growth rates (10, 15), although it is not clear if it is the porphyrin, the iron, or both components which are required. The iron requirements of epimastigotes were evaluated by prior depletion of hemin stores. Sequential passage of parasites in LIT devoid of hemin prolonged the generation time and reduced the final yield. After six passages in the absence of hemin there was complete failure of replication (data not shown). Epimastigotes partially hemin-depleted by three passages in hemin-deficient medium resumed a normal rate of growth when inoculated into LIT containing hemin (Fig. 5). Hemin-depleted epimastigotes inoculated into hemin-deficient medium had an initial rate of growth similar to those in the complete medium. However, after 5 d the hemin-deficient medium failed to maintain a normal rate of growth and the final yield was reduced from 73×10^6/ml to 25×10^6/ml (Fig. 5). When the iron chelator desferrioxamine B was added to the hemin-deficient medium, an even

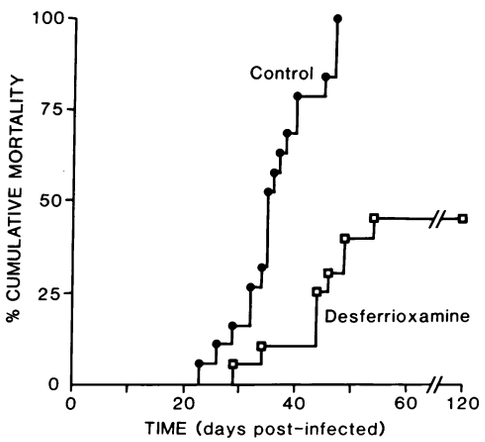

Figure 4. Effects of iron depletion on *T. cruzi* infection in C_3H mice. C_3H mice were infected with 10^3 parasites and the course of infection was monitored. Groups of mice received either PBS (●), on day 6, or 10 mg desferrioxamine B on day 6 and 7 (□). PBS-treated mice were fed a normal diet, and desferroxamine-treated mice received an iron-deficient diet.

*Table II. Effect of Iron on the Course of T. cruzi Infection of C_3H Mice**

Mouse group	Peak parasitemia	Mortality rate	*P* value by chi-square	Mean time to death	*P* value by *t* test
	log 10 parasites±SD/ml	*(dead/infected [%])*		*days±SD*	
Control	6.54±0.39	19/19 (100%)	—	36.5±6.8	—
Desferrioxamine B	6.33±0.16	9/20 (45%)	≤0.001	43.7±7.7	≤0.025

* All mice were infected with 10^3 parasites. After 5 d, mice either received PBS or desferrioxamine (see Methods).

greater reduction in growth rate and yield were observed (Fig. 5). Thus, epimastigotes of *T. cruzi* required two sources of iron for optimal growth. Heme was essential and in its absence a requirement for chelatable iron was demonstrated. As compared with desferrioxamine B, referrated desferrioxamine B did not reduce the growth rate of hemin-deprived epimastigotes (Fig. 5), thus demonstrating that desferrioxamine B had inhibited growth by chelation of iron. Desferrioxamine B when added to the complete medium had no influence on the growth rate of epimastigotes.

Discussion

Inoculation of *T. cruzi* into B_6 mice produced a subacute infection with a transient parasitemia and a 23% mortality rate. The course of infection was characterized by a biphasic hypoferremic response and a transient anemia. The intensity of the anemia and of the secondary hypoferremia correlated with the intensity of the parasitemia. Exogenous iron was administered in a form that is specifically delivered to the intracellular stores of the RES (13) and this provoked a significant increase in the mortality rate of *T. cruzi* infection. Conversely, depletion of RES iron stores with desferrioxamine B (14) and an iron-deficient diet provided complete protection of B_6 mice from death as a result of infection from *T. cruzi*. Furthermore, similar iron depletion protected the highly susceptible C_3H mouse strain by reducing the mortality rate from 100 to 45% and also by prolonging the survival time in iron-depleted C_3H mice that died. *T. cruzi* epimastigotes were shown to have a growth requirement for both heme iron and chelatable iron. Thus, the influence of iron excess or depletion correlated with the influence of iron availability on the growth rate of *T. cruzi* epimastigotes.

Although *T. cruzi* transits through extracellular spaces in the form of a trypomastigote, growth occurs strictly within cells in the form of amastigotes (7). During the acute stage of infection, injury to host tissues results predominantly from the replication of amastigotes (7). In vitro, *T. cruzi* assumes the epimastigote form. Metabolic events in epimastigotes and amastigotes are usually quite similar (16). Thus, the observed epimastigote requirement for iron is a reliable indicator for a similar iron requirement in amastigotes. Therefore, the enhanced mortality

*Table III. Effect of Iron Administration or Depletion on Weight, Hematocrit, and Content of Iron in Serum and Tissues of Uninfected B_6 Mice**

	Control	Iron-Dextran	Desferrioxamine
Weight (*g±SD*)	20.8±1.3	21.3±0.7	21.4±0.6
Hematocrit (*%±SD*)	47.3±1.0	46.2±1.6	48.0±1.4
Serum iron (*μg/dl±SD*)	140.7±12.3	120.3±19.0	116.6±55.6
TIBC (*μg/dl±SD*)	381.0±23.5	372.3±8.7	408.5±21.7
Transferrin iron saturation (*%±SD*)	37.2±3.1	32.3±4.5	28.4±12.2
Hepatic iron (*μg/mg tissue±SD*)	0.077±0.032	0.453±0.040	0.040±0.014
Splenic iron (*μg/mg tissue±SD*)	0.233±0.035	0.513±0.188	0.197±0.038

* Uninfected B_6 female mice were treated with PBS, iron dextran, or desferrioxamine B (see Methods). The assays were performed on samples obtained from uninfected mice 53 d after treatment.

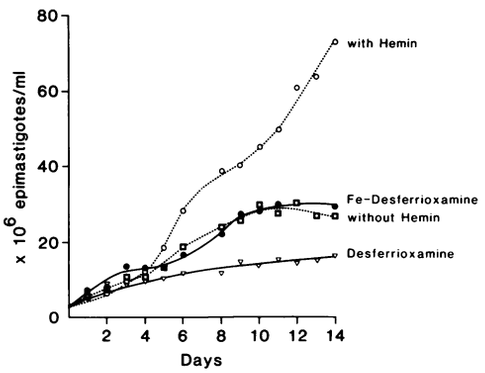

Figure 5. Influence of iron availability on growth of *T. cruzi* epimastigotes in vitro. Axenic cultures of epimastigotes were passaged thrice in hemin-deficient LIT containing 10% FCS. Hemin-depleted epimastigotes were either inoculated into complete medium (○) or into test media deficient in hemin and the growth rates were monitored. The test media all contained 10% FCS and were formulated as follows: LIT without hemin (□), LIT without hemin containing desferrioxamine B (▽), LIT without hemin containing referrated desferrioxamine B (●).

in iron-dextran-treated mice resulted from stimulation of growth of the parasite and hence, increased pathogenicity. Conversely, the reduced mortality in iron-depleted mice resulted from starvation of the parasite for iron with a restriction on its growth rate.

Although the treatments had dramatic effects on the mortality rates, the effects on the parasitemia were modest. While investigating the effects of drug therapy on murine Chagas' disease, Cover and Gutteridge (17) have also observed that the outcome of infection with *T. cruzi* was poorly correlated with the level of parasitemia. Thus, it is suggested that the outcome of infection with *T. cruzi* is determined not by the number of parasites in the circulation but by the tissue load of actively replicating intracellular amastigotes.

All pathogenic microorganisms require iron for optimal growth and virulence (1). The hypoferremic response to systemic infection is protective because it reduces the availability of iron to extracellular pathogens (1). Hypoferremia is predominantly the result of sequestration of iron within the intracellular stores of the RES (4, 5). Thus, during a hypoferremic response, there is potentially an increased availability of iron to intracellular pathogens lodged within the RES.

Clinical investigations in Africa have shown that subjects with depleted iron stores are protected against several intracellular pathogens (6). Furthermore, iron-dextran specifically produces an increase in the intracellular RES iron levels (13), whereas desferrioxamine B specifically chelates and removes intracellular iron rather than the iron bound to transferrin (14). Thus, desferrioxamine B and an iron-deficient diet protected against intracellular *T. cruzi* by a reduction in the intracellular iron stores, whereas iron-dextran produced a detrimental effect by augmenting intracellular iron stores.

Iron depletion that is severe enough to seriously impair erythropoiesis usually depresses several host immune responses and this may increase susceptibility to infection (1, 18). The tissue iron levels in uninfected mice receiving desferrioxamine B were only modestly decreased, the serum iron concentration was not significantly altered, and erythropoiesis was normal. Hence, infected mice treated with desferrioxamine B likely had sufficient iron stores to maintain normal immune responses. All mice infected with *T. cruzi* had an unexplained transient anemia. In the iron-depleted infected mice, the anemia was more pronounced and lasted longer. Thus, in the iron-depleted mice, it is likely that the rate of erythropoiesis was transiently limited by a restricted availability of iron. After recovery from the infection, the hematocrit returned to a normal level in all three groups of infected mice. Hence, the iron stores in the desferrioxamine B-treated group, although reduced, were again adequate to meet the needs of normal erythropoiesis.

A reduction in the iron stores has produced beneficial results in several experimental infections (19). Furthermore, administration of exogenous iron has similarly enhanced the pathogenicity of several infectious agents, both extracellular (1, 2) and intracellular (1, 20, 21). The mortality rate of normal or immune mice infected with *Mycobacterium tuberculosis* was enhanced by exogenous iron (21). During the course of infection, the replication of *M. tuberculosis* is largely inside phagocytic cells of the RES (22). Thus, our results confirm Kochan's observation (21) that exogenous iron increases the pathogenicity of infection with some intracellular parasites.

Mice infected with *T. cruzi* clearly show a biphasic hypoferremic response composed of a decrease in serum iron concentration and an increased iron-binding capacity. Iron availability would thus seemingly be more restricted for extracellular than for intracellular pathogens. Indeed, the maximal hypoferremic response coincided with the peak parasite replication, and thus, the intracellular parasites likely profited from the host hypoferremic response.

Although there are some conflicting reports (18), it appears that mild iron deficiency will protect against several human and experimental intracellular pathogens (1, 6, 20). Thus, there may be an optimal level of storage iron that will meet physiologic requirements of the host while restricting the rate of proliferation of intracellular pathogens. In mice, a 50% reduction in hepatic iron stores has conferred greater resistance against *T. cruzi,* while providing enough iron for normal erythropoiesis and host defences.

In conclusion, mice infected with *T. cruzi* had a biphasic hypoferremic response. Treatment of mice with exogenous iron enhanced the mortality rate of *T. cruzi* infection, whereas depletion of iron with desferrioxamine B and an iron-deficient diet was protective. The effects of treatment correlated with the in vitro growth requirements of *T. cruzi* for iron. Thus, it was suggested that the physiologic hypoferremic response potentially increased the amount of iron available to *T. cruzi;* exogenous iron further increased the iron available to the parasite; and desferrioxamine B and an iron-deficient diet reduced the availability of iron. Furthermore, the pathogenicity of *T. cruzi* correlated with its growth rate and with the amount of iron available within intracellular stores of the host.

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