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Immunological control of chronic *Trypanosoma brucei gambiense* in outbred rodents

P. DIFFLEY, J. O. SCOTT

Summary

Recent human isolates of *Trypanosoma brucei gambiense* generally fail to become or remain patent in laboratory rodents. The purpose of this study was to determine if this was due to acquired immunity and if so which immunosuppressive method was the most efficient in raising parasitemia levels. Prior to infection, rats and mice were immunosuppressed by treatments with cobra venom factor, anti-lymphocyte sera, hydrocortisone acetate, cyclophosphamide; by splenectomy; or by lethal X-irradiation. While no parasites were detected in the blood of most of the untreated rodents for 30 days postinfection, all immunosuppressive procedures resulted in patent parasitemias in at least fifty percent of the treated animals. The most effective method, lethal X-irradiation, consistently caused fulminating infections typical of acute African trypanosomiasis. Cyclophosphamide had the same effect as X-irradiation in rats but was less effective in mice. Splenectomy allowed fulminating first peak parasitemias in two-thirds of the rodents while cobra venom factor and anti-lymphocyte sera in general allowed only low first peaks of parasitemia that were resolved within 10 days of infection. Hydrocortisone acetate allowed low grade and sporadically patent infections throughout the 30-day study. To determine if in untreated rodents, the parasites were eliminated or maintained in a subpatent state, rodents infected for 30–45 days were immunosuppressed with cyclophosphamide. Fifty percent of rats and thirty percent of mice developed patent parasitemias within one week of immunosuppression suggesting that a majority of animals had eliminated the infection. The doubling time for *T. b. gambiense* in normal and immunosuppressed rodents was determined to be two times a day, one-half the rate of more virulent *Trypanosoma brucei* spp. isolates. The parasite growth rate, therefore, is the most likely factor in the effective host control of this parasite during the first peak of parasitemia. This experimental host-parasite relationship may be a model for the study of chronic African

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trypanosomiasis caused in humans by *T. b. gambiense* and in native ungulates by *T. b. brucei* and *T. b. rhodesiense*.

Key words: chronic African trypanosomiasis; *Trypanosoma brucei gambiense*; immunosuppression; cyclophosphamide; anti-lymphocyte serum; cobra venom factor; splenectomy, hydrocortisone acetate; lethal X-irradiation.

Introduction

African trypanosomiasis runs two different courses in humans and in other mammals (reviewed by Hoare, 1972). There is the acute disease caused by *Trypanosoma brucei brucei* and *Trypanosoma brucei rhodesiense* which has high levels of parasites in the blood and which is lethal within a year. There is also the chronic disease caused in humans by *Trypanosoma brucei gambiense* and presumably in native African ungulates by all trypanosome species. In the chronic disease, there are few if any parasites in the peripheral blood. Humans survive many years before central nervous system involvement induces the fatal sleeping sickness syndrome. The acute infection is the most often studied since it is most often encountered in laboratory rodents. Recent isolates of *T. b. gambiense* generally do not become or remain patent in the blood of adult laboratory animals but weanling rodents have been used to establish stabilates (Gray, 1972). The purpose of this study was to determine if the control of the *T. b. gambiense*-infection was immunologically based, if that immunological control included elimination of the parasite, and which immunosuppressive method was the most effective in allowing patent parasitemias.

Materials and Methods

Parasites

A *T. b. gambiense* line, DTR 20, initiated from TREU 1309 kindly provided by Prof. B. M. Honigberg, was used in this study. The history of isolation of TREU 1309 is described by Gray (1972). As reported, adult rats maintained low grade, avirulent infections even after 20–30 passages. The acute disease could only be established in 8–10 day old rats after 4–46 passages. After receipt in this laboratory, the stabilate was passaged twice in weanling rodents before cryopreservation in 10% glycerol. Infections in normal adult rodents (Diffley, 1978 and present study) indicate that this stabilate retains the parasitemia levels and avirulence associated with those recently isolated from humans.

Laboratory infections

Outbred adult (4 mo.) ICR mice (TIMCO Breeding Labs) and Sprague Dawley rats (Texas Tech University Vivarium) were used for all experimental infections. Infections were initiated by intraperitoneal injections of cryopreserved trypanosomes (10^4 /animal). Infections were monitored at 24-h intervals by microscopic examination of tail blood. Levels of parasitemia were estimated according to the method of Herbert and Lumsden (1976).

Immunosuppression

Several methods were employed. Rabbit anti-mouse or rat lymphocyte sera (ALS) are lymphocytotoxic and delay allograft rejection (Sell, 1969). The sera were administered via the intraperitoneal route (2 ml to rats and 0.25 ml to mice as recommended by the manufacturer, Microbiological Associates) one day before and one day after infection.

Cobra venom factor (CVF) depletes C3–C9 components by activation of the alternate pathways (Brai and Olser, 1972) which may reduce the induction (Pepys, 1974) or the function of antibody (Nelson, 1966). Administered in dosages recommended by the manufacturer (Cordis Laboratories), 200 units CVF/kg body wt were administered via the intravenous route one day before and one day after infection.

Cyclophosphamide (CY) is a radiomimetic drug (Gabrielson and Good, 1967). It has been used to exacerbate acute *Trypanosoma brucei brucei* infections (Smith et al., 1982; Vickerman et al., 1976). One day before infection, rats were injected via the intraperitoneal route (200 mg/kg body wt); mice at dosages ranging from 200–800 mg/kg. Two commercial sources for CY were used, Mead Johnson and Sigma. The CY was placed in sterile saline at 4° C for at least 2 wk prior to immunosuppression. To determine if *T. b. gambiense* remained in the host in a chronic, subpatent state, CY (200 mg/kg) was administered to rodents up to 45 days postinfection.

Hydrocortisone acetate (HC) from Sigma is lymphocytotoxic and inhibitory to lymphocyte and macrophage function (Cupps and Fauci, 1982). It was administered at 400 mg/kg body wt via the intramuscular route 2 days before and 2 days after infection. This same regimen (Diffley, 1978; Diffley and Honigberg, 1978) and a different regimen (Luckins, 1972) have been used to immunosuppress rodents before infections with African trypanosomes.

Splenectomy is a quick and inexpensive form of immunosuppression that is known to exacerbate other parasitic infections such as amebiasis (Ghadirion and Meerovitch, 1982). Rodents were infected one day after splenectomy.

Lethal X-irradiation (Daria et al., 1982) has been used to exacerbate acute African trypanosomiasis (Balber, 1972; Luckins, 1972) and to establish *Trypanosoma vivax* in rodents (Gee and Shah, 1980). Rodents were exposed to a total of 900 rads whole body irradiation (linear acceleration-proton source) one day before infection with *T. b. gambiense*.

Results

Pre-infection immunosuppression

Data for the first 10 days of infections with *T. b. gambiense* in rats are depicted in Fig. 1. Two thirds of untreated rats, infected with 10^4 parasites, failed to develop patent parasitemias during the entire course of the 30-day study. After HC, ALS, or CVF-treatment, about two-thirds of the rats had patent infections within the first 10 days of infection. These animals were capable of remitting the parasitemia by 9 days postinfection presumably as immune components and functions were restored. Low numbers of parasites were detected occasionally in one half of these treated rats from 5–30 days postinfection. Splenectomy caused fulminating and lethal first peak parasitemias in two-thirds of the rats. The other splenectomized rats remitted the first peak, were sporadically patent in infection, but survived the 30-day test period. Both CY-treatment and X-irradiation consistently allowed fulminating infections and death of the hosts with 10 days postinfection.

The immunosuppressive-regimens were repeated in mice with similar results (Fig. 2). Untreated mice had subpatent infections and CVF, ALS, and

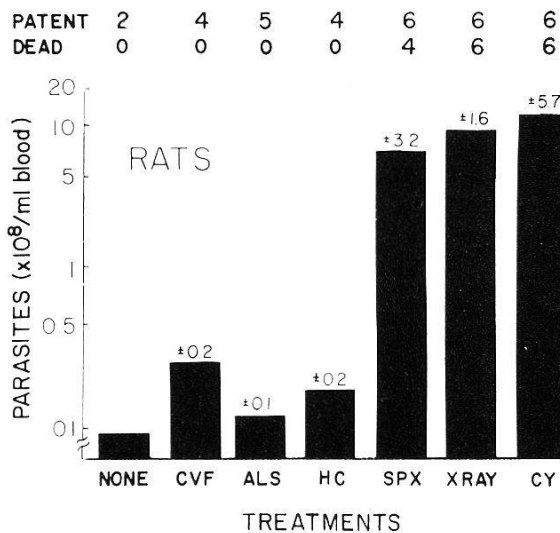


Fig. 1. The effects of immunosuppression of rats prior to infection. Depicted are the average first peak parasitemias (\pm s.d.) of 6 rats/group which were untreated or treated with cobra venom factor (CVF), anti-rat lymphocyte serum (ALS), hydrocortisone acetate (HC), lethal X-irradiation (X-RAY), or cyclophosphamide (CY) or were splenectomized (SPX) prior to infection with 10^4 *T. b. gambiense*. Also depicted are the number of patent infections and host deaths recorded during the first 10 days postinfection.

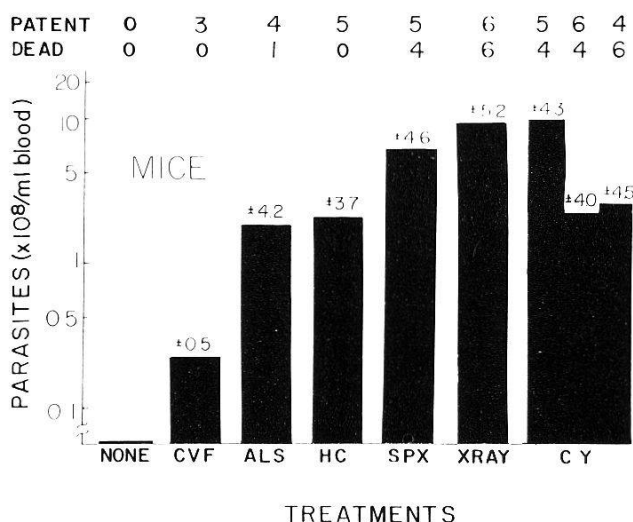


Fig. 2. The effects of immunosuppression of mice prior to infection. Depicted are the average first peak parasitemias (\pm s.d.) for 6 mice/group which were untreated or treated with cobra venom factor (CVF), anti-mouse lymphocyte serum (ALS), hydrocortisone acetate (HC), lethal X-irradiation (X-RAY), or cyclophosphamide (CY) at 3 dosages (200, 400, 800 mg/kg) or were splenectomized (SPX) prior to infection with 10^4 *T. b. gambiense*. Also depicted are the number of patent infections and host deaths recorded during the first 10 days postinfection.

HC were inconsistent in their immunosuppressive effects. Both ALS and HC allowed patent infections higher in mice than in rats. Mice treated with ALS or CVF remitted the first peak of parasitemia and in general did not have detectable numbers of trypanosomes for the duration of the 30-day study. Two-thirds of the mice treated with HC retained patent parasitemias and died during the

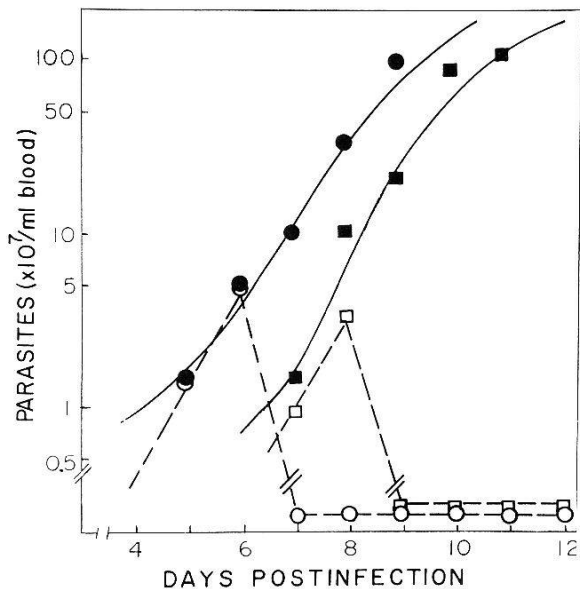


Fig. 3. Average *T. b. gambiense* (DTR20 stabilate) infections in mice that remitted (--○--) and mice that did not remit the first peak of parasitemia (—●—) and rats that remitted (--□--) and did not remit (—■—) the peak of parasitemia.

course of the study. Splenectomy resulted in a single fulminating, lethal first peak of parasitemia in four mice, the other two surviving the test period. X-irradiation caused complete immunosuppression and high levels of parasitemia resulting in death of the host.

The effect of CY-treatments upon infection differed between rats and mice. Cyclophosphamide, administered at sublethal dosages, caused high levels of parasitemia in 5 mice of which only one survived. Increasing the level of CY to lethal levels in the mouse appeared to affect parasite growth. The radiomimetic and/or cytotoxic effect of CY, apparently affected both host and parasite. Both commercial sources of CY worked equally well.

The average first peaks of parasitemia in lethally-immunosuppressed and in non-lethally treated and normal rodents are depicted in Fig. 3. A comparison of growth rates indicated the same trypanosome doubling time (2 times a day) in rats and mice and in lethally-suppressed and nonlethally-suppressed rodents.

Post-infection immunosuppression

Once it was determined that the first peak parasitemias were controlled by acquired immune effector mechanisms, rodents were suppressed to determine if infections were maintained in a chronic, subpatent state or were completely eliminated. Rats and mice in which no parasites were detected from at least 5 days postinfection, were treated with CY (200 mg/kg) at various times. From the data presented in Table 1, it was concluded that about 50% of rats and about 30% of mice harbored subpatent infections for at least 30–45 days.

In the case of rats, the probability of having a patent infection after CY-treatment at 30 days was closely correlated to the presence of a patent first peak

Table 1. Post-infection immunosuppression

CY-treatment on post-infection day	No. of patent parasitemias/total no. tested	
	rats	mice
10	5/5	–
15	2/5	2/5
30	11/19	4/20
45	–	2/7

of parasitemia. Of the rats without a patent first peak, 7/8 showed no parasites after CY-treatment. Of the rats with a patent first peak, 10/11 had observable infections after CY-treatments. This correlation could not be established, however, among mice. One-half of these hosts that had patent infections after CY-treatment at 30 days, did not have patent first peaks.

Survivors were periodically monitored for patent parasitemias over a 6-month period. No deaths were recorded and no parasites were detected in the blood of any survivor.

Discussion

It was concluded from this study that low grade infections in rodents caused by *T. b. gambiense* are controlled by acquired immune responses and not by innate immune factors. At 30 days postinfection one-half of the rats and one-third of the mice maintained subpatent infections under immunological control. This suggests that a majority of rodents had completely eliminated the infection or at least severely restricted the number of bloodstream form trypomastigotes.

Lethal X-irradiation and high doses of CY were very effective and consistent methods for eliminating this immunological control. Splenectomy caused fulminating parasitemias in two-thirds of infected rodents. Hydrocortisone, ALS and CVF were the least effective. While anti-mu chain serum has been found to be quite effective in eliminating immunological control over African trypanosomiasis (Campbell et al., 1977) its cost for anyone who had to purchase the antibody would be prohibitive. Inbred mice have also been reported to vary in susceptibility to acute African trypanosomiasis (e.g. Levine and Mansfield, 1981) and to the low grade, chronic *T. b. gambiense*-infections (Perez and Diflely, unpublished results). Still, it would be less expensive to purchase outbred rodents and immunosuppress them prior to infection if one wished only to collect large numbers of parasites for cloning, cryopreservation or experiments. It is hoped that this information will be helpful to those attempting to maintain fresh human isolates of *T. b. gambiense* in rodents (e.g. Beckers et al., 1981;

Gray, 1972). It must be noted, however, that if trypanosome isolates are injected in low numbers and divide very slowly, immunosuppression of hosts will not have immediate results. The hosts will die of or overcome immunosuppression before the parasites reach patent levels. In this case, several passages of large amounts of blood through immunosuppressed rodents may be required for the inocula to reach levels that will result in patent parasitemias.

It was also concluded from this study that the *T. b. gambiense* DTR20 stabilate, like the more virulent *T. brucei* subspecies isolates can develop massive parasitemias that result in the death of their (immunosuppressed) hosts. Not surprisingly, pathogenesis and death of the host during acute disease is related to parasite load. Virulence of African trypanosomes, therefore, is dependent upon inocula (either the number of parasites in the initial injection or the number of heterologous variants remaining after remission) and upon growth rates. For example, *Trypanosoma brucei rhodesiense*, DTR11.1 stabilate, doubles its population four times a day (until reaching a density of 10^8 /ml blood) in rats (Diffley et al., 1980) and in mice (unpublished results). Given a high enough dose of trypanosomes, rats cannot control the first peak. Lower inocula result in remission of over 98% of the parasites. The *T. b. gambiense* DTR20 stabilate used in this study divides only two times a day in rodents and therefore can be immunologically controlled before the infection overwhelms the host. Since the generation of variants is related to time and parasite numbers, the low growth rate further insures a smaller number of variants after the first and subsequent peaks of parasitemia.

It is intriguing that this *T. b. gambiense* stabilate remains subpatent after the first peak of parasitemia and appears to be eliminated in over 50% of rodents by 30 postinfection days. This could be due to: low parasite growth rates that cannot replace parasites eliminated by variant-specific immune responses, or to an acquired, cross-variant immune response that eliminates or metabolically-controls the trypanosome, such as ablastin's effect upon *Trypanosoma lewisi* (Patton, 1975). The basis for this "benign" host-parasite relationship involving African trypanosomes is currently being explored.

Acknowledgments

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