Trypanosoma lewisi Infection in the Rat: Effect of Adenine

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ABSTRACT The reproductive activity of Trypanosoma lewisi was strikingly enhanced and sometimes prolonged for 2 days when sublethal doses of adenine were given to rats. The drug was effective when given for 5 or 7 days in the diet and/or intraperitoneally, starting one or two days before infection through two days after infection. Peak parasitemia was also enhanced. In terms of host immunity, daily sublethal doses of adenine given to rats within two days before or after infection with T. lewisi significantly depressed a hitherto unrevealed natural immunity to reproduction of the trypanosomes, but only slightly modified the development of acquired immunity.

Ablastin is an antibody elaborated by the rat that inhibits reproduction of its blood parasite, Trypanosoma lewisi (1). Ablastin, in addition, decreases carbohydrate metabolism of the trypanosomes (2), protein and nucleic acid synthesis (3), and some enzymes (4). At peak parasitemia, an early trypanolysin sweeps many of the trypanosomes from the blood, and a later one ends the infection (5, 6). Sublethal doses of adenine (oral or parentral) produce toxic effects in dogs, mice, and rats such as renal damage (7-10) and possible derangements of nucleic acid metabolism (7). Adenine also inhibits growth of mammalian cells in vitro at concentrations of 10^-4 M or higher (11). In view of these effects, excess adenine might be assumed to interfere with the product ion of antibodies by inhibiting DNA synthesis in rapidly proliferating antibody-forming cells. Thus, flooding the rat with adenine before the start of ablastin formation might inhibit synthesis of ablastin so that T. lewisi, when introduced into the rat, would divide more prolifically. Flooding the rat with adenine after ablastin is formed might decrease further formation of ablastin to such an extent that T. lewisi, already introduced into the rat and partially inhibited from dividing, would resume reproduction. The first of these situations, but not the second one, was encountered in the current work. In addition, adenine, when introduced early in the infection, delayed the appearance of the early trypanolysin.

MATERIALS AND METHODS

The strain of white rats, designated SD/Anl[Anl 63], was started from germfree Sprague-Dawley weanling rats that were purchased from A. R. Schmidt Co., Inc., Madison, Wis., in 1963. In 1964, the rats suffered a hemorrhagic disease associated with a possible vitamin K deficiency (12), but were healthy and free of respiratory infections and external and internal animal parasites at the time the current experiments were performed in the fall of 1965. Ten series of three female rats were used: each rat weighed 120-230 g when infected intraperitoneally with trypanosome-containing blood from donor infected rats. Series 1 through 7 were given adenine; the 3 control series were not. All rats were housed in air-conditioned rooms at a temperature of 22°C, humidity of 40%, and 12-hr changes of light and dark.

The strain of T. lewisi is the same one used by Moulder (2) and Taliaferro and Pizzi (3). It has been maintained in rats by blood passage in the Department of Microbiology at the University of Chicago. Each rat in the current study was infected intraperitoneally with 10^9 T. lewisi per 100 g of rat.

Blood films were made from the rats at 9 a.m. and 5 or 8 p.m. daily for 10 or more days after their infections became patent. The slides were stained with May-Gruenwald (0.3 g in 100 ml of methyl alcohol) Giemsa stain (Sargent). The slides were flooded with May-Gruenwald for 1 min; an equal amount of distilled water was added quickly but carefully; after 1 min the slides were decaent and placed in Coplin jars that contained Giemsa diluted with 15 parts of distilled water for 2 hr, washed in tap water, and dried.

The stained blood films yielded two measures. (a) Parasite counts were calculated as the number of trypanosomes per mm^3 of blood after obtaining ratios of trypanosomes to red cells on each stained slide. Hemacytometer counts of red cells were made twice a week. (b) Reproductive activity on each stained slide was obtained as a percentage of dividing and short trypanosomes in random samples of 100-300 trypanosomes. Short forms were those measuring less than 20 μm, as determined by camera lucida drawings at a magnification of X3000 (3, 13).

Series 1 through 4 and their respective control series were fed a diet (obtained from Nutritional Biochemicals, Cleveland, Ohio) consisting of “vitamin free” casein, 18%; vegetable oil, 3%; salt mixture-w, 4%; sucrose, 75%; and an adequate vitamin fortification mixture (1 kg/100-lb diet). Each rat, caged separately, obtained its food by going through a short tunnel: the food was offered during each 24-hr period at hourly intervals one twenty-fourth of 20 g of the diet, with or without 100 mg of adenine, in small pans via an automatic apparatus that worked on the principle of a Ferris wheel. The apparatus accommodated 12 rats at a time. Before the actual test, each rat was starved for 2 days and was then given the diet free of adenine for 2 days. Residual food was weighed each day to ascertain the amount of food and adenine eaten.

Series 5 through 7, which were given adenine intraperitoneally, and their control series were fed Wayne Lab Blox
(Allied Mills, Peoria, Ill.). These rats, in groups of six, were kept in plastic cages, equipped with stainless steel tops containing a feeder and two water bottles.

Also studied were the effects of adenine on blood stream forms of *T. lewisi* grown in vitro at 37°C. Details of the culture method have been described (14). The complete medium consisted of normal rat serum, lactalbumin hydrolysate, yeast extract, and Hanks' balanced salt solution that contained various amounts of adenine. In each experiment, concentrations of the purine in the complete medium ranged, in doubling amounts, from $2.34 \times 10^{-4}$ M to $1.5 \times 10^{-4}$ M. After inoculation with adult trypanosomes, the cultures were incubated overnight (17-18 hr) at 37°C. A sample was then removed from each flask, spread on a glass microscope slide, and stained with Giemsa stain. Reproductive activity was measured as described above.

Adenine (Calbiochem, Los Angeles) did not readily dissolve in 0.85% saline solution. Therefore, the stock solution (400 mg of adenine per 100 ml of warm 0.85% saline solution) was shaken three times for periods of 30 min at room temperature, with intervals of 1.5 hr at 6°C between. This solution, which was used within a week, was kept at 6°C; aliquots of it were heated to body temperature just before injecting it into the rats with a 5-ml syringe (23-gauge needle).

### RESULTS

**Untreated infected controls**

Characteristic features of the *T. lewisi* infection, after the intraperitoneal injection of trypanosomes, are shown in the mean-control plots in Fig. 1. Trypanosomes, after appearing in the blood—usually on day 2—rapidly reproduced and increased in number for several days, then decreased in number thereafter. In terms of host immunity, a blastin, which develops and inhibits reproduction of the trypanosomes in about a week, is aided by the formation of trypanolysins (1-6). The early blastin was not as potent in the current work as has been previously reported (1,3).

**Sublethal doses of adenine in the diet and injected intraperitoneally**

Series 1-4 were offered adenine in their diet before or after infection with 10- to 12-day-old *T. lewisi*. The rats did not

![Fig. 1. Mean plots of reproductive activity (A, C, E) and parasite counts (B, D, F) from 3 groups (3 rats each) given daily doses of adenine starting 2 days before, the day of, and 2 days after infection with *T. lewisi*, and similar data from two untreated control groups of 3 rats each. Note, in the adenine-treated rats as compared to the controls, enhanced reproductive activity and intense parasitemia as well as the maintenance of peak parasitemia for two additional days.](image-url)
like the adenine-supplemented diet; what little was eaten was confined almost exclusively to intervals between 4 p.m. and 6 a.m. During a 5- or 7-day period of treatment, the rats ate 14 ± 1 mg of adenine per 100 g of rat per day. This amount was supplemented by the intraperitoneal injection of 4 mg of adenine per 100 g of rat at 9 a.m. and at 1 and 3 p.m. The rats were weighed every day, and varied between 152 and 190 g in series 1 and its control series and between 190 and 230 g in series 2, 3, and 4 and their control series.

The three rats of series 1 were given adenine for 7 days, from 2 days before to 5 days after infection with *T. lewisi*. As shown by mean percentages, dividing and short forms (Fig. 1A) markedly increased in the treated, as compared to the control rats, especially on days 3 (13.7 vs. 6%), 4 (12.3 vs. 3%), and 5 (10 vs. 2%). In addition, they continued to be present through the morning of day 7 (1.3%) in the treated rats but disappeared during day 5 in the controls. Concomitantly, the mean parasite count was markedly enhanced in the adenine-treated group (Fig. 1B). The decline in mean reproductive activity from 13.7 to 6.7% in 8 hr on day 3 in the adenine-treated group may have been due to the cyclic nature of division during the early stages of reproductive activity. This idea is reinforced by a corresponding dip in mean reproductive activity from 6 to 4% during the same interval in the control series. Enhanced reproduction in this, and in other adenine-treated series, was probably brought about by more intense division per trypanosome. Multiplying-dividing rosettes, however, were not found to be more numerous—probably because they were so scarce (<1%) in all populations.

The three rats of series 2 were given adenine for 5 days, starting on day 0 of their infection. As shown by mean percentages, dividing and short forms markedly increased in the treated as compared to the control rats (Fig. 1C). The increase started on day 2.5, reached a peak on day 3 (17.3 vs. 5.7%) and was maintained through day 5 (9.3 vs. 2.7%). During this time, as would be expected, the mean parasite count was higher in the treated than in the control series (Fig. 1D).

The three rats of series 3 were treated with adenine for 5 days, starting on day 2 of their infection. When the effect of treatment appeared 2.5 days later (4.5 days after infection), dividing and short forms (Fig. 1E) were more than 3 times as prevalent as in controls for 2 days (means of 9–1.4% vs. 2.7–0.2%). They disappeared on the next day in both series.

The pronounced reproductive activity on days 5 and 6 of the treated infections was reflected by a higher mean parasitemia in the treated than in the control series (Fig. 1F).

Adenine was deferred until day 3 in series 4. It was without any appreciable effect although given for 5 days. In the treated rats, there was no extra surge of reproduction and no relapse of division, although trypanosomes were examined from day 3 through day 15, i.e., for 8 days after adenine was stopped. In addition, parasite counts were within the control range.

Formation of the early lysin appeared to be slightly depressed in series 1, 2, and 3, as evidenced by delays of 2 days in the initial disappearance of trypanosomes. As shown in Figs. 1B, D, F, trypanosomes began to decrease on days 8, 7, and 7 in the treated series and on days 6, 5, and 5 in the control series.

In the first three series influenced by adenine, enhanced reproductive activity and persistence of trypanosomes could perhaps be partly attributed to weight loss and anemia as a result of the toxic and unpalatable nature of adenine. Thus, of the 20 g of food offered per 24-hr day, the treated rats ate less than the untreated rats (2.8 ± 0.2 vs. 7.4 ± 0.2 g per 100 g of rat). As a consequence, they began to lose weight 2 days after adenine was started and showed a decrease of 5–15% about 2 days after adenine was stopped. Controls gained in weight throughout the experiment, and on day 5 weighed about 7% more than they did on day 0. Somewhat similarly, anemia occurred in the treated series. As compared to the normal red-cell count in controls, 7–10 million per mm³ blood, the count began to drop on the 4th or 5th day of adenine treatment, reached a low point of 5–6 million cells per mm³ 1–3 days later, and then returned to the normal value in about 2 weeks, i.e., about 3 weeks after adenine was started. These symptoms undoubtedly reflected other pathological changes (7–10, 15).

**Sublethal doses of adenine injected intraperitoneally**

Series 5 through 7, which weighed 120–150 g, were given adenine before or after infection with 4- or 5-day-old *T. lewisi*. The control series received trypanosomes alone. Adenine was given intraperitoneally at the rate of 4 mg/100 g of rat every 6 hr for 5 days. Larger amounts were lethal. Data from these series in general corroborated the findings in the preceding series. On the one hand, mean reproductive activity in series 5, when adenine was started the day before infection, was strikingly higher than in the untreated control group from day 2 through day 4 (11–21.5% vs. 4–9.3%) and continued to be slightly higher on day 5 (7 vs. 5%) and day 6 (2 vs. 0%). The mean parasitemia also reached a higher peak on day 6, but the decline was not appreciably delayed. The failure of adenine to depress the early lysin was no doubt associated with the smaller amounts of adenine received in this series than in series 1.

In series 6, when adenine was started on the day after infection, the mean reproductive activity of *T. lewisi* was higher than in the control series from day 4 through day 6 of the infection (12.5–19% vs. 0.5–7%). In addition, on days 7 and 8, 3.8 and 0.8% dividing forms were found in treated series 6, whereas none was found in the control group. The mean parasitemia also reached a higher peak on day 8 but declined in a normal manner. On the other hand, series 7, when adenine was started 4 days after infection, was not different from the control group. No division of trypanosomes was seen from 7 through 18 days after the start of the infection, in either the treated or control series. Examinations were discontinued thereafter as trypanosomes had disappeared from all but two of the rats within the preceding 1 or 2 days.

Weight of the rats was maintained during treatment with adenine while control rats gained about 15%. Red-cell counts in the treated rats varied slightly less than in series 1 through 4.

**Adenine in vitro**

The inhibiting effects of adenine on reproduction of *T. lewisi* in vitro are shown by the averaged results of two experiments in Fig. 2. Trypanosomes after 17–18 hr contact with adenine at 37°C at the lowest concentration tested (2.34 × 10⁻³ M), showed practically the same result as that found in control
cultures, i.e., about 90% of the forms were dividing or short. As the concentration of adenine was increased to $1.5 \times 10^{-3}$ M, however, adenine progressively inhibited reproduction of the trypanosomes until it was virtually completely inhibitory at the highest concentrations tested. At none of the concentrations tested did adenine exert a stimulatory effect.

**DISCUSSION**

The most striking feature of the current results is that large doses of adenine, when given at certain times, depress the immune response, as evidenced by a significantly enhanced peak reproductive activity of *T. lewisi* in the rat (Fig. 1A, C, E). Thus, reproductive activity at peak parasitemia was about twice that of controls when sublethal doses of adenine were administered from 2 days before to 2 days after infection. In addition, reproductive activity was at times prolonged (Fig. 1A), and the parasitemia was elevated (Fig. 1B, D, and F). Such a combination of effects has only been reported for *T. duttoni* in mice subjected to such extreme stress as splenectomy and blockade (16), or whole-body x- or neutron-irradiation, with or without blockade (17, 18). Prolonged reproduction and elevated parasitemia without enhanced parasitemic rates have, however, been reported for *T. lewisi* in rats under varied circumstances, such as splenectomy and blockade (16), x-irradiation (18–20), and administration of salicylates and related compounds (21), cortisone (22), and dexamethasone (23). It is further of interest that 6-mercaptopurine, which is not a natural metabolite although it is structurally similar to adenine, not only depressed the immune response of the rat but also adversely affected *T. lewisi* as evidenced by a depressed parasitemia (24).

From such comparative results with immunodepressant substances, it was earlier concluded (16–19) that the mouse possesses a natural as well as an acquired immunity to its specific trypanosome, whereas the rat possesses only an acquired immunity to its specific trypanosome. The enhanced reproductive activity demonstrated for *T. lewisi* in the current study, however, suggests that the rat also possesses a natural immunity to *T. lewisi*. The following two lines of evidence lend added support to this conclusion: (a) Blood-stream forms of *T. lewisi* do not reproduce at a maximal rate in the rat since they reproduce at a significantly higher rate in vitro than in vivo (ref. 14; see Fig. 1A, C, E vs. Fig. 2). (b) Adenine evidently does not have a direct stimulatory effect on reproduction of *T. lewisi in vivo* because its effect is so strongly inhibitory on *T. lewisi* grown at 37°C in vitro (Fig. 2). This relationship, however, does not rule out the unlikely possibility that adenine induces the release of a growth stimulant in vivo. Characteristics of the natural immunity are not clear. A humoral factor, however, seems not to be present or, if it is present, it has a short half-life since intense reproduction of *T. lewisi in vitro* occurs in the presence of 40% serum from normal rats (14). That a humoral factor is not the basis for the natural immunity in mice toward *T. duttoni* seems equally true because reproduction of the parasites in splenectomized and blockaded mice is unaffected by the injection of normal mouse serum (6).

Our results further indicate that the immunodepressant effect of adenine, which has not been reported previously as far as we are aware, is directed mainly against natural immunity of the rat toward *T. lewisi* and only slightly against acquired immunity. Thus, in rats treated with sublethal amounts of adenine before or early in their infection (series 1–3, 5, and 6), as compared to untreated controls, the initially increased rate of reproduction and accompanying enhanced parasitemia were followed by no appreciable delay in the appearance of ablastin (Fig. 1A, C, E) and by only a slight delay in the appearance of the early lysin (Fig. 1B, D, F). Furthermore, in rats similarly treated with adenine later in their infection, there was no recurrence of reproductive activity or lengthening of the infection (series 4 and 7). This sequence was more or less expected in series 4 and 7, however, because ablastin had already been synthesized in sufficient amounts to begin to inhibit reproduction and was probably supplemented for a few more days before adenine began to be effective. Such negative results were also obtained when rats (20) and mice (18) were x-irradiated from 5 to 14 days after infection with trypanosomes. Acquired immunity, nevertheless, might have been more depressed if adenine had been continued for periods longer than 5 or 7 days.

The foregoing differentiating effects between natural immunity and acquired immunity indicate that an animal, subnormal in health, may be naturally susceptible to infection, but if infected can develop an acquired immunity. That the adenine-treated rats were under par was indicated by their weight loss and anemia. Other pathological effects, such as kidney damage, proteinuria, and uremia (7–10, 15) were undoubtedly also present since weight loss alone does not enhance the reproductive rate of *T. lewisi*; this has been studied by the caloric intake of infected rats restricted by pair-feeding (25). Although the immunosuppressive effect of adenine might be indirectly accounted for by derangements in protein and nucleic acid biosynthesis brought about by uremic intoxication, it is tempting to speculate that excess adenine acts directly by interfering with nucleic acid metabolism in vivo as it is believed by Aronow (11) to do in tissue culture.

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