Suppression of Antibody Response to *Leptospira biflexa* and *Brucella abortus* and Recovery from Immunosuppression After Berenil Treatment

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Zebu cattle infected with either *Trypanosoma congolense* EATRO 1800 or *Trypanosoma vivax* EATRO 1721 had suppressed humoral immune responses to *Leptospira biflexa* injected intravenously and to attenuated *Brucella abortus* injected subcutaneously. *T. congolense* infections were more suppressive than *T. vivax* infections. In cattle infected with *T. vivax*, the suppression of immune responses to both bacterial immunogens was abrogated when the animals were treated with Berenil at the time of antigen administration. In cattle infected with *T. congolense*, simultaneous Berenil treatment at the time of vaccination abolished the suppression of immune response to *L. biflexa*, and lessened but did not abrogate the suppression of immune response to *B. abortus.*

Suppression of the immune response associated with infectious diseases of humans and animals has been recognized as a major contribution to the pathogenicity of many infectious organisms. This effect has been demonstrated in several hemoproteozal diseases such as malaria (5, 18), babesiosis (1, 13), theileriosis (17), and trypanosomiasis (5, 11). While immunosuppression in laboratory animals infected with trypanosomes is well documented, its full significance in domestic animals has not yet been determined, although studies on immune responses to viral and bacterial antigens in cattle (6, 14, 15, 19) and in goats (L.S.P. Griffin, Ph. D. thesis, Cambridge University, 1977) infected with *Trypanosoma congolense* or *Trypanosoma vivax* have demonstrated immunosuppression.

In view of the wide use of vaccines and the widespread vaccination campaigns against cattle diseases throughout Africa, including areas of endemic trypanosomiasis, information on the immunosuppressive effect of *T. congolense* and *T. vivax* on humoral immune responses was regarded as being of practical importance. Thus, experiments were set up to investigate (i) whether immunosuppression occurred in cattle infected with *T. congolense* or *T. vivax* when the animals were immunized with bacterial antigens 25 days after onset of infection and (ii) whether simultaneous trypanocidal therapy on the day of vaccination had an effect on the immunosuppressive state.

**MATERIALS AND METHODS**

**Animals.** Sixty Boran steers (*Bos indicus*) aged between 9 and 10 months were used. During the experimental periods the animals were housed together in one large fly-proof barn, each group in a separate pen. The animals were given a bale of hay for every three animals per day. This was supplemented with 3 kg of ranch cubes per animal per day. They had free access to mineral salt lick and water.

All animals were vaccinated against rinderpest, foot-and-mouth disease, leptospirosis (Grippotyphosa-Hardjo-Pomona), and anthrax before the experiment. They were also dewormed with two doses of Ranzole (Merck Sharp & Dohme, B.V. Haarlem, Netherlands) at 3-week intervals. During the experiment all the animals were given a weekly injection intramuscularly of 1 ml per 25 kg of body weight of Combict (200,000 IU of benzylpenicillin + 250 mg of dihydrostreptomycin [Pfizer Corp., Brussels]) to prevent secondary bacterial infections.

**Trypanocidal therapy.** Diaminazine aceturate (Berenil, Farwetere Hoechst, Frankfurt, Germany) was dissolved in sterile distilled water and administered intramuscularly at a dosage of 7.0 mg per kg of body weight. Each animal received one injection.

**Trypanosomes.** *T. congolense* EATRO 1800 and *T. vivax* EATRO 1721 were used after passage through 1- to 2-week-old calves. Both *T. congolense* and *T. vivax* were maintained in liquid nitrogen before passage through the calves.

All infections with trypanosomes were established by intravenous injection of a blood sample containing 10³ trypanosomes. The blood sample was collected from the donor calf at the first rising parasitemia. The
blood sample was diluted in phosphate-saline-glucose to obtain the desired trypanosome concentration.

Parasitemia was assessed by microscopic examination of stained thin smears. This was done by multiplying the trypanosome-leukocyte ratio observed in smears by the total leukocyte count. Trypanosomes and leukocytes were counted in 400 oil-immersion (×1000) fields or in 100 fields if the number of trypanosomes exceeded those of the leukocytes. Total leukocyte count was measured by using a Coulter Counter model ZB1 (Coulter Electronics Ltd., Hialeah, Fla.).

*Leptospira biflexa.* *L. biflexa* (F1) was isolated from a water trough of a pasture at this institute and was maintained in Korthof medium at 30°C.

Preparation of *Leptospira* immunogen. Korthof medium was prepared in 200-ml bottles which were inoculated with *L. biflexa* and incubated at 30°C for 5 days. The culture was centrifuged at 30,000 × g for 30 min. The sediment was washed once in normal saline (0.85% NaCl) and then suspended in normal saline. The volume was adjusted until the optical density was 1.80 at 410 nm in a Beckman 2D spectrophotometer. The suspension was kept at 4°C until required. Each animal was injected intravenously with 20 ml of the suspension prepared as outlined above.

*Leptospira* antigen for agglutination. *L. biflexa* was grown in Korthof medium for 5 days at 30°C. The culture was stored at 4°C until used. The whole culture was used as the agglutination antigen.

Microagglutination test. Microagglutination was carried out by modifying the method of Cole and co-workers (1). V-plates instead of the flat-bottom plates were used. Serum inactivated at 56°C for 30 min was diluted in phosphate-buffered saline (pH 7.2) by an automatic microdilutor (Cooke Engineering Co., Ltd.). Volumes of 0.025 ml were used. An equal volume of antigen was added to each serum dilution. The plates were covered with sealing tape (Cooke Laboratory Products) and gently shaken to mix the contents. The plates were incubated in a 30°C incubator for 2 h. The control wells contained the buffer and antigen only.

The plates were placed on the stage of a dark-field microscope (Leitz) equipped with a long-working distance 10× objective (10/0.22 nm, Leitz Wetzlar Inc.) and 10× eye pieces, and the wells were examined for agglutination. The endpoint in a positive test was the highest dilution showing agglutination followed by a well in which no agglutination could be detected.

Vaccination against brucella. Freeze-dried *Brucella abortus* vaccine (S19, living), a product of Tasman Vaccine Laboratory Ltd. (batch no. 1492), was obtained through the Kenya Veterinary Laboratories. It was reconstituted according to the instructions, and 2 ml was given to each animal subcutaneously.

Serum agglutination and antibody against *B. abortus.* The serum agglutination test was performed according to *Standard Laboratory Techniques for the Diagnosis of Brucellosis* (9). Antigen was obtained from Central Veterinary Laboratory, New Haw, Weybridge, Surrey, England.

Statistics. Serum antibody titers were converted to dilution counts (8) or log2 and analyzed by the split-plot-in-time method (16).

Experimental design. A total of 60 Boran steers were divided at random into three groups of 20 animals each. Two of the groups were infected with trypanosomes: one with *T. congolense* (EATRO 1800) and the other one with *T. vivax* (EATRO 1721). The remaining 20 animals were used as controls. The 20 animals in each group were randomly subdivided into 2 subgroups of 10 animals each. One subgroup in each of the three groups was treated with Berenil 25 days after infection with *T. congolense* or *T. vivax*, and at the same time all animals were immunized with *L. biflexa* and *B. abortus*.

The animals were bled daily for determination of parasitemia and collection of serum. The sera were kept at −20°C until the experiment was over and then tested for agglutinating antibodies against *L. biflexa* and *B. abortus*.

RESULTS

Establishment of *T. congolense* and *T. vivax* infection. Parasitemia was detected 5 days after infection in both groups and remained patent until treatment or termination of the experiment. The average daily parasitemia (log2) is recorded in Fig. 1.

Immune response to *L. biflexa.* The mean antibody titers (log2) to *L. biflexa* for all groups are plotted in Fig. 2 and 3. Both of the infected groups had lower antibody titers than the control group (Fig. 2). The titers of both infected groups were significantly lower than those of the control group (*P* < 0.001). The titers of the *T. congolense*-infected group were also significantly lower (*P* < 0.001) than those of the *T. vivax*-infected group.

Figure 3 shows a comparison among treated groups. It is obvious that there was recovery of the antibody response in the previously infected groups after Berenil treatment. The previously *T. vivax*-infected group mounted a somewhat better antibody response (*P* < 0.001) than the control groups. Berenil treatment was found to be slightly depressive in the uninfected controls as compared with the untreated controls.

Immune response to *B. abortus.* The mean titers of antibodies to *B. abortus* are shown for all groups in Fig. 4 and 5.

The group infected with *T. congolense* and immunized with *B. abortus* 25 days after infection had significantly lower mean antibody titers than the control group (*P* < 0.001) (Fig. 4). The group infected with *T. vivax* and immunized 25 days after infection also had significantly lower mean antibody titers than the control group (*P* < 0.001) (Fig. 4), but only after 2 weeks.

The effect of Berenil treatment on the immune response to immunization with *B. abortus* was demonstrated by measuring the serum antibody levels of groups infected with *T. congolense* and treated, infected with *T. vivax* and treated, and treated and untreated controls (Fig. 5). The antibody titers of the group previously
infected with *T. congolense* were significantly lower than those of the control groups (*P* < 0.001). The mean antibody titers of the group previously infected with *T. vivax* were not significantly different from those of the two control groups. The mean antibody titers of the two control groups also did not differ.

**DISCUSSION**

In this work it has been shown that infection

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**FIG. 1.** *Comparison of daily mean parasitemia (log_{10}) of cattle infected with T. congolense (x) and T. vivax (O).***

**FIG. 2.** *Mean serum antibody titer (log_{2}) in cattle immunized intravenously with L. biflexa 25 days after infection with T. congolense (x) or T. vivax (O). Comparison with uninfected controls (O).***

**FIG. 3.** *Mean serum antibody titer (log_{2}) in cattle which were simultaneously treated with Berenil and immunized intravenously with L. biflexa 25 days after infection with T. congolense (O) or T. vivax (O). Comparison with both Berenil-treated (O) and untreated (x) controls.*
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25 counts) of controls (○) or T. vivax (×). Comparison with uninfected controls (●).

**Fig. 4.** Mean serum antibody titer (dilution counts) of cattle immunized subcutaneously with B. abortus 25 days after infection with T. congolense (○) or T. vivax (×). Comparison with uninfected controls (●).

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10 20 30
0 2 4 6 8
DILUTION COUNTS
DAYS POST VACCINATION WITH Br: ABORTUS S 19

**Fig. 5.** Mean serum antibody titer (dilution counts) in cattle which were simultaneously treated with Berenil and vaccinated with B. abortus 25 days after infection with T. congolense (○) or T. vivax (●). Comparison with both Berenil-treated (○) and untreated (×) controls.

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Suppressor cells have been proposed as the cause of immunosuppression in rodent African trypanosomiasis (7). It is possible that the failure of the Berenil-treated T. congolense-infected group of animals to fully recover their humoral response to B. abortus was due to the residual effect of suppressor cells. This would suggest that T. congolense induces specific suppressor cells to B. abortus but not to L. biflexa since the immune response to L. biflexa was not affected in this way. Such a condition appears unlikely.

It has now been shown in mice (10) infected with T. congolense that immunoglobulin-positive lymphocytes are decreased and “null cells” are increased. Lymphopenia has also been demonstrated in Zebu cattle infected with T. congolense EATRO 1800 (G. Maxie, personal communication). This might explain the failure of Berenil-treated T. congolense animals to fully regain immune responsiveness to B. abortus, whereas they did regain the entire response to L. biflexa. An alternative explanation may be that trypanosomes can limit S.19 multiplication and thus influence the level and duration of agglutinin production. However, previous reports on immunosuppression in cattle vaccinated with inactivated organisms or toxoids (6, 15, 19) render this suggestion implausible. It is possible to envision that an antigen administered intravenously in one single dose has a better opportunity of contacting more antigen-reactive cells than antigen which is administered subcutaneously and is dependent on the multiplication of the organism.

The spontaneous recovery of immune responsiveness after Berenil treatment suggests that
live trypanosomes may be necessary for the immunosuppressive effects. This could be effected through factors produced by live trypanosomes; it could be the trypanosomes themselves or an essential nutrient needed for antibody production being competed for by the live trypanosomes and the immunocompetent cells. Thus, at this level of infection, treatment of Zebu cattle with Berenil will overcome the immunosuppressive effect of *T. congolense* and *T. vivax* infections on the humoral immune response of these animals, and, in situations in which depression of the humoral immune response is responsible for vaccination failure, simultaneous Berenil treatment may be of assistance.

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LITERATURE CITED


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Immunosuppression in Bovine Trypanosomiasis: Suppression of Antibody Response to \textit{Leptospira biflexa} and \textit{Brucella abortus} S19 and Recovery from Immunosuppression After Berenil Treatment

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Volume 26, no. 3, p. 822: The title as originally printed was incorrect. The correct title appears above.

Action of Bacterial Lipopolysaccharide on the Respiration of Mouse Liver Mitochondria

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Volume 27, no. 1, p. 104, column 2, line 14: “complex VI” should read “complex II.”