Suppression of Cell-Mediated Immunity in Experimental African Trypanosomiasis

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Adult New Zealand white rabbits were experimentally infected with a parasitic African hemoflagellate, Trypanosoma congoense, and were subsequently tested for in vivo and in vitro aspects of cell-mediated immune function. Chronically infected rabbits were sensitized to mycobacterial protein and skin-tested with purified protein derivative; all infected animals demonstrated much milder skin-test responses to antigen than control groups. Similarly, peripheral blood lymphocyte responses in vitro to purified protein derivative and, as well, to phytohemagglutinin were markedly suppressed. Supernatant fluids of antigen-stimulated lymph node cell cultures from T. congoense-infected rabbits failed to demonstrate migration inhibitory factor activity but did possess normal levels of blastogenic factor activity. An active infection was necessary for demonstration of suppressed immune responses, and components present in infected rabbit serum were apparently not responsible for the observed abnormalities. Suppression of T-lymphocyte subpopulations may well explain the occurrence of numerous immunological aberrations arising during human and animal infections with the African trypanosomes.

Aberrations in the immunological responses of animals infected with the pathogenic African trypanosomes have been reported. These include the demonstration of autoantibodies to normal tissue antigens (22, 27, 33), a rise in heterophile agglutinins and rheumatoid factor-like antibodies (14, 17), grossly elevated levels of nonparasite-specific immunoglobulin M (25, 28, 31), elevated C-reactive protein levels (34), and the possible occurrence of immune complex disease (9, 11, 23) in experimentally infected animals. Furthermore, lower levels of specific antibody occur subsequent to immunization with sheep erythrocytes in trypanosome-infected animals than in normals (10); a suppression of experimental allergic neuritis is seen in trypanosome-infected rabbits (2); and increased susceptibility to secondary infection has been noted in infected humans (9, 10).

The foregoing observations suggest a loss of function and internal control of immune responses in trypanosome-infected animals. The thymus-derived (T) lymphocyte is intimately associated with control or expression of many such responses (1, 20, 30); since there have been no previously reported tests for suppression of T-lymphocyte function in these animals, we chose to analyze both in vivo and in vitro aspects of cell-mediated immunity in trypanosome-infected rabbits.

MATERIALS AND METHODS

Animals. Adult New Zealand white female rabbits (3.0 to 4.0 kg body weight) were used for experimental infections. Albino rats were used for routine passage of trypanosomes. All animals were caged individually and were provided with food and water ad libitum.

Trypanosomes. The strain of Trypanosoma congoense used was provided by Frans C. Goble (CIBA Pharmaceutical Co.) and has been maintained by storage in liquid nitrogen (16). Parasites were removed from frozen storage, thawed, and injected into rats; terminally ill rats with fulminating parasitemias were bled by cardiac puncture, and their blood was passed through a diethylaminoethyl-cellulose column (21) to separate trypanosomes from all blood cells. The parasites were washed three times in Hanks balanced salt solution (HBSS), and 10⁶ viable organisms were injected subcutaneously into rabbits to initiate infections. A control group of animals was injected subcutaneously with 10⁶ nonviable (heat killed) trypanosomes.

Immunization. Immunization procedures were initiated 3 weeks after inoculation with trypanosomes. At this time infected rabbits, as well as the control animal groups, were sensitized to mycobacterial protein by the administration of an emulsion of complete Freund adjuvant (CFA; Difco) and saline. A total of 0.3 mg of mycobacteria per kg of body weight was administered by injecting 0.1-ml amounts of CFA into the hind footpads, and at multiple intradermal sites.

Skin tests. Twenty-one days after sensitization, rabbits were skin-tested intradermally with graded doses of purified protein derivative (PPD, 1.0 to 50.0
µg; Parke-Davis) in 0.1-ml volumes of HBSS. Both immunized and control groups of animals were observed for erythema and induration at the sites of injection.

Stimulation of PBL. Heparinized (10 IU/ml) blood was taken by cardiac puncture from sensitized rabbits as well as from controls on the day of skin testing. Peripheral blood lymphocytes (PBL) were harvested from whole blood by a modification of the Hypaque-Ficoll technique, as we previously reported (24). These cells were cultured at concentrations of 2 x 10^6 PBL per 2.0 ml of RPMI-1640 (supplemented with penicillin-streptomycin, Tylocoll, L-glutamine, and 10% fresh heat-inactivated autologous plasma) in glass tubes (16 by 125 mm) fitted with stainless steel closures. For control purposes, selected cultures of normal rabbit PBL were supplemented with 10% infected rabbit plasma instead of autologous plasma, and some cultures of infected rabbit PBL received 10% normal rabbit plasma supplementation. Optimal doses of phytohemagglutinin (PHA-P; 5 µg/ml; Difco) or PPD (5 µg/ml) were added to PBL cultures in 0.1-ml volumes of HBSS, and the cells were incubated for 72 h at 37 C in an atmosphere of 5% CO2 in air. Approximately 22 h before culture termination, 1 µCi/ml of [3H]TdR was added. The amount of isotope incorporated into deoxyribonucleic acid was determined in comparison with controls and the results were expressed as the stimulation index (SI) as follows: SI = (mean counts/min in stimulated cultures)/(mean counts/min in control cultures).

RESULTS

Course of infection. The disease progressed in trypanosome-infected rabbits as we have described in our earlier studies (22, 23). Briefly, all animals developed chronic infections characterized by the appearance of edematous sites about the face, ears, and appendages by 3 weeks, and small localized patches of necrosis occurred at these sites after 4 to 6 weeks of infection. Except for splenomegaly, gross pathology was not evident in any of the internal organs at the time of testing, and none of the animals appeared to be severely debilitated by the disease. Few or no trypanosomes were observed in peripheral blood samples during infection. Control animals receiving nonviable trypanosomes did not demonstrate any of the signs or symptoms of an infection.

Immunization. Granulomatous responses to the intradermal CFA injections were normally severe for uninfected rabbits; trypanosome-infected rabbits, however, routinely displayed much milder granulomas at the sites of injection.

Skin tests. Skin test results are presented diagrammatically in Fig. 1. Immunized uninfected rabbits developed erythematous, indurated lesions at PPD skin test sites. These lesions became more pronounced in intensity up to about 48 to 72 h postinjection, and then gradually diminished. Sensitized, infected rabbits, however, displayed minimal erythema and induration at the injection sites which rapidly subsided at 24 h. Nonsensitized controls developed no significant reactions at skin test sites.

Stimulation of PBL. PBL from nonimmunized and immunized uninfected rabbits responded to PHA stimulation by incorporating consistently high levels of [3H]TdR into deoxyribonucleic acid (SI = 45 to 65). In contrast, trypanosome-infected rabbit PBL responses to PHA were consistently lower (SI = 13 to 25). Similarly, trypanosome-infected rabbit PBL responses to PPD after immunization were strikingly low (SI = 1 to 2) in comparison with responses by uninfected immunized rabbit PBL (SI = 10 to 18). Representative results of PBL
stimulation by PHA and PPD are presented in Fig. 2. PBL from control rabbits which were injected with nonviable trypanosomes before sensitization to tuberculoprotein exhibited normal in vitro responses to both PHA and PPD. Also, supplementation of PBL cultures from immunized uninfected animals with infected rabbit plasma did not substantially alter responses to PHA or PPD, and the addition of normal rabbit plasma to cultures of PBL from infected immunized rabbits did not abolish the observed suppressed responses to mitogen or antigen (Table 1).

Lymphokine assays. Supernatant fluids from PPD-treated LNC cultures of uninfected immunized rabbits routinely inhibited guinea pig PEC migration in the indirect MIF tests (Table 2). In contrast, fluids of PPD-treated LNC cultures derived from infected immunized rabbits did not exhibit any MIF activity. However, both infected and uninfected immunized rabbit LNC cultures produced comparable levels of BF subsequent to exposure to PPD (Table 2).

DISCUSSION

Immunosuppression has been intimately associated with several protozoan diseases, including malaria, leishmaniasis, and toxoplasmosis (4, 5, 12, 13, 15, 18, 19, 26, 32). The nature of the immunosuppression in these diseases has not been well defined but is thought to involve, at least in part, deficiencies in cell-mediated immunity.

Data presented here demonstrate that depression of cell-mediated immunity occurs in experimental African trypanosomiasis. Skin test responses to antigen (PPD) were less marked in infected immunized rabbits than in uninfected immunized rabbits. Similarly, the responses of PBL from infected animals to PPD

![Fig. 1](http://iai.asm.org/)  
**Fig. 1. Suppressed skin reactions to intradermal injections of PPD in T. congolense-infected rabbits.** Symbols: △, normal rabbits; ▲, infected rabbits sensitized to tuberculoprotein 21 days prior to skin testing with 10 μg of PPD; ○, normal control rabbits; ●, infected unimmunized control rabbits. Each point in the figure represents the mean responses of six rabbits; the range of values for the infected versus uninfected immunized rabbit responses does not overlap at any point.

![Fig. 2](http://iai.asm.org/)  
**Fig. 2. Suppressed responses of PBL from T. congolense-infected rabbits to PHA and PPD.** (Immunized animals were sensitized to tuberculoprotein 21 days prior to testing; ○, uninfected rabbits; ▲, infected rabbits.)

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<th>Table 1. Effects of normal and infected rabbit plasma on the ability of PBL from immunized rabbits to respond to PHA and PPD</th>
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* Mean values ± S.E.
* Sensitized to tuberculoprotein by prior injection with CFA.
and a T-lymphocyte-specific mitogen (PHA) were lower than responses obtained with PBL from uninfected animals; and there was no detectable MIF production by infected rabbit LNC cultures in comparison with uninfected rabbit cultures. The fact that there was no apparent difference in blastogenic factor production suggests that only part of the cell-mediated immune system, or certain subpopulations of T-lymphocytes, may be affected.

Although suppressed skin-test responses and suppressed T-lymphocyte responses to antigen and mitogen do not necessarily imply a more generalized T-cell deficiency state, such a state may well explain the aberrations in T-cell-dependent immune responses noted by others to occur during trypanosome infections. For example, depressed T-helper cell and T-suppressor cell functions (1, 8, 20, 29) may be in part responsible for the apparently uncontrolled synthesis of nonparasite-specific immunoglobulin M and pathological immunoglobulins, the suppressed antibody responses to SRBC, and the appearance of immune complex disease. Proper testing of this hypothesis requires closer analysis of both T-dependent and T-independent B-lymphocyte functions in trypanosomiasis.

The mechanism or mechanisms by which trypanosome infections cause suppression of cell-mediated immunity is at present unknown. We have considered several possibilities. The first was that some physicochemical trait of the trypanosome cell was responsible. However, when rabbits were injected with nonviable parasites, PPD skin tests and in vitro lymphocyte responses to PPD and PHA were normal. An active, ongoing infection is therefore necessary for immunosuppression to occur. This was also found to be true with suppressed anti-SRBC responses in infected rabbits (unpublished data).

A second possibility was that some serum factor (e.g., antibody or immune complexes) arising during infection was causing the suppression. However, when the in vitro PBL tests were run with culture medium supplemented with normal or preinfection plasma rather than plasma taken from the infected animals, PBL responses remained suppressed. Also, infected rabbit plasma failed to "block" or affect normal rabbit PBL responses in vitro.

Another possibility was selective T-cell depletion during infection. Indeed, when we examined infected rabbit lymph nodes and spleen histologically, we found evidence of lymphocyte depletion in thymus-dependent regions; primary follicles and germinal centers (B-cell areas) were intact and well developed in contrast. We are currently attempting to determine whether T/B cell ratios are altered in peripheral blood.

Our data do not rule out a defect at the level of the macrophage cell which may contribute to a T-cell (and B-cell) deficiency in infected animals. Although work by others (9) suggests that phagocytic ability of macrophages from trypanosome-infected animals is unimpaired, other macrophage-associated functions have not been critically analyzed. We are currently examining these aspects of the problem.

Of interest would be a comparison of our model of T. congolense-induced immunosuppression with the responses of rabbits infected with the Brucie group trypanosomes, T. brucei and T. rhodesiense. We have noted in previous work (23) that rabbits infected with T. congolense apparently develop intensive antibody-mediated hypersensitivity responses to parasite antigen, but fail to demonstrate any cell-mediated responses; these findings contrast with the work of others who report that delayed-type hypersensitivity responses occur in rabbits to Brucie group trypanosome antigens (35). We point out that the course of infection is significantly different in Congolense group infections (intravascular) as compared to Brucie group infections (extravascular and intravascular), and that the nature of the immunosuppression may also be inherently different.

In summary, we report here that both in vivo and in vitro correlates of cell-mediated immunity are depressed in rabbits infected with the African trypanosome T. congolense. Suppression of T-lymphocyte functions may well explain the occurrence of numerous immunological aberrations in African trypanosomiasis. Several possible mechanisms for this suppression are currently being evaluated.
ACKNOWLEDGMENTS

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LITERATURE CITED


