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Autoimmunity in Experimental *Trypanosoma congolense* Infections of Rabbits[1]

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Autoimmunity in rabbits with experimental *Trypanosoma congolense* infections was investigated. Complement-fixing (CF) and precipitating autoantibodies to normal allogeneic and autologous tissues were found in the sera of all infected rabbits tested; the titers of CF autoantibody occurring during infection were significantly higher than normally occurring titers of autoantibody in pre-infection serum samples. Autoantibody did not cross-react with trypanosome antigens, and Wassermann antibody was not detected in normal or infected rabbit sera. Passive transfer of autoantibody to normal rabbits did not produce observable pathology or death. Physicochemical methods of analysis revealed that the autoantibody was exclusively of the immunoglobulin M class. That cell-mediated autoimmunity to normal tissue antigens did not occur during *T. congolense* infections was shown by histological analyses, skin tests, migration inhibitory factor, and skin reactive factor tests.

The causes of histopathology and death in rabbits with experimental *Trypanosoma congolense* infections are not known. Rabbits infected with *T. congolense*, a parasitic African hemoflagellate of veterinary importance, undergo a chronic disease in which few trypanosomes appear in peripheral blood and in which extravascular or intracellular forms of the parasite are not common (32). There is no convincing evidence that trypanosomal toxins, physiological imbalances due to metabolite consumption by trypanosomes, or direct action of the parasites on cells are primary factors in the pathology and death of infected animals (17, 33).

Clinicopathological data obtained in our laboratory (*unpublished observations*) suggest that the edema, necrosis, and neutrophil and monocyte accumulation within affected tissues of *T. congolense*-infected rabbits may have an immunological etiology. This paper reports on the occurrence of autoimmunity in infected rabbits.

MATERIALS AND METHODS

Experimental animals. Adult male and female albino rabbits, 2.5 to 3.5 kg body weight, were used for experimental infection. The animals were caged individually and had food and water available ad libitum. Albino rats (0.2–0.5 kg) were used for routine passage of trypanosomes.

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Trypanosomes. The strain of *T. congolense* was provided by Frans C. Goble (CIBA Pharmaceutical Co.) and was maintained by storage in liquid nitrogen (20). Albino rats were injected with the liquid nitrogen-preserved trypanosomes; blood was taken by cardiac puncture from terminally ill rats, diluted in pyrogen-free (PF) saline to contain 5×10^6 parasites/ml, and inoculated into albino rabbits. Each rabbit received 1 ml of infected rat blood subcutaneously.

Course of infection. Trypanosome infections were permitted to progress for 6 weeks after inoculation. A curative dose of homidium bromide (Boots Pure Drug Co., Ltd.) was administered at 6 weeks; the dosage used was 10 mg/kg of body weight given subcutaneously as a 1% solution on 2 consecutive days.

Preparation of tissue antigens. Tissues from normal and recovered rabbits (6 weeks after homidium bromide treatment) were taken aseptically and quick-frozen in liquid nitrogen for storage at −68 C. Brain, liver, heart, and kidney were routinely collected in this manner and were thawed for extraction just before testing. Tissues were homogenized on a 1:5 (w/v) basis in triethanolamine-buffered saline (TBS, *p*H 7.3) for complement fixation (CF) tests, in PF saline for gel diffusion and skin tests, and in minimal essential medium or minimal essential medium-suspension culture (MEM or MEM-S, *p*H 7.3, to which 15% normal allogeneic rabbit serum, 100 mM fresh L-glutamine, and 100 IU of penicillin per ml were added after extraction; Grand Island Biological Co.) for cell cultures. The crude extracts obtained from Waring Blendor homogenization were centrifuged at $5{,}000 \times g$ for 15 min at 4 C, and the supernatant fluids were collected and analyzed for protein concentration by biuret analysis (9). For CF tests, the tissue antigens

in TBS were diluted to the following protein concentrations (mg/ml): brain, 1.15; liver, 0.55; heart, 0.15; and kidney, 0.20. At these concentrations, the tissue antigens were reactive but not anticomplementary in the CF tests. The tissue antigens used for other techniques were diluted to the protein concentrations desired and filter-sterilized, if necessary. All tissue antigens were frozen at −68 C until used.

Collection of sera. Blood was taken by cardiac puncture from rabbits just before inoculation with trypanosomes, and at weekly intervals thereafter until 12 weeks postinoculation (6 weeks after homidium bromide treatment). Serum was collected by conventional techniques and heat-inactivated. Normal and infected rabbit sera were occasionally taken for cell culture media or passive injection procedures. These sera were collected in the same manner but were filter-sterilized. All sera were stored at −20 C until used.

Tests for CF and precipitating antibody to normal rabbit tissues. All sera taken before, during, and after infection were tested for CF activity with normal allogeneic or autologous tissue antigens. The CF procedure was an adaptation of the technique used by Kent and Fife (26) and the Department of Serology, Walter Reed Army Institute of Research (Fife, *personal communication*) in which twofold dilutions of the serum to be tested are prepared in 0.3 ml of TBS. Tissue antigen and five complement 50% hemolytic units ($5C'H_{50}$), each in 0.3 ml of TBS, were added to the tubes, resulting in a final volume of 0.9 ml/tube. Tubes were incubated at 4 C for 16 to 18 hr. Following incubation, 0.6 ml of an optimally sensitized sheep erythrocyte (SRBC) suspension was added to each tube, and all tubes were incubated at 37 C for 30 min. Tubes were then centrifuged, and the supernatant fluids were compared to previously prepared standards simulating varying degrees of hemolysis. Serum titers in this CF procedure were based on an endpoint expressed as the reciprocal of that serum dilution giving 50% hemolysis in the presence of tissue antigen, $5C'H_{50}$, and optimally sensitized SRBC. Appropriate antigen, serum, complement and erythrocyte controls were included in each test. Gel diffusion tests of infected-rabbit sera with tissue antigens were also performed. Petri dishes (100 by 15 mm) were filled with 20 ml of borate-buffered Ionagar no. 2, and wells 5 mm in diameter were punched in the solidified gel; distance between adjacent wells was 2 to 4 mm. Wells were filled with 0.1 ml of undiluted tissue antigen or serum, and the plates were read after incubation at room temperature for 24 hr and then after incubation at 4 C for 1 week.

Immunoglobulin nature of the autoantibody. Pooled infected-rabbit serum (samples taken 6 weeks postinoculation) was treated with 0.2 M 2-mercaptoethanol (5) and retested for CF activity with normal allogeneic tissue antigens. Infected-rabbit serum was also fractionated on a Sephadex G-200 column with phosphate-buffered saline (*p*H 7.4 with 0.02% sodium azide preservative) as an eluant. The effluent stream was monitored at 280 nm with an LKB Uvicord II ultraviolet analyzer equipped with an LKB recorder. Fluid corresponding to each peak was collected and dialyzed against TBS to the original serum sample volume with a Diaflo cell (XM-50 membrane; Amicon, Inc.). These fractions were analyzed for immunoglobulin content by gel diffusion tests and immunoelectrophoresis with antisera monospecific for immunoglobin G, A, and M (IgG, IgA, and IgM; Miles Laboratories, Inc.), and were tested for CF activity with normal allogeneic tissue antigens. In addition, serum was subjected to ultracentrifugation in linear gradients of 10 to 40% (w/v) sucrose in 0.15 M NaCl (50). Gradients were formed in cellulose nitrate tubes [0.5 by 2 inch (1.27 by 5.08 cm) Beckman] to a final volume of 4.5 ml/tube. Infected-rabbit serum samples were diluted 1:4 in 0.15 M NaCl and layered onto the sucrose in 0.5-ml volumes; a Spinco SW-39L rotor containing the tubes was spun at 4 C in a Beckman L2-65B ultracentrifuge for 18 hr at 35,000 rev/min. Following centrifugation, the contents of the tubes were fractionated on an Isco density gradient fraction collector with a 48% sucrose chase solution. Effluent from each tube was analyzed at a wavelength of 254 nm. Fluid corresponding to each peak was collected and dialyzed against TBS to the original serum sample volume with a Diaflo cell. These fractions were analyzed for immunoglobulin content by gel diffusion and immunoelectrophoresis with antisera monospecific for IgG and IgM and were tested for CF activity with tissue antigens.

Passive transfer of autoantibody. Pooled serum from infected rabbits was injected into normal rabbits in an attempt to reproduce the pathology observed during the course of infection. Twenty-five ml of serum was injected intraperitoneally, 5 ml was injected subcutaneously on the sides of the rabbits, and several intradermal injections of 0.1 ml were given on the back. Normal serum served as a control. All animals were watched closely for several weeks for any signs of pathology. In addition, pooled normal- or infected-rabbit serum was mixed with equal volumes of the various tissue extracts; these mixtures were injected intradermally in 0.1-ml amounts into the shaved skin of normal rabbits. The sites of injection were observed at 4, 8, 24, and 48 hr for any evidence of a skin reaction.

Absorptions of infected-rabbit sera with trypanosome extracts. Trypanosomes were separated from infected rat blood with a diethylaminoethyl cellulose column as described by Lanham and Godfrey (29) with phosphate-saline-glucose buffer (*p*H 8.0, ionic strength 0.217) as an eluant. The parasites were then hypotonically lysed and sonically treated for 30 sec at 20 kHz with a Bronwill BP-1 sonic oscillator. Equal volumes of infected-rabbit sera (1:8 in TBS) and trypanosome extract (2.0 mg of protein/ml) were incubated at 37 C for 30 min and then at 4 C overnight. The absorbed samples were centrifuged at 12,000 × *g* for 30 min at 4 C. The supernatant fluids from these tubes were again absorbed with trypanosome extract and were tested for CF activity with normal allogeneic tissue antigens and trypanosome extract. Results were compared to unabsorbed control sera which had been similarly treated.

Venereal Disease Research Laboratory tests. Sera from normal and infected rabbits were examined by

Venereal Disease Research Laboratory (VDRL) tests for reactivity to determine if Wassermann antibody was present. For the test, 0.03 ml of serum from normal or infected rabbits was added to 0.01 ml of the VDRL antigen mixture (Lederle Laboratories) on a slide and rotated for 4 min at approximately 160 to 180 rev/min. Slides were read with a microscope at 40 × for flocculation. A saline control and a positive control (known syphilitic serum) were included.

Skin tests. Normal, infected, and recovered rabbits were skin-tested with various concentrations of tissue antigens prepared in PF saline. Tissue antigen injections contained 0.8, 0.4, and 0.08 mg of protein/0.1 ml of saline. All rabbits received 0.1 ml of the antigens intradermally on their shaved backs. Results of the skin tests were read at 4, 8, 18, 24, 48, and 72 hr.

Histological analyses of infected rabbit organs. Samples of brain, liver, heart, and kidney were collected from normal and infected rabbits. All tissues were washed, dehydrated, and infused with and embedded in paraffin in the usual manner (22). Embedded tissues were sectioned 7 μm thick, mounted on slides, and stained with hematoxylin and eosin. Tissues were examined for cellular infiltration, the presence of trypanosomes, and any other differences from normal rabbit tissues.

Test for migration inhibitory factor. The procedure used for detection of migration inhibitory factor (MIF) was derived from previously reported techniques (6, 47). Normal and infected rabbits were given 50 ml of Marcol 52 (light mineral oil; Humble Oil and Refining Co.) intraperitoneally 2 days prior to collection of peritoneal exudate cells (PEC). On the day of collection, rabbits were exsanguinated by cardiac puncture, and their PEC were collected into sterile centrifuge tubes. Cells were washed twice in Hanks balanced salt solution (HBSS) and suspended in MEM to give 5×10^7 viable PEC/ml. Sterile capillary tubes were filled with the PEC from normal and infected rabbits, plugged with sterile clay, and centrifuged in a sterile hematocrit head. The capillary tubes were then scored and broken at the cell-medium interface and placed into sterile Sykes-Moore chambers; sterile silicone vacuum grease (Beckman) was used to hold the cell-packed tubes in position. The chambers were sealed and filled with MEM containing allogeneic brain antigen (4.0 and 0.4 mg of protein/ml). Controls consisted of the chambers with normal PEC, chambers filled with MEM containing no antigen, and a positive control. The positive controls were PEC that had been incubated with 25 μg of concanavalin A (Calbiochem) per ml in MEM for 30 min at 37 C and then washed twice before being packed into capillary tubes and incubated in MEM. All samples were done in triplicate, and the chambers were incubated for 24 hr at 37 C. After incubation, the areas of PEC migration were determined from photographic enlargements of the test chambers. Experimental PEC migration values were compared to control values, and the migration index was calculated (12).

Test for skin reactive factor. The skin reactive factor (SRF) procedure was adapted from Bloom and Bennett (6) and Schwartz et al. (42). Spleens were removed from the normal and infected rabbits used in the MIF studies, and the spleen cells (SPC) were teased apart in HBSS with dissecting needles and strained through several layers of sterile gauze into centrifuge tubes. SPC were washed twice in HBSS, counted, and suspended in MEM-S to give 5×10^8 viable SPC/ml. One ml of SPC inoculum was added to 2.0 ml of MEM-S containing brain antigen (0.4 mg protein/ml) in sterile plastic tubes (12 by 75 mm; Falcon Plastics). Negative controls consisted of normal SPC cultures plus SPC incubated in MEM-S without antigen, and positive controls consisted of SPC minimally stimulated with 15% fetal calf serum in MEM-S. All cells were incubated for 24 hr at 37 C in an atmosphere of 5% CO_2 in air. Following incubation, SPC were centrifuged and the culture supernatant fluids were removed to storage at −20 C. On the day of testing, the culture fluids were thawed and injected intradermally in 0.1-ml amounts into the shaved backs of guinea pigs. The sites of injection were observed for erythema and induration every 4 hr up to 24 hr, and then again at 48 hr.

RESULTS

Course of infection. Infected rabbits developed edematous sites about the face, ears, and extremities after 2 weeks of infection; some rabbits were unable to hold their edematous ears erect. Edema progressed to necrosis at about 3 to 4 weeks. The necrotic areas were primarily located on the ears, about the eyes and nose, on appendages, and the genitals. Lesions increased in intensity until cure with homidium bromide or death. Few or no trypanosomes were observed in the animals' blood at any time during infection, although blood was infective for rats. Most organs of infected rabbits appeared normal upon examination with a macroscope, but grossly enlarged spleens and occasional cortical congestion of the kidneys were observed. Homidium bromide treatment resulted in complete recovery of the rabbits. Examination of histological sections of the brain, liver, heart, or kidney of infected rabbits revealed no mononuclear cell infiltration. No pathology was observed by microscopy in any of the organs except kidney where glomeruli and tubules were damaged and were sometimes filled with an eosinophilic substance. No trypanosomes were observed in the sections.

CF reactivity of infected-rabbit sera with tissue antigens. Most rabbits exhibited low titers (8–64) of complement-fixing antibody to autologous or normal allogeneic tissue antigens prior to infection. Autoantibody titers to the tissue antigens increased in all rabbits (up to 512) after infection. The antibody responses were greatest to brain and were less to liver, heart, and kidney. Titers with autologous tissues were the same as with normal allogeneic tissues. CF titers fell to pre-

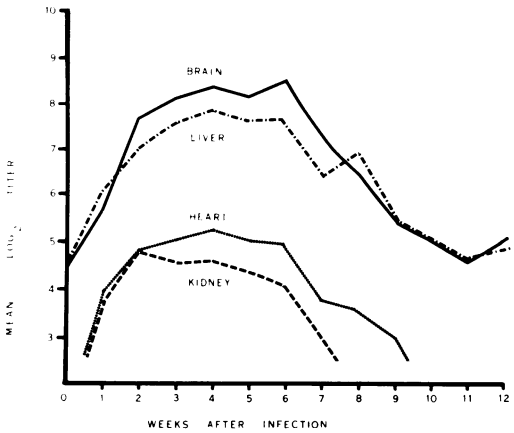

FIG. 1. *Mean complement-fixation titers of sera from Trypanosoma congolense-infected rabbits with normal allogeneic tissue antigens.*

infection levels after homidium bromide treatment. All controls were uniformly negative throughout the series of tests. The mean complement-fixing antibody responses to normal allogeneic tissues are depicted diagrammatically in Fig. 1.

Gel diffusion tests with tissue antigens. Infected rabbit serum contained precipitating antibody to tissue antigens (Fig. 4). Single faint or diffuse lines of precipitation were observed with brain, heart, and kidney antigens at their optimal dilutions. Liver antigen sometimes gave two bands. Succinct lines of precipitation could not be obtained when all of the tissue antigens were tested at their optimal concentrations with a serum sample; this precluded the testing of tissue antigens for similar or cross-reacting antigenic components. Individually, however, the tissue antigens were shown to have no cross-reactivity with trypanosome antigens.

Immunoglobulin nature of the autoantibody response. CF activity with tissue antigens of pooled 6-week infected rabbit serum was reduced by mercaptoethanol treatment (Table 1). Pooled infected-rabbit serum exhibited three protein peaks after gel filtration (Fig. 2). Fraction 1, peak 1 was in the void volume of the Sephadex G-200 fractionated sera; CF activity with tissue antigens occurred only in fraction 1. Gel diffusion and immunoelectrophoretic analyses of the protein peak fractions revealed that fraction 1 contained IgM, fraction 2 had IgG and trace amounts of IgA, and fraction 3 had some IgG. Serum samples from infected rabbits were separated into four protein-containing fractions by density gradient ultracentrifugation (Fig. 3). Gel diffusion and immunoelectrophoretic analyses revealed that

TABLE 1. *Complement-fixation activity of pooled infected-rabbit serum treated with 0.2 M 2-mercaptoethanol*

Serum	Titer against	
	Brain	Liver
Mercaptoethanol-treated	32	<32
Untreated control	256	128

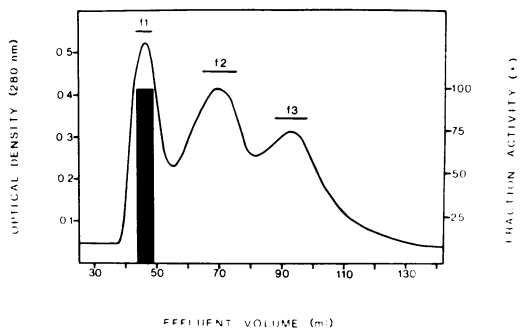

FIG. 2. *Fractionation of infected rabbit serum on a Sephadex G-200 column; fraction complement-fixation activity with tissue antigens.*

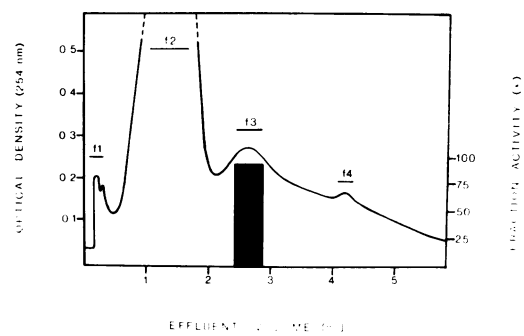

FIG. 3. *Fractionation of infected rabbit serum by ultracentrifugation in a linear sucrose gradient; fraction complement-fixation activity with tissue antigens.*

IgG occurred in peak 2 and that IgM was in peak 3 of the profiles. CF activity with tissue antigens occurred only in peak 3 of infected-rabbit serum samples.

Passive transfer of autoantibody. Normal rabbits that received injections of pooled normal or infected rabbit serum did not die or develop gross external pathology of the type occurring in trypanosome-infected rabbits. No pathology was observed at the sites of injection of tissue-antigen-antibody complexes.

Absorptions of sera. Absorption of serum from infected rabbits with crude trypanosome extract did not remove CF activity for tissue antigens

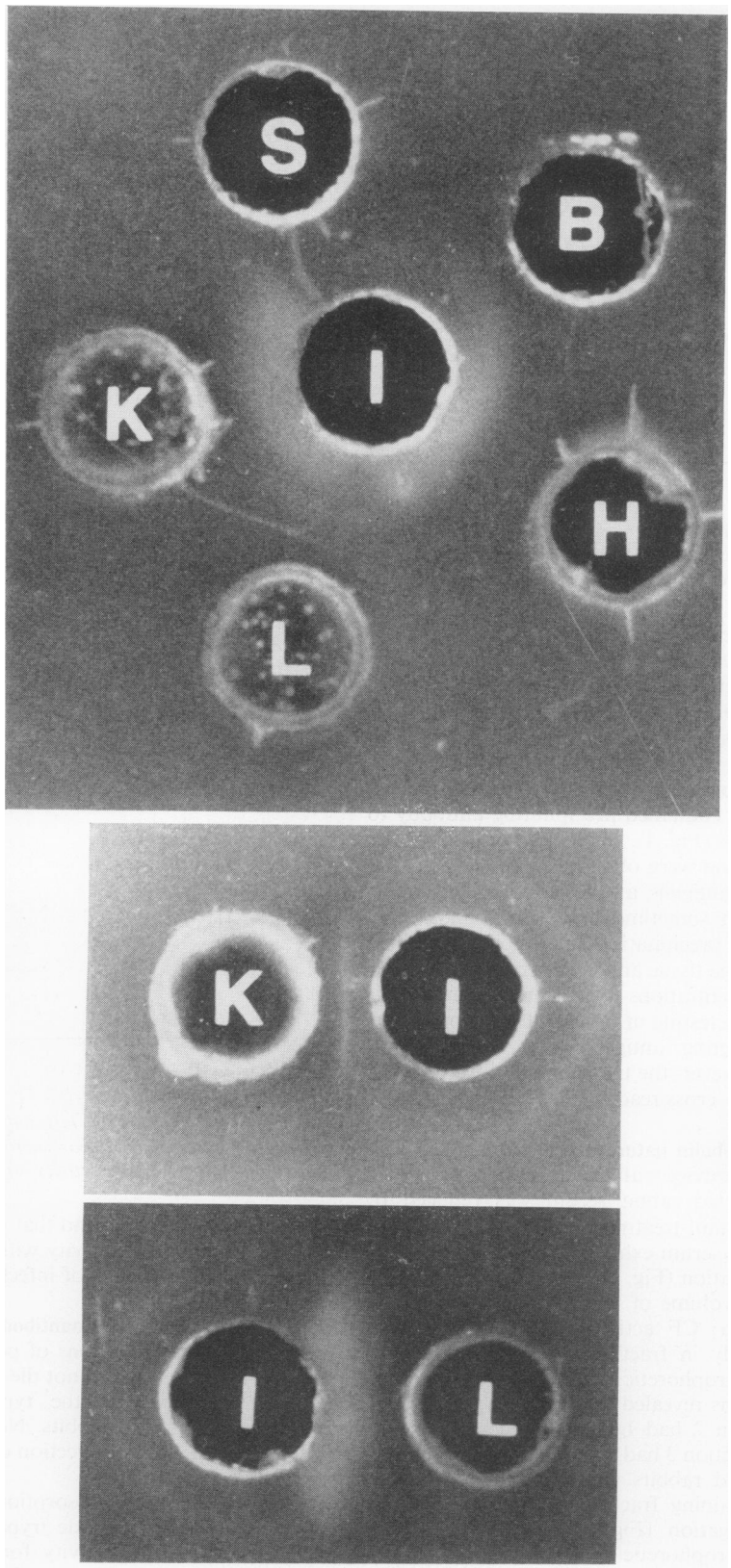

FIG. 4. *Immunodiffusion patterns of infected rabbit serum (I) tested with tissue antigens (B = brain; H = heart; K = kidney; L = liver; S = saline control).*

but did remove CF activity for trypanosome extract (Table 2).

VDRL tests. Sera from normal and infected rabbits were uniformly VDRL-negative; controls with known syphilitic serum were positive.

Skin tests. Normal, infected, and recovered rabbits did not develop skin reactions to intradermal injections of tissue antigens.

MIF tests. Normal and infected-rabbit PEC migrated similarly in the presence of medium or brain antigen. Concanavalin A-treated PEC failed to migrate or migrated very little from the capillary tubes. Representative photographs of MIF test migration patterns are shown in Fig. 5.

SRF tests. Supernatant fluids from normal- and infected-rabbit SPC cultures containing brain antigen or MEM-S did not elicit skin reactions greater than positive controls. All results indicate that there was no greater SRF response to brain antigen than to controls containing no antigen.

TABLE 2. *Complement fixation (CF) activity of pooled infected-rabbit serum absorbed with trypanosome extract*

Serum	Titer against		
	Brain	Liver	Trypanosome extract[a]
Absorbed	256	128	<64
Unabsorbed control	256	128	2,048

[a] Optimal dilution for CF test is 0.13 mg of protein/ml of triethanolamine-buffered saline.

DISCUSSION

Autoimmunity occurs in animals infected with various microorganisms (1–3, 5), and changes supportive of autoimmunity have been previously reported in animals with African trypanosomiasis. Enhanced erythrocyte sedimentation rates, spontaneous autoagglutination of red cells, and adhesion of trypanosomes to erythrocytes, leucocytes, and platelets have been observed (8, 13, 14, 16, 18). Several of these phenomena have been related to the adsorption of serum globulins or antitrypanosome antibody plus complement onto blood cell surfaces; the globulin adsorption alters

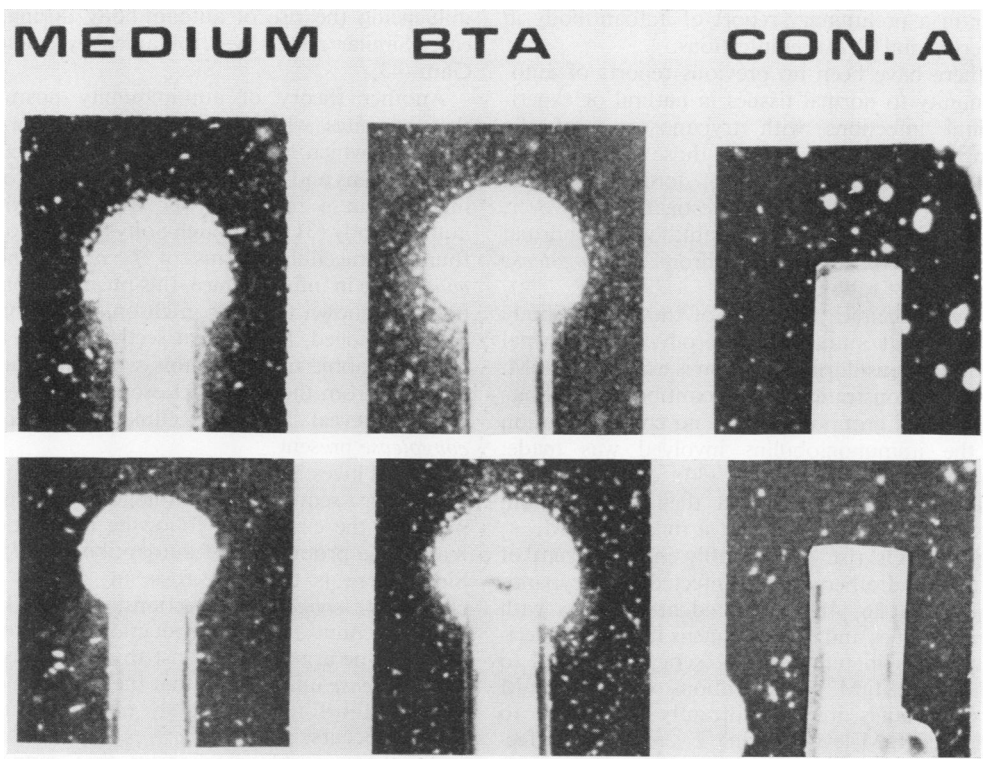

FIG. 5. *Representative migration inhibitory factor test patterns of peritoneal exudate cells (PEC) from normal (top line) and infected (bottom line) rabbits with medium, brain tissue antigen (BTA; 0.4 mg of protein/ml of MEM), and concanavalin A-treated PEC (Con. A; 25 μg/ml of MEM).*

membrane net electrical charge and thus causes cell clumping and increased erythrocyte sedimentation as well as attachment of trypanosomes (40, 49). Several authors have suggested that the adsorption of immunoglobulins onto erythrocytes may contribute to erythrophagocytosis resulting in the anemia of trypanosomiasis (51). In addition, immunoconglutinin and cold agglutinins to erythrocytes arise in the sera of some trypanosome-infected humans and animals (23, 45). Other than as a cause of anemia, none of these reactions has been implicated in the pathology of trypanosomiasis.

Autoantibody to normal tissues has been found in experimental infections with the Brucei group African trypanosomes. Muschel et al. (37) and Seed and Gam (43) have demonstrated that CF autoantibodies to liver and other tissues are produced in rabbits infected with the trypanosomes of humans, *T. gambiense* and *T. rhodesiense*. Tests for cell-mediated autoimmunity were not performed in these studies, and no role was established for the autoantibody since passive transfer of serum was not reported. Recently, MacKenzie and Boreham (34) have demonstrated the possible occurrence of precipitating antibody to rabbit liver in a preliminary report of autoantibody in experimental *T. brucei* infections.

There have been no previous reports of autoimmunity to normal tissues in natural or experimental infections with trypanosomes of the Congolense or Vivax groups, those trypanosomes that are serious pathogens of domestic animals. Results presented in this investigation, however, clearly demonstrate that autoantibodies to normal tissue antigens arise during chronic *T. congolense* infections of rabbit.

Physicochemical analyses of the autoantibody indicate that autoantibody produced to normal tissue antigens during infection is exclusively IgM. In the demonstrations of autoantibody in rabbits with Brucei group infections, no characterization of the immunoglobulins involved was made. MacKenzie and Boreham (34) did show heat lability of autoantibody in their investigation, however, and suggested that it might be IgM.

IgM levels rise considerably in the serum of humans and other animals infected with trypanosomes (10, 36, 41). Repeated absorptions with trypanosomes and other antigens for which specificity exists in infected host sera have failed to reduce the IgM concentrations (19, 21). IgM autoantibodies may significantly contribute to enhanced IgM levels during *T. congolense* infections, although this remains to be tested by effective absorption procedures. One must be prepared to accept the possibility, however, that in certain animals induced to form specific antibody the total gamma globulin increase may not be immunoglobulin specifically directed against the inducing antigen (4, 15, 38).

Landsteiner and Van der Scheer (28) demonstrated Wassermann antibody production in rabbits following injections of phenolized *T. equiperdum*; consequently VDRL tests were performed on rabbit sera used in this investigation for autoimmunity. Negative VDRL results show that Wassermann antibody was not produced by rabbits as a result of infection with *T. congolense*. Therefore the observed autoimmune reactions were not Wassermann-type reactions with a lipid moiety of extracted tissues, and the autoantigens of this study were not similar to cardiolipin.

Several theories on the induction of autoimmunity during infectious disease have been formulated. One theory is that some microbial antigens are antigenically similar to host antigens and that antibody produced to the microbial antigens cross-reacts with host antigens (3, 11, 25). We could not remove serum CF activity for tissues by absorbing with trypanosome extract. This indicates that antitrypanosome antibody cross-reactive with tissue antigens was not a factor influencing the rise of autoantibody during disease. Similar results were obtained by Seed and Gam (43).

Another theory of autoimmunity postulates that parasites which are intracellular (e.g., viruses) or which have intracellular stages acquire host antigens and influence host antibody-producing cells in a hapten-carrier effect to produce autoantibody (31). Although Soltys and Woo (44) found intracellular forms of *T. brucei* and *T. congolense* in infected mice, this phenomenon has not been shown to occur in chronic infections of rabbits. Indeed, histological sections taken from infected rabbit tissues in this work and similar evidence from the work of Losos and Ikede (32) do not reveal any intracellular forms of *T. congolense* present.

Several investigators have postulated that intracellular or "sequestered" autologous antigens exposed to the circulation following tissue trauma may cause production of autoantibodies (7, 11). Since there is tissue necrosis in animals with chronic *T. congolense* infections, such a theory could account for the production of autoantibodies. One argument against this mechanism in *T. congolense* infections is that the autoantibodies arise well before any grossly observable tissue necrosis occurs.

Another possible mechanism that cannot be eliminated in this study is that direct microbial action or metabolic products alter host tissues

enough to cause autoantigenicity and that the resultant autoantibody cross-reacts with normal tissues in in vitro tests (3).

An interesting theory of thymic-dependent (T) lymphocyte and bursal-equivalent (B) lymphocyte homeostatic control of autoimmunity has been proposed (2, 46) in which T-lymphocytes may control such B-lymphocyte functions as the production of autoantibodies; under certain circumstances, including infectious disease, T-lymphocyte control over B cells may be altered or broken resulting in autoantibody formation. Thus, the rise in autoantibody, as well as an absence of cell-mediated autoimmunity, may reflect T-lymphocyte disfunction during trypanosome infection.

In this investigation, passive transfer of infected-rabbit sera or sera mixed with tissue antigens failed to reproduce the disease or its signs and external pathology. Although these results suggest that *T. congolense*-induced autoantibody does not have immunopathologic capacity, such an assumption may be false. One cannot preclude the possibility that the autoantibodies may produce "inapparent" pathologic changes such as the blocking of membrane sites that are physiologically or pharmacologically important recognition sites for enzymes or hormones (30), cytotoxicity for certain immunologically important cells as lymphocytes or neutrophils (48), or the coating of certain tissues to prevent adequate nutrient and gas exchange with the blood. One would expect, however, that if vital functions were blocked, visible pathology should result eventually.

Conversely, the autoantibody may actually be beneficial to the host. Paterson et al. (39) have reported that CF antibrain IgM autoantibody can protect rats against the cell-mediated effects of experimental allergic encephalomyelitis. Boyden (7) suggests that some autoantibodies may be part of a physiological clearing mechanism for cellular debris released during tissue damage. In any event, the consequence or role of autoantibody formed during chronic *T. congolense* infections of rabbit remains to be elucidated.

In vivo and in vitro tests were used in this work in an attempt to detect cell-mediated sensitivity (classical delayed-type hypersensitivity) to normal brain and other tissue antigens. That cell-mediated responses to brain antigens do not occur during chronic *T. congolense* infections of rabbits was shown by the negative skin test and MIF and SRF test results. Autoimmunity in these infections, then, is probably limited to production of autoantibody against normal tissue antigens. This finding was substantiated by examination of histological sections of infected-rabbit organs which revealed that there were no mononuclear cell infiltrates.

Although there are no reports in the literature of cell-mediated autoimmunity in African trypanosomiasis, there is histopathological evidence for it. Several investigators (24, 27, 35) have reported encephalitis and mononuclear cell foci within tissues of man and other animals infected with Brucei group trypanosomes. Comparable lesions have not been recorded in animals with Congolense group trypanosome infections, and this may be partially due to an absence of central nervous system involvement of animals infected with the Congolense group parasites.

In summary, rabbits infected with the pathogenic African hemoflagellate *T. congolense* produced elevated titers of autoantibody to normal tissue antigens. The autoantibody response did not arise as a cross-reaction with parasite antigens, and Wassermann antibody was not a complicating factor. Physicochemical analyses revealed that the autoantibody immunoglobulin class was exclusively IgM. Cell-mediated autoimmunity to normal tissue antigens was not detected.

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