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ARTICLE

Pathogencity, Histopathology and Immunoperoxidase Staining of Trypanosomes in *Trypanosoma congolense* Infected Balb/C Mice

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SUMMARY

Thirty six BALB/C mice were divided into three groups of twelve each (10 infected and 2 control) and cyclically infected with three genetically distinct types of *Trypanosoma congolense (Savannah; SV, Riverine/Forest; RF, and Kenya Coast; KC)*. Parasitaemia, haematology and gross and microscopic lesions were evaluated, including detecting trypanosomes in tissues by immunohisto-chemistry. Mice cyclically exposed to SV type trypanosomes had a higher infection rate (70%) and a shorter prepatent period (6 days) than those exposed to the other two types (60% and 30% infection rates and 27 and 13 days prepatent periods for RF and KC types, respectively). In each group, there was only one parasitaemic peak leading to anaemia and a moribund state. The mice were euthanized in extremis 11 -16 days for SV, 27 -38 days for RF and 18-20 days for KC. The main gross lesions were markedly enlarged lymph nodes in all infected groups as well as splenomagaly and renal petechial haemorrhages in the RF group. Histopathological changes consisted of mononuclear cell infiltration (lymphocytes, plasma cells, and macrophages) in the tissues of infected mice. The reaction was severe in the RF group, moderate in the SV group and mild in the KC group. Immunoperoxidase staining demonstrated trypanosomes in blood vessels of the tissues of the infected mice in all the three groups more clearly than in H&E sections. The results of this study have indicated differences in the pathogenicity and pathology of the three genetic types of *T. congolense* in BALB/C mice. The results have also shown the potential use of immun-operoxidase staining for improved detection of *T. congolense* parasites in the vasculature of the tissue sections of infected animals.

KEYWORDS: Immunoperoxidase staining, pathogenicity, histopathology, *T. congolense*, BALB/C mice.

INTRODUCTION

Genetic diversity has been established within *Trypanosoma congolense* (Broden, 1904) which belong to the Nannomonas subgenus of trypanosomes. *Trypanosoma congolense* has been established to consist of a complex of three distinct genetic types that corresponded with different geographical locations in Africa; Savannah Riverine/forest and Kenya Coast types (Gashumba, 1985; Nyeko *et al.,* 1990; Majiwa *et al.*, 1985; Majiwa, 1992). These developments have led to the accurate taxonomic definition of the Nannomonas trypanosomes (Dukes *et al.*, 1991). The initial investigation on the pathogenicity of the types of *T. congolense* was carried out in sheep and pigs (Mohammed, 1991) and was also evaluated in cattle (Bengaly *et al*, 2002b) and mice (Bengaly *et al.*, 2002a). The mouse model, especially inbred mouse strains including BALB/C, seems to serve as important and valuable tools in trypanosomosis research involving different aspects of the disease in domestic animals and humans (Antoine-Moussiaux *et al.*, 2008). In *T. congolense* infections, the organisms attach to endothelial cells and localize in capillaries and small blood vessels. *Trypanosoma brucei* and *T. vivax* also invade tissues and result in tissue damage in several organs (Connor and Van Den Bossche, 2004).

The use of improved diagnostic techniques in order to get a better understanding of the pathogenesis of trypanosome infections has been reported in different infected animal models. Such investigations mostly aimed at determining the presence of trypanosomes in blood circulation following both cyclical and mechanical infections in different animal models. Inoculation of metacyclics of *T. congolense* in sheep led to the development of a local skin reaction in which large number of the trypanosomes were demonstrated following initial multiplication of the parasites in the lymph nodes draining the affected site 5-6 days

after inoculation and prior to the detection of the trypanosomes in the blood circulation (Luckins *et al.,* 1994). *T. congolense* and *T. vivax* affect domestic animals in Africa and are known to be mainly restricted to the vascular system (Losos and Ikede, 1970, Connor and Van Den Bossche,2004) and they have the tendency to cause changes to the capillary endothelium caused by a combination of factors including products of the parasite, immune complexes, cytokines, and vasoactive amines leading to increased vascular permiability (Connor and Van Den Bossche, 2004). However, *T. vivax* has been demonstrated to show greater tendency towards tissue invasion compared to *T. congolense* (Connor and Van Den Bossche, 2004) resulting in disseminated intravascular coagulation. *T. brucei* has been reported to be tissue invasive based on the evaluations of histopathological sections stained with Haematoxylin and Eosin or Giemsa stain (Losos and Ikede, 1970; Goodwin, 1970).

Microscopic lesions associated with the cardiovascular system and the lymphoid tissues have been observed with extensive infiltration of tissues by mononuclear cells. In *T. congolense* infections, there is aggregation of the parasites in blood vessels of the heart, skeletal muscles, and brain (Ikede, 2007) resulting in dilatation of the microvasculature, oedema, and structural wall changes in vessels (Connor and Van Den Bossche, 2004). Shi *et al.,* (2004, 2005) investigated the detection of trypanosomal antigens in the Kupffer cell of *T. congolense* infected mice while in *T. cruzi* infected mice, the antigens were evaluated using immuno-peroxidase method (Grogl and Kuhn, 1985). Human chagasic infection with *T.cruzi* was evaluated also using the immunoperoxidase method in order to demonstrate the parasite antigens in tissues associated with mega-oesophagus in affected patients (Lages-Silva *et al.,* 2001). The present study was designed to use histopathological and peroxidase antiperoxidase immunohisto-chemical staining techniques to evaluate tissue response of inbred BALB/C mice to three genetically distinct types of *T. congolense*. The pathogenicity of the types was also compared.

MATERIALS AND METHODS

Trypanosome stocks

Three distinct genetic types of *Trypanosoma congolense* (Young and Godfrey, 1983; Gashumba, 1985, 1986; Majiwa *et al.,* 1985; Knowles *et al.,*1988) were used to infect BALB/C mice (Table I)

Tsetse Flies

Laboratory reared teneral *Glossina morsitans morsitans* (line 1.6) (Maudlin, 1982; Maudlin and Dukes, 1985; Maudlin *et al.,* 1986) flies were used to infect BALB/C mice based on the method of Welburn and Maudlin (1987).

Two groups of 50 female *G. m. morsitans* (line1.6; Maudlin and Dukes, 1985) were in each case kept in two separate cages of 25. One group of 50 was infected with stabilates of the SV and the second group of 50 with RF type of *T. congolense*. Another batch of 60 separated in two cages of 30 female *G. m. morsitans* FX9 flies were infected with the KC type of *T. congolense*. The flies were checked for infective metacyclic trypanosomes 3 weeks after receiving an infective feed. Infected flies were identified through the use of the glass probe method after they had been separated into individual cages. Positive flies were separated into individual cages and used to cyclically infect the mice according to the three different groups. After successful feeding, the infected flies were dissected and their midguts and proboscides examined for trypanosomes (Table II).

Mice

Inbred BALB/C mice of either sex and weighing approximately 10 gm were obtained from the University of Bristol mouse colony at Langford. The mice were kept 3 to 5 in clean cages and fed Biosure GLP grade diet (Special Diet Services, Cambridgeshire) with water supplied *ad-libitum*. The mice were divided into three groups of 10 each and cyclically infected with the three distinct types of *T. congolense*. Two mice were used as uninfected controls for each of the three groups. The clinical effects of the infections were monitored in the mice for up to six weeks. The infected mice were euthanised *in-extremis* using halothane (Rhone May and Baker) in a glass chamber and samples of their heart blood, tissues, and organs were obtained for haematology, histopathology and immunohisto-chemistry.

Rabbits

Six New Zealand White male rabbits (Regal

Rabbits Ltd) weighing between 3 and 3.5 kg were obtained and kept at the University of Bristol Animal House. They were maintained on Rabbit and Guinea-pig Diet (Beekay Foods) and water was supplied *ad-libitum*. The rabbits were segregated into three groups of two and inoculated with irradiated trypanosomes from the three types of *T. congolense* for the purpose of producing polyclonal antibodies needed for the immunoperoxidase staining.

Rats

Male Wister Rats (Harlan Olac Ltd) weighing approximately 200 gm were used to grow and harvest the three types of *T. congolense* used to produce stabilates and in the experimental infection of the BALB/C mice. The rats were kept 1 or 2 per cage and fed Biosure Diet with water supplied *ad-libitum*. The rats were immuno-suppressed using cyclophosphamide administered at the rate of 100mg/kg body weight, intraperitoneally. At the peak of parasitaemia, the rats were euthanized using halothane and the heart blood obtained through a mid ventral incision over the sternum to expose the chest followed by a cardiac puncture using a sterile needle and syringe.

Preservation of Infected Blood

Rodent blood infected with the three types of *T. congolense* were preserved during harvesting in blood tubes containing sodium salt of heparin at the rate of 5units per milliliter of infected blood

Preparation of Antigen for Rabbit Immunization

Diethylaminoethyl cellulose (DEAE; DE52, Whatman Biosciences Co.)anion-exchanger was prepared according to the method of Lanham and Godfrey (1970). It was equilibrated and the pH adjusted to 8.0 using10% H_3PO_4 solution. It was allowed to settle after five washings. The column of DE52 was used to separate pure trypanosomes from the blood of the three different groups of infected Wister Rats according to the three types of *T. congolense*. The trypanosomes were then re-suspended in phosphate saline glucose (PSG) 6:4 in aliquots of 1 ml and lethally exposed to 60,000 rads of x-irradiation in a modified method of Morrison *et al.* (1982). The effectiveness of the irradiation was determined by inoculating cyclo-phosphamide immunosuppressed mice and observing them for 5 weeks. The rabbits were immunized by intravenous inoculation with the three types of irradiated *T. congolense*. Just prior to the initial inoculation and 6 weeks after the booster dose, 20ml of ear vein blood were collected from each rabbit and the serum separated and stored at - 70°C in aliquots of 0.5 ml until used as the primary antiserum in the immunoperoxidase staining.

Parasitological and Haematological Evaluations

Parasitemias were monitored by wet blood films under light microscopy using a x40 objective. The films were made by placing a drop of the tail blood from each mouse on a clean glass slide to which a cover-slip was placed. Trypanosome numbers were estimated by the matching methods of Herbert and Lumsden (1976) with the counts being recorded in logarithmic form. The microhaematocrit centrifugation technique (mHCT; Woo, 1970; Murray *et al.,* 1977) was used to detect very low parasitemias. Blood samples were obtained from each mouse following euthanasia through a cardiac puncture and placed in 1 ml ethylene diaminetetraacetic acid (EDTA) tubes (Teklab). The total red blood cell (RBC) counts, the white blood cell (WBC) counts, haemoglobin (Hb) concentrations, and the packed cell volumes (PCV) were determined using a Coulter haemocytometer counter and a System 9000 (Baker Instruments Corporation, USA) automated cell counter.

Tissue Processing

T. congolense infected BALB/C mice from the three groups were euthanised *in-extremis* using halothane and clipped to a dissection board on dorsal recumbency. At necropsy, the liver, spleen, kidney, lung, heart, brain and superficial lymph nodes were removed and preserved in neutral buffered formalin for 24 hours. They were trimmed, appropriately labelled, loaded into cassettes, and processed using a 24 hour cycle in a Leica Histokinette® automatic tissue processor (Reichert-Jung). Following processing, the tissues were embedded with paraffin wax using a TissueTek® Embedder (Ames Co. Ltd). Tissue sections, 4 to 6 micrometer thick, were cut using a Leitz base sledge 1400 microtome. The sections were mounted on glass slides, dried over a hot plate and stored for histopathologic and immunoperoxidase staining.

Histological Staining of Tissues

The mounted tissue sections were conventionally stained using the haematoxylene and eosin (H & E; Stevens, 1977) method and examined under the light microscope x 100 magnification (using oil immersion).

Immuno histochemical Procedure

The method of Bourne (1983) for formalin fixed, paraffin embedded tissues was used. The primary antiserum was the polyclonal anti-*T. congolense* antibody produced in the rabbits. The secondary antiserum was a commercially obtained sheep anti-rabbit immunoglobulins (Sigma Chemical Co). Prior to the application of the primary antiserum, normal swine serum was added to the slides and incubated in a humid chamber for 30 minutes in order to minimize false-positive staining due to non-specific collagen binding. Di-aminobenzidine (DAB) tetrahydrochloride (Polysciences Inc., USA) was prepared by dissolving 10 mg in 20 ml of Tris buffer, pH 7.4. To 10 ml of the DAB solution was immediately added 2 drops of 30% hydrogen peroxide before use in order to block endogenous tissue peroxidase activity. Horseradish peroxidase and rabbit anti-horseradish peroxidase (Dakopatts) were used at 1:100 and 1:200 dilution for the peroxidase-antiperoxidase (PAP) reaction. In order to assist in preventing tissues coming off the slide during the staining procedure, poly-L-lysine treatment (Bourne, 1983) was also employed. The slides were counterstained with haematoxylin after which each slide was dehydrated in graded alcohol, cleared in xylol, and mounted in DPX.

RESULTS

Clinical Observations

The infected mice became overtly ill with signs of inappetance, lethargy, rough hair, and rapid respiration observed between days 11-14 post-infection (*pi*) in the SV, days 27-38 *pi* in the RF, and days 18 - 20 *pi* in the KC infected mice. One mouse each from the SV and RF groups died overnight by days 3 and 6 *pi*, respectively, and no abnormal lesions were observed.

Parasitaemia

Not all the challenged mice became infected; seven, six, and, three became parasitaemic with the SV, RF, and KC types of *T. congolense*, respectively. The mean prepatency period in the SV type infected mice was 8.4 days ±2.2 which was significantly lower ($p<0.05$) than in the RF infected (26.5 days ±7.7) and the KC infected mice (17.3 days ±0.7). However, the mean peak of parasitaemia appeared on day 16 *pi* in the SV infected mice as compared to days 32 and 20 *pi* in the RF and the KC infected mice, respectively. All the mice in the three groups had only one peak of parasitaemia following which they became moribund and were euthanized *in extremis*. The mean parasitaemia in the SV group of mice was $10^{5.4}$ on day 6 *pi* which rose to a peak of $10^{8.7}$ on day 16 *pi* when the mice were *in extremis*. The mean parasitaemia in the RF infected mice was $10^{5.4}$ on day 27 *pi* with a peak of $10^{7.8}$ on day 32 *pi*; the mice were *in extremis* on day 33 *pi* with a mean parasitaemia of $10^{7.5}$. In the KC infected group, the mean parasitaemia was $10^{5.4}$ on day 13 *pi* which rose to a peak of $10^{8.4}$ on day 20 *pi* when the mice were *in extremis* (Fig.I)

TABLE I: Stocks of the three distinct types of *T. congolense* used in infecting the BALB/C mice

Trypanosome Stock	Type:	Original Host:	Country [a]:	Year [b]:
T. congolense JG1 (MBOI/KE/83/JG1) [c]	Savannah	Cow	Kenya	1983
T. congolense TSW103 (MSUS/LR/77/TSW103-A)	Riverine/Forest	Pig	Liberia	1977
T. congolense WG84 (MOVS/KE/81/WG84)	Kenya Coast	Sheep	Kenya	1981

a = Place of isolation
b = Year of isolation
c = Recommended coding system (WHO 1986)

TABLE II: Infection rates of the 3 types of *T. congolense* in laboratory reared female G. m. morsitans

	Number of flies			Infection rates (%)		
T. congolense type:	Infected	+ve on probe	Dissected*	Midgut	Labrium	Hypopharynx
SV	50	15	39	74.1	48.8	36.1
RF	50	11	38	76.6	55.3	37.2
KC	60	5	33	66.7	46.4	29.1

Key: * = some flies have died in the cages during the course of the experiment

TABLE III: Grading of tissue changes in BALB/C mice infected with the three types of *T. congolense*

	T. congolense Infection Type:		
Tissue/Organ:	*Savannah*	*Riverine Forest*	*Kenya Coast*
Lymph nodes [a]	++	++	++
Spleen [a]	++	+++	++
Liver	++	+++	+
Myocardium	++	+++	++
Kidney	+	+++	+
Lung	+	++	+
Skeletal muscles	+	+	±
Brain [b]	+	+++	±

Key: [a] = includes degree of lymphoid hyperplasia/depletion??.
[b] = includes concentration of trypanosomes in brain capillaries detected using immunoperoxidase (PAP) staining.
± = very mild response; + = mild response; ++ = moderate response; +++ = severe response

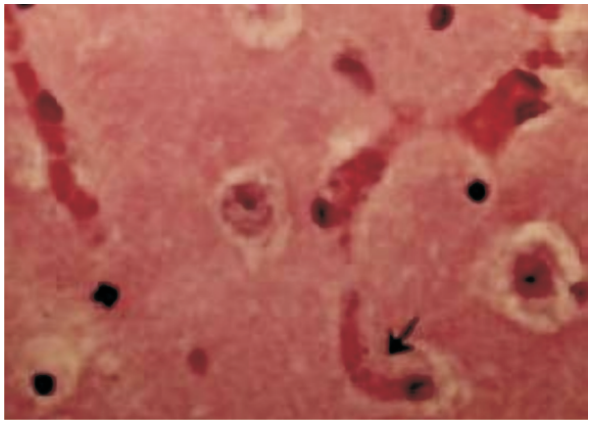

Plate 1: Brain capillary congestion in a mouse infected with the RF T. congolense (H&E stain x250). The dark coccoid bodies between red blood cells (arrow) are nuclei of trypanosomes.

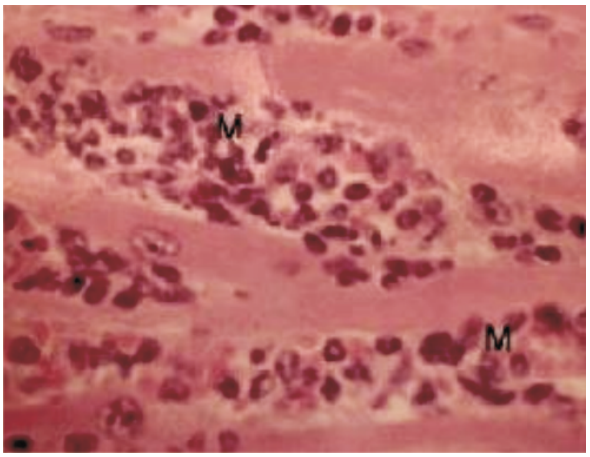

PLATE 2: Mononuclear cell infiltrates (M) in the myocardium of a mouse infected with the RF *T. congolense* (H&E stain x250)

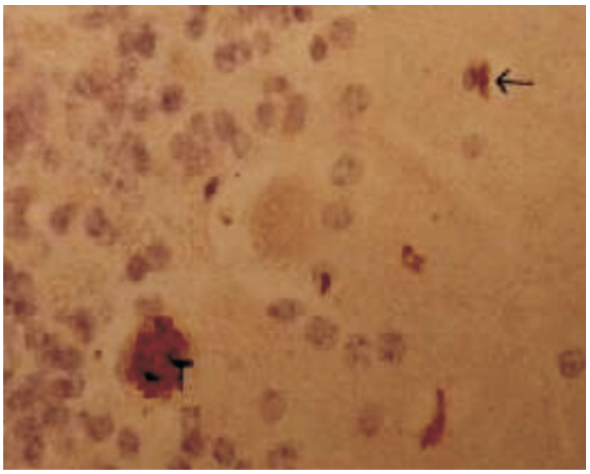

PLATE 3: Trypanosomes (T) in the cerebellar capillary of a mouse infected with the SV T. congolense. Note one trypanosome (arrow) attached to a capillary endothelial cell (immunoperoxidase staining x 250).

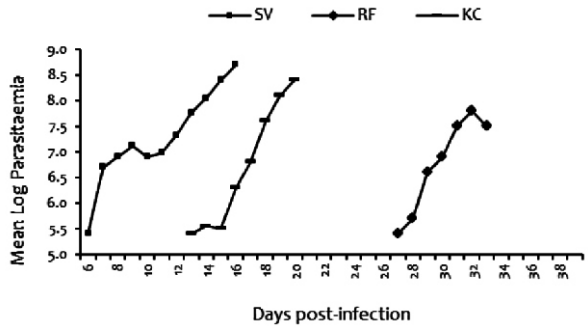

FIG. 1: Mean log parasitaemia in Balb/C mice infected with the Savannah (SV), Riverine/Forest (RF), and Kenya Coast (KC) types of *T. congolense*

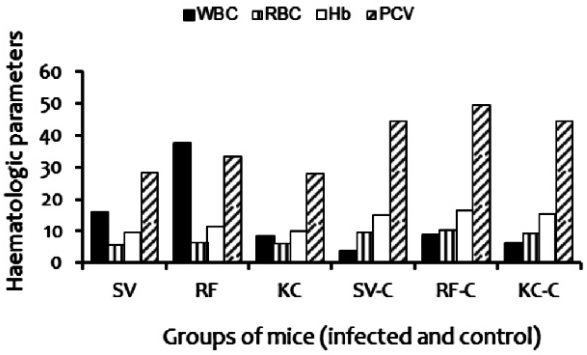

FIG. 2: Mean haematologic parameters (WBC x 10^9/L; RBC x 10^{12}/L; Hb g/dL; PCV%) in Balb/C mice infected with the Savannah (SV), Riverine/Forest (RF), and Kenya Coast (KC) types of *T. congolense* and in the control (SV-C, RF-C, and KC-C) mice

Haematological parameters

The mean (±SD; standard deviation) erythrocyte (RBC) counts were 5.5 ±1.21 x 10^6 ml^{-1} ,6.3 ±0.40 x x 10^6 ml^{-1}, and 6.2 ±0.77 x 10^6 ml^{-1}, for the SV, RF, and KC infected mice, respectively. The mean RBC counts in the respective controls were 9.5 ±0.43 x 10^6 ml^{-1},10.4 ±0.46 x 10^6 ml^{-1}, and 9.2 ±0.22 x 10^6 ml^{-1}. The decrease in RBC counts was statistically significant ($P<0.05$) when the values were compared with the respective controls. Similarly, the mean Hb concentrations were 9.5 ±1.66 g dl^{-1}, 11.3 ±0.99 g dl^{-1}, and 9.9 ±1.16 g dl^{-1}, in the SV, RF, and KC infected mice and were also statistically significantly different ($P<0.05$) from the corresponding control values of 14.9 ±1.05 g dl^{-1}, 16.3 ±0.40 g dl^{-1}, and 15.3 ±0.40 g dl^{-1}. The mean PCV values for the SV, RF, and KC infected mice groups were 28.3 ±4.30%, 33.6 ±0.95%, and 28.0 ±2.49%, respectively. Changes in the PCV values were statistically significant ($P<0.05$) when compared with the respective control values of 44.5 ±0.50%, 49.5 ±3.05%, and 44.8 ±0.35% (Fig. 2). Changes in leukocyte (WBC) counts showed the mean WBC

counts of 16.1 ±7.47 x 10^9 L^{-1},16.1 ±15.34 x 10^9 L^{-1}, and 8.2 ±3.61 x 10^9 L^{-1} for the SV, RF, and KC infected mice, respectively. The mean WBC counts in the respective controls were 3.7 ±0.99 x 10^9 L^{-1},8.7 ±4.10 x 10^9 L^{-1}, and 6.3 ±0.99 x 10^9 L^{-1}. The increase in WBC counts was statistically significant ($P<0.05$) when the value in mice infected with RF type was compared with the values in the other two groups of infected mice. Statistically significant ($P<0.05$) differences were also obtained when the mean WBC counts were compared with the respective controls (Fig.2).

Pathological changes

Gross lesions observed included mild paleness of the carcass in one of the RF infected mice, while the carcass condition in the SV, KC, and control mice were apparently normal. The superficial lymph nodes were markedly enlarged in all the three infected groups of mice. The lymph nodes in the control mice were apparently normal. The spleen in five of the RF infected mice was severely enlarged but was moderately enlarged in the SV and KC infected mice. The liver was moderately enlarged in all the RF infected mice while it was apparently normal in the SV and KC groups of infected mice. The kidneys in three of the RF infected mice had diffused petechial haemorrhages while the kidneys in the SV and KC infected mice were apparently normal. The carcass, spleen, liver, lymph nodes, and kidneys in the control mice were apparently normal.

Lymph nodes

Histopathological sections of the lymph node revealed marked follicular lymphoid hyperplasia consisting of both small and large lymphocytes in the three mice infected with the KC type of *T. congolense*. The follicles of the lymph nodes of five of the six mice infected with the RF type showed marked proliferation of lymphoid cells while the medullary cords contained large numbers of plasma cells and erythrophagocytes. The lymph nodes of the mice infected with the SV type showed mild to moderate hyperplasia and with expanded medullary cords. Trypanosomes were detected in the lymph nodes of mice from the three groups of infected mice using the immunoperoxidase staining but not so with the H & E sections.

Spleen

Massive splenic hyperplasia with expanded lymphoid follicles and moderate erythrophagocytosis occurred in the spleen of 6 mice infected with the RF type. All the SV infected mice had erythrophagocytosis and moderate splenic hyperplasia. The spleen of the three mice infected with the KC showed only a mild hyperplastic response with erythrophagocytosis. Immunoperoxidase staining revealed trypanosomes in the spleen of mice infected with the RF and SV types of *T. congolense*. Only a few trypanosomes were stained in the case of mice infected with the KC type. Very few trypanosomes were detected within the congested splenic sinusoids with the H & E sections in the SV and RF groups of infected mice.

Liver

Severe portal vascular infiltrations consisting of lymphocytes, macrophages, and plasma cells occurred in the liver of five mice infected with the RF type while one had moderate infiltrates. The hepatic sinusoids of the mice were distended by erythrophagocytes, plasma cells, and haematopoietic blast cells. Focal hepatocyte necrosis also occurred in this group. In mice infected with the SV type, all seven with infection showed moderate lymphocytic infiltrations around the hepatic portal vessels. Mild perivascular infiltrates occurred in the liver of the three mice infected with the KC type of *T. congolense*. The immunoperoxidase staining showed trypanosomes located in the portal vessels in all the three infection groups. However, trypanosome nuclei were detected with the H & E sections in the SV and RF infected mice.

Heart

The myocardium of the mice infected with the SV type of *T. congolense* showed mild mononuclear cell infiltrates. However, three mice infected with the RF type had marked myocardial infiltrates consisting of lymphocytes and macrophages with marked separation of myocardial fibers in presence of mild degenerative changes (Plate 1), while another three had moderate infiltrates. Mild infiltrates occurred in the myocardium of the three mice infected with the KC type. Trypanosomes were stained with immunoperoxidase stain and located within the lumen of the myocardial vessels in the RF and SV infected mice. Similarly,

the nuclei of trypanosomes were detected in the myocardial vessels with the H & E sections in four SV, six RF, and one KC infected mice.

Brain

The brain of five mice infected with the RF type of *T. congolense* showed capillary congestion and in one mouse, nuclei of trypanosomes were observed in between the erythrocytes (Plate 2) while these did not occur in any of the SV infected, apart from moderate congestion in three. None of the three infected with the KC showed severe or moderate congestions. Immunoperoxidase staining revealed large number of trypanosomes in the lumen of brain capillaries in the SV infected (Plate 3) and the RF infected mice. A few trypanosomes were identified in the capillaries of the meninges, cerebellum and cerebrum in the KC infected mice with the H & E sections.

Lungs

Mononuclear cell infiltrates occurred in the lungs of all mice infected with the RF and SV types of *T. congolense* with marked congestion in the six RF infected mice. Congestions also occurred in the lungs of the three KC infected mice with aggregates of lymphocytes and macrophages. Trypanosome nuclei were detected in the expanded pulmonary septum in the SV, RF and one of the KC infected mouse. The immunoperoxidase staining revealed trypanosomes within the lumen of pulmonary vessels in all the three groups of infected mice.

Kidneys

Mild to moderate focal and diffuse infiltrations of lymphocytes, plasma cells, and macrophages occurred in the interstitium of the renal medulla, cortex, and in the glomerular areas of four mice infected with the SV type of *T. congolense*. Similar infiltrates were observed in three of the RF and one of the KC infected mice too. Immuno-peroxidase staining revealed trypanosomes in the renal capillaries in all the three groups of infected mice. No trypanosomes were detected with the H & E sections in the kidney vessels in the three groups of infected mice.

Skeletal Muscles

Moderate mononuclear cell infiltrates occurred in the skeletal muscles of the six mice infected with the RF and three of those infected with the SV types of *T. congolense*. Only mild infiltrates occurred in the skeletal muscles of the three mice infected with the KC type of *T. congolense*. Immunoperoxidase staining revealed the presence of trypanosomes in the capillaries of the skeletal muscles in the RF and SV type infected mice.

Tissue reactions following the experimental infections were graded and presented in table III.

DISCUSSION

The demonstration of genetic diversity within *T. congolense* (Young and Godfrey, 1983) and its subsequent characterization into three genetically distinct types (Gashumba *et al.,* 1988) suggested the types may also differ in their behavioural properties. This led to an investigation to determine any possible differences in the pathogenicity of the different types in sheep and pigs (Mohammed, 1991). *T. congolense* is a salivarian trypanosome that is restricted to Africa where it is mainly transmitted cyclically by tsetse flies (*Glossina* spp). It is a monomorphic trypanosome pathogenic to domestic ruminants (cattle, sheep, and goats), equine, and to a lesser extent, pigs (Connor and Van Den Bossche, 2004).

Many reports on the pathogenicity of trypanosome infections were based on non-cyclical infection of animals through inoculation of bloodstream forms of the parasites including *T. brucei*, *T. vivax*, and *T. congolense* (Losos and Ikede, 1970,1972). Many investigations in rodents were based on intraperitoneal inoculation of trypanosome infected blood (Jennings *et al.,* 1974; Brown and Losos, 1977; Jennings *et al.,* 1978) rather than through tsetse transmission. However, in the pathogenesis of tsetse-transmitted infections, the multiplication of the parasites at the site of bite results in a local skin reaction (chancre) which occurs prior to the development of parasitemia (Roberts *et al.*, 1969; Emery and Moloo, 1981; Akol and Murray, 1982; Taiwo *et al.,* 1990). The trypanosomes initially multiply at the bite site and subsequently get to the circulation via the lymphatics. Thus, when bloodstream forms of trypanosomes are inoculated intraperitoneally, the dermal phase of trypanosome development which occurs in cyclical infection does not feature in the pathogenesis of the infection.

However, this may become significant in relationship to the prepatent periods when cyclical and mechanical inoculations are compared. The mean prepatent periods obtained in this study were 6, 27, and 13 days in the SV, RF, and KC infected mice, respectively. Thus, mice infected with the SV type succumbed much earlier to the infection while the mice with the RF type of *T. congolense* infection had the longest mean prepatent period. Mean prepatency ranging between 13 to16 days were observed in sheep inoculated with metacyclic forms of *T. congolense* (Luckins *et al.,* 1994). However, in another study in which BALB/C mice were infected with the three genetic types of *T. congolense* through intraperitoneal inoculation, the average prepatent periods ranged between 3 to 5 days (Bengaly *et al.,* 2002a). When zebu cattle were experimentally inoculated with the three types of *T. congolense* (Bengaly *et al.,* 2002b), the average prepatent period was 7-11 days (Bengaly *et al.,* 2002b). Therefore, the prolongation of the prepatent period observed in this study may be attributable to cyclical infection through the tsetse flies. For example, the varying infection rates in the three groups of mice and the difference in the percentage of flies positive on probing (Table II) would suggest differences in the vectoral capacity of the flies for the three types of *T. congolense*. Further studies in this area might be helpful.

Based on changes observed in the haematological parameters, the mice infected with the RF type of *T. congolense* had relatively higher mean erythrocyte counts, mean Hb concentrations, and mean PCV values compared to mice infected with the SV and KC types. The disease pattern in the mice with the RF type of infection developed a subacute type of infection indicated by longer mean prepatency, relatively milder anaemias compared to infections with other two types. The nature of the infection in the mice infected with the SV was relatively more acute while in the case of the KC type, mild to moderate changes were observed. It may be suggested that the ability of the RF mice to better initiate compensatory response by the erythropoietic system resulting in the production of new RBCs, might have been responsible for the longer survivability of the mice in this group. In *T. congolense* infections in cattle, the stimulation of the erythropoeitic system was observed to occur as one of the positive initial response to anaemia (Dargie, 1978). However, SV and KC mice were dead before RF mice were even patently infected, perhaps because RF flies did not transmit an infective dose until later. Once parasitaemic, RF mice died after one peak like the other groups.

Histopathological evaluations of the tissues of the three groups of BALB/C mice revealed a more severe response in the tissues of mice cyclically infected with the RF type with respect to mononuclear cell infiltrations and hyperplasia in the lymph nodes and spleen of infected mice. The mean prepatent period was also longer in mice infected with the RF compared to the other two groups of mice infected with the SV and KC types. Furthermore, leukocytosis was more severe in mice infected with RF compared to similar responses in the other two groups of mice. The moderate increase in erythrophagocytic activity which occurred in the spleen, lymph node, and liver of mice infected with the RF type of *T. congolense*, and the increased stimulation of the lymphoid organs characterized by marked lymphoid hyperplasia that occurred in the RF type infected mice could very much be due to the prolongation of the infection, probably due to relatively lower infecting dose of trypanosomes compared to the SV and the KC types which exhibited moderate and milder lymphocytic responses. The severe mononuclear cell infiltrations observed in mice infected with the RF type could be attributed to the significantly higher mean leukocyte counts observed compared to the other two groups of mice infected with the other types of *T. congolense*. The expanded activity of the cells of the mononuclear phagocytic system are known to be the main agents involved in erythrophagocytosis of affected erythrocytes in trypanosome infected animals (Connor and Van Den Bossche, 2004).

Judging from the time flies first fed on the mice, the RF strain was probably least pathogenic because RF infected mice lived longer than those infected with SV and KC. The prolonged infection probably resulted in a more severe mononuclear inflammatory response but less anaemia compared to the other two types. Despite the moderate differences that occurred in the histopathological changes between the

three groups of infected mice, the PAP immunohistochemical staining could detect trypanosomes in the blood vessels in various tissues and organs of the three infected groups of the mice. The PAP test could not demonstrate the presence of trypanosomes outside the bounds of the microvasculature which is in line with previous reports indicating *T. congolense* as restricted to the vasculature and not tissue invasive (Losos and Ikede1970; Connor and Van Den Bossche, 2004). The immunoperoxidase staining in mice infected with *T. congolense* has however shown the potential use of the technique to identify the location of the parasites. In another investigation in rats, *Trypanosoma evansi* was demonstrated in tissue sections using an abidin-biotin peroxidase (Sudarto *et al.,* 1990). When the tissues were stained with H&E only, trypanosomes were poorly visible. This is in conformity with the finding in this study in which the immunoperoxidase test revealed clearly the intravascular presence of trypanosomes in the tissues of the infected mice. In a similar investigation in mice and cattle (Bengaly and others, 2002a, 2002b), the pathogenic picture of the three types of *T. congolense* had been shown to differ significantly even though the infection method was through mechanical inoculation of the parasites.

CONCLUSION

The results of this study have indicated some differences in the behaviour of the three genetic types of *T. congolense* in BALB/C mice in which infection with the RF type showed a longer prepatent period and a more severe mono-nuclear cell infiltrations in various tissues and organs compared to the changes that occurred in mice infected with the SV and KC types. However, the infection in the SV infected mice appeared to be more acute while that in the KC type infected mice was moderate. The results have also indicated that immunoperoxidase tests can effectively be of value in identifying the location of trypanosomes in the tissues of infected BALB/C mice.

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