Parasite Immunology 1993: **15:** 101–111

Antibody responses to invariant antigens of *Trypanosoma congolense* in cattle of differing susceptibility to trypanosomiasis

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SUMMARY

*Five trypanotolerant N'Dama (*Bos taurus*) and five susceptible Boran (*Bos indicus*) cattle were challenged by tsetse flies infected with* Trypanosoma congolense *IL 13-E3. These animals had experienced five previous infections with* T. congolense, *each terminated by drug therapy. Immunoblotting and ELISA were used to determine isotype and specificity of antibody responses to trypanosome invariant antigens. Both IgM and IgG1 were elicited, but the IgG1 responses were directed against a greater diversity of antigens. A 69 kD antigen was the major invariant antigen which elicited IgM antibodies in both breeds, but the N'Damas also responded with high levels of specific IgG1. Analysis of isotypic responses to whole trypanosome extract also revealed lower levels of IgG1 and higher levels of IgM in the Borans than in the N'Damas, suggesting that a dysfunction in the switch from IgM to IgG might occur in infected Boran cattle. A 33 kD antigen appeared to elicit only IgG1. Sera from all five N'Damas and the two Borans which were most resistant to the disease reacted with this antigen prior to and following re-infection. Furthermore, during the primary* T. congolense *infection in the same animals, anti-33 kD antibodies were detectable in all five trypanotolerant N'Damas, but in none of the five susceptible Borans. Thus, the presence of antibodies to the 33 kD antigen of* T. congolense *appeared to be associated with a capacity to control the disease.*

Keywords *trypanotolerance, N'Dama, Boran cattle,* Trypanosoma congolense, *invariant antigens, antibody responses*

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Received: 27 September 1991
Accepted for publication: 20 July 1992

INTRODUCTION

Indigenous taurine breeds of West Africa, mainly the N'Dama and the West African Shorthorn, exhibit a degree of resistance to trypanosomiasis, termed trypanotolerance. Trypanotolerance is genetically determined, but the degree to which it is manifested depends on physiological, environmental and nutritional parameters. It is characterized by an ability to limit both parasite growth and trypanosome-induced pathology, in particular anaemia (Murray, Morrison & Whitelaw 1982, Trail, d'Ieteren & Teale 1989). Although the parasite load has an effect on the severity of the syndrome, the degree of anaemia and the level of parasitaemia are not directly correlated in trypanotolerant breeds (Authié & Pobel 1990, Paling *et al.* 1991b). Thus, these two parameters appear to be controlled by separate mechanisms.

It is generally accepted that parasitaemia is controlled by anti-VSG antibody response, stimulated in response to each variant (reviewed in Roelants & Pinder 1984). Following antibody-mediated destruction of each wave of trypanosomes, a wide variety of invariant antigens are released. It has been suggested that these antigens may be involved in inducing the pathology associated with the disease (Mansfield 1990). It has been proposed for malaria that immune recognition of such parasites 'toxins' could protect the host against the pathological effects of infection (Playfair *et al.* 1990). Similarly, an effective immune response to invariant trypanosome antigens may be an important mechanism of resistance to the disease. During *T. brucei* infection in N'Dama and West African Zebu cattle, animals of both breeds which controlled the disease produced antibodies to at least one of three invariant antigens of *T. brucei* (Shapiro & Murray 1982). Thus, immune recognition of invariant antigens may be involved in control of pathogenesis and associated with resistance to the disease.

In the field, cattle are under permanent tsetse fly challenge and are repeatedly infected with various sero-

demes of trypanosomes. It is therefore important to determine their immune responses during sequential infections. Trypanotolerant cattle control rechallenge infections with homologous trypanosome clones more efficiently than trypanosusceptible cattle (Williams *et al.* 1991, Paling *et al.* 1991a). In addition, in four sequential heretologous challenges, the overall mean PCV in a group of N'Dama progressively and significantly increased following each infection (Paling *et al.* 1991b). The present study was designed to determine whether superior recognition of invariant antigens by trypanotolerant cattle occurs in rechallenge infections. Isotypic responses to invariant antigens were characterized in a group of N'Dama and Boran cattle undergoing their sixth *T. congolense* infection. In addition, their primary-infection serum was retrospectively analysed to confirm associations revealed in the rechallenge infection.

MATERIALS AND METHODS

Experimental design

In order to compare immune responses of cattle of differing susceptibility to trypanosomiasis, a series of experimental infections with *T. congolense* has been conducted at ILRAD since 1985, in Boran (Zebu) cattle (*Bos indicus*) and N'Dama cattle (*Bos taurus*). The sequence of infections and the clones of *T. congolense* used in the various experiments have been reported previously (Williams *et al.* 1991).

The analysis of serological responses reported here (analysis 1) was carried out during a rechallenge experiment conducted in 1988 (Williams *et al.* 1991). Briefly, five Boran and five N'Dama cattle matched for age (4 years) and sex, were used. These animals had previously been challenged by tsetse infected with clones from four different serodemes of *T. congolense*, on five occasions. At the end of the fifth experiment, cattle were treated with a trypanocidal drug. The sixth infection, which is reported here, was administered 10 months later. Animals received five bites by *Glossina morsitans centralis* infected with *T. congolense* IL 13-E3, a clone from the same serodeme as that used in the second challenge 32 months previously.

A more limited analysis (analysis 2) was performed retrospectively on sera from the same five N'Dama and five Boran collected during their primary infection with *T. congolense* ILNat 3.1, in 1985 (Paling *et al.* 1991a).

In both experiments, parasitaemia and packed red cell volume (PCV) were monitored daily using standard procedures (Murray, Murray & McIntyre 1977) and sera were collected at intervals prior to and during the infection. Cattle were treated with 7 mg/kg diminazene aceturate (Berenil, Hoechst, Frankfurt, Germany) if their PCV fell to 15%, to prevent possible death.

Preparation of trypanosome lysate

A stock of *T. congolense* (IL C-49), which was unrelated to any of the clones used for infection of cattle, was used for detection of antibodies to invariant antigens throughout the study. IL C-49 was originally isolated from a cow in Transmara, Kenya, and was passaged in rodents (Wellde *et al.* 1974). A stabilate was inoculated into irradiated rats. Trypanosomes were isolated from infected blood on DEAE-cellulose (Whatman Biosystems Ltd., Maidstone, Kent, UK) according to the method of Lanham & Godfrey (1970) and washed three times in phosphate saline glucose (pH 8·0). Crude lysates were prepared by freeze-thawing the pellets in the presence of protease inhibitors (leupeptin (10 μg/ml), 1 mM phenylmethylsulphonylfluoride (PMSF), 0·2 mM N-tosyl-L-phenylalanine chloromethyl ketone (TPCK), 0·05 mM N-alpha-p-tosyl-L-lysine chloromethyl ketone (TLCK)) and were stored at −70°C in aliquots of 5×10^8 trypanosomes (approx 3 mg of total protein) in 100 μl.

Immunoblotting

Lysates were rapidly thawed, made up to 1 ml in distilled water and immediately boiled for 3 min in an equal volume of 100 mM Tris-hydrochloride pH 6·8, containing 4% (w/v) sodium dodecyl sulphate (SDS), 20% (v/v) glycerol, 8% (v/v) 2-mercaptoethanol, and 0·01% bromophenol blue. Lysates were then loaded onto a 7·5–15% polyacrylamide gradient gel, 1·5 mm thick and 13 cm wide. Stock solutions and buffers were essentially those described by Laemmli (1970). Prestained molecular weight markers (Amersham Int., Amersham, UK) included myosin (200 000), phosphorylase b (92 500), bovine serum albumin (69 000), ovalbumin (46 000), carbonic anhydrase (30 000), trypsin inhibitor (21 500) and lysozyme (14 300). Proteins were separated using constant voltage (20 V per cm of gel), then transferred from SDS-PAGE gels onto nitrocellulose membranes (pore size, 0·45 um; Schleicher & Schuell, Dassel, Germany) according to the method described by Towbin, Staehelin & Gordon (1977). The transfer onto nitrocellulose was performed overnight at 10 V followed by 20 min at 70 V, in 25 mM Tris, 200 mM glycine, and 0·1% SDS buffer containing 20% methanol. Unbound sites on the nitrocellulose membranes were blocked with 10 mM Tris-hydrochloride containing 5% skimmed milk (pH 7·4) for 30 min prior to each incubation. Nitrocellulose membranes were cut into strips and incubated for 2 h with

selected bovine sera diluted 1:100. Strips were then incubated with appropriate dilutions of monoclonal antibodies (MoAbs) IL-A59, IL-A30, IL-A66, IL-A74, specific for bovine immunoglobulin light chains and the μ, γ1 and γ2 chains, respectively (Naessens *et al.* 1988, Williams *et al.* 1990). Finally, horseradish peroxidase-conjugated goat anti-mouse Ig was used to reveal the position of antigen-antibody complexes. This conjugate, which was a gift from Professor K.Lindqvist, ILRAD, had been prepared following the method described by Wilson & Nakane (1978) as modified by Lindqvist, Gathuma & Kaburia (1982).

Diaminobenzidine tetrahydrochloride (0·5 mg/ml in 10 mM phosphate-EDTA) or 4-chloro-1-naphthol (0·5 mg/ml in 200 mM sodium chloride, 50 mM Tris-hydrochloride, pH 7·4; both from Sigma Chemical Co., Poole, UK) were used as chromogens with hydrogen peroxide (0·01%) as the substrate. After the first two incubation steps, the strips were washed rapidly in PBS containing 0·1% (v/v) Tween 20 (Riedel-de Haen AG, Seelze, Germany) then given three 10 min washes in PBS, and then blocked again for 30 min. After incubation with the conjugate, washes were done in the buffer used for dilution of the chromogen.

ELISA on total trypanosome lysate

An enzyme-linked immunosorbent assay (ELISA) was used to measure specific IgG1, IgG2 and IgM responses to invariant *T. congolense* proteins during the course of infection. A lysate was prepared from stock IL C-49 as indicated above, then centrifuged at 10 000 *g* for 30 min at 4°C. The supernatant was filtered through a 0·22 μm filter. The filtrate was diluted in 50 mM carbonate buffer, pH 9·6, and used to coat flat-bottomed microplates (type M129A, Dynatech, Plochingen, Germany) overnight at 4°C. The coating antigen was titrated and the optimal dilution selected. The coated plates were used within 24 h. Prior to utilization they were washed rapidly with PBS-0·1% Tween 20 (washing solution). Bovine sera diluted 1:300 in washing solution were added to the plates for 2 h at 37°C. Each serum sample was tested in duplicate and each test was repeated once. After incubation the plates were washed five times and incubated for 1 h at 37°C with an appropriate dilution of bovine isotype-specific MoAb (IL-A30, IL-A66, or IL-A74) in washing solution. The plates were washed and incubated for 30 min with 1:2500 dilution of peroxidase-conjugated sheep anti-mouse Ig (Amersham Int., Amersham, UK). After washing, the substrate (hydrogen peroxide, 0·01%) was added to each well together with the chromogen (2,2′-azino bis (3-ethyl)-benthiazoline-6-sulphonic acid (ABTS, Sigma, Poole, UK), 250 μg/ml in 50 mM citric acid buffer, pH 4·0). The coloured reaction was read 45 min later at a wavelength of 414 nm, using a Titertek Multiskan micro-ELISA auto-reader (Type MCC 340, Flow, Oy, Finland). Results were expressed as the difference between sample absorbance and the mean absorbance of wells treated with three control sera from naïve cattle.

Antigen purification by electroelution from polyacrylamide gels

In order to measure antibody responses to selected immunodominant antigens identified by immunoblotting, these antigens were purified from lysates of *T. congolense* stock IL C-49 by electroelution, according to the method described by Hunkapiller *et al.* (1983) with modifications. Lysates, as prepared for Western blot analysis, were run on 7·5–15% SDS-polyacrylamide gels. After staining with Coomassie blue, bands of selected molecular weight were excised from the gels. Electroelution was performed using an ECU-40 electroeluter (Scientific Co., Del Mar, CA., USA) for 17 h at 12 mA, in buffer containing 50 mM ammonium bicarbonate, 0·1% SDS, and 1 mM dithiothreitol. The eluates were dialysed for 24 h against 10 mM ammonium bicarbonate buffer containing 0·005% SDS. The purity of each eluted fraction was assessed by Coomassie blue staining after SDS-PAGE. Western blots of the eluted proteins were probed with known positive bovine sera to check for the presence of the required antigen.

ELISA on electroeluted antigens

The electroeluted antigens were used to coat ELISA plates. The isotype-specific antibody responses to these antigens were measured in ELISA using 1:100 or 1:50 dilutions of the bovine sera.

Statistical analysis

Student's *t*-test was used in analysis 1 to compare prepatent periods in the two breeds. Measurements of parasitaemia and ELISA results from sera collected between 0 and 45 days after detection of parasitaemia, were subjected to an analysis of variance factorial design with repeated measures (Winer 1971). Comparisons were made between cattle breeds for parasitaemia and levels of specific IgM and IgG1, as well as breed-time interactions which compared rates of change between breeds. Probability levels of less than 0·05 were regarded as significant.

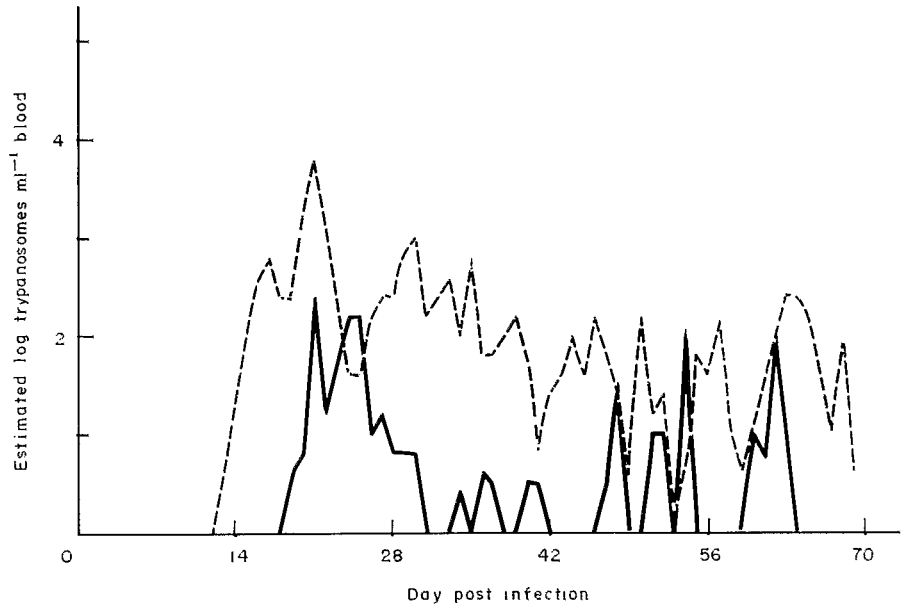

Figure 1. Changes in parasitaemia following rechallenge with *T. congolense* IL 13-E3 in N'Dama (——) and Boran (- -) cattle. Each graph represents the mean from five cattle monitored daily.

RESULTS

Analysis 1

Parasitaemia

The time between infection and detection of parasites in blood (i.e., the prepatent period) was longer in N'Dama (20·4±0·9 days) than in Boran cattle (15·6±3·1 days, $P<0·02$). Significant breed-time interactions occurred; the N'Damas experienced only one parasitaemic wave followed by intermittent parasitaemia (Figure 1). All five N'Dama cattle, and three out of the five Boran cattle (no. 16, 17, 23) self-cured 3 to 6 months after infection (parasites were no longer detectable in peripheral blood).

Anaemia

The average PCV in the N'Damas prior to the rechallenge was 36%±4%, and it remained above 30% throughout the experiment. The PCV in Boran cattle started decreasing when trypanosomes were detected in blood (Figure 2). It fell continuously for the first 4 weeks of parasitaemia, and thereafter stabilized. However, variations in the degree of anaemia were observed between individual animals. Animals no. 21 and 22 developed the most severe chronic anaemia which required drug therapy on days 75 and 119 post inoculation, respectively. Two Borans (no. 17, 23) had more moderate anaemia (lowest PCV reached was 20%, on four and two occasions, respectively) and recovered spontaneously. The last Boran (no. 16) was more anaemic than no. 17 and 23 (a PCV of 20% or less was reached on twenty occasions) but did not require drug therapy.

Antibody responses

1. *Kinetics of responses to trypanosome invariant antigens*

The results are presented with reference to the number of days post detection of parasitaemia (DPD) rather than to the number of days post inoculation (DPI), since the prepatent period varied for each animal and the specific antibody response was always detected at or shortly after the onset of parasitaemia.

Prior to the rechallenge, yet 10 months after the previous infection had been terminated with diminazene aceturate treatment, all ten animals had detectable levels of IgG1 antibodies to trypanosome antigens (Figures 3 & 5).

The kinetics of trypanosome-specific IgM responses, as determined by ELISA, are shown in Figure 3. In both breeds of cattle, there was an increase in the specific IgM titres which reached a peak 10 DPD and thereafter declined. However, the IgM levels remained consistently higher in the Borans than in the N'Damas ($P<0·01$). Immunoblotting showed that the IgM response was mainly directed against antigens with a molecular mass (Mr) of 65 to 70 kilodalton (kD). No difference in the pattern of antigen recognition by IgM was observed between the two breeds (Figure 4a).

Specific IgG and IgM levels increased simultaneously during the rechallenge. There was a major IgG1 response

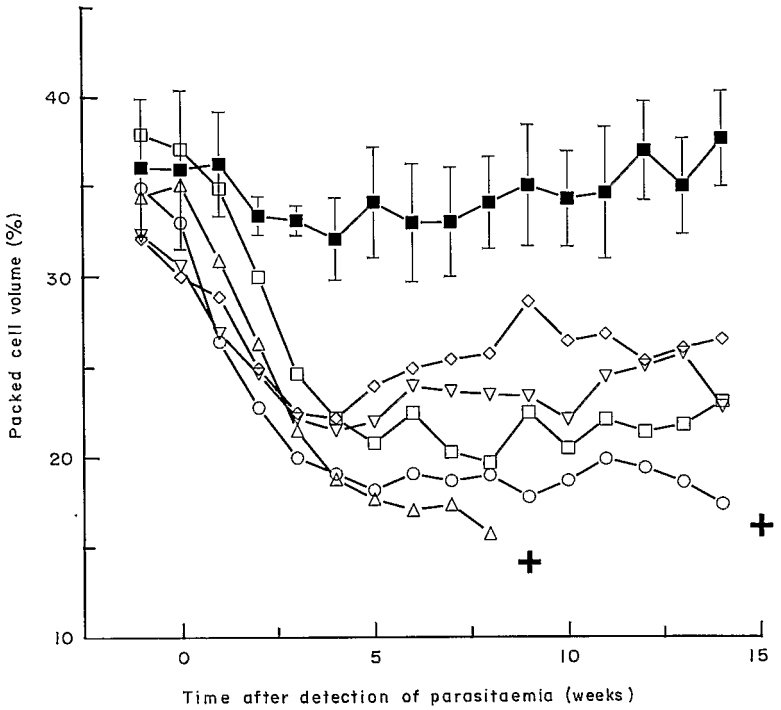

Figure 2. Sequential changes in packed red cell volume (PCV) following rechallenge with *T. congolense* IL 13-E3 in N'Dama and Boran cattle. Data collected daily for one week were averaged for each animal. Data points for N'Dama cattle (—■—) represent the mean group values. Data points for Boran cattle represent individual values: Boran 16 (—□—), 17 (—▽—), 21 (—△—), 22 (—○—), 23 (—◇—). Plus signs indicate drug therapy.

to trypanosome invariant antigens in both groups, as shown by immunoblotting (Figure 4b) and by ELISA employing a *T. congolense* lysate as antigen (Figure 3). Specific IgG1 levels reached a peak between 15 and 20 DPD, by which time IgG1 antibodies directed against the majority of invariant antigens were detectable. Thereafter, IgG1 levels progressively decreased until day 50 (Figure 3), but remained higher than the pre-rechallenge levels for several months (data not shown). The N'Damas showed a significantly higher IgG1 response than the Borans ($P<0{\cdot}05$). In N'Damas 4 and 10, which had the highest titres of specific IgG1, these antibodies were also directed against a greater diversity of antigens (Figure 5).

In both breeds, the IgG2 response was weak and was

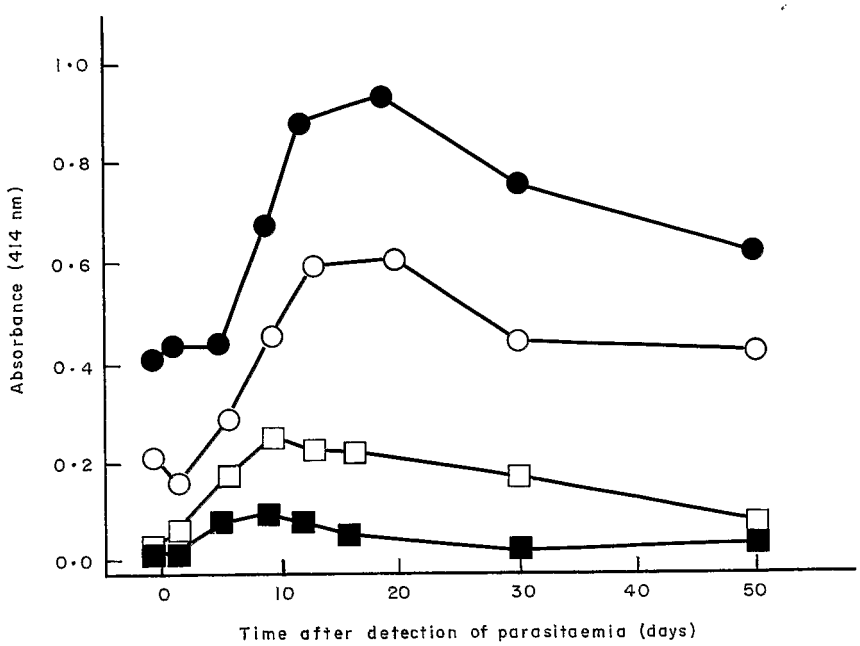

Figure 3. Kinetics of specific IgM (—■—, —□—) and IgG1 responses (—●—, —○—) in N'Dama (solid symbols) and Boran cattle (open symbols) following rechallenge with *T. congolense* IL 13-E3. The antigen used in ELISA was a lysate of *T. congolense* IL C-49. Each point represents the mean of five cattle.

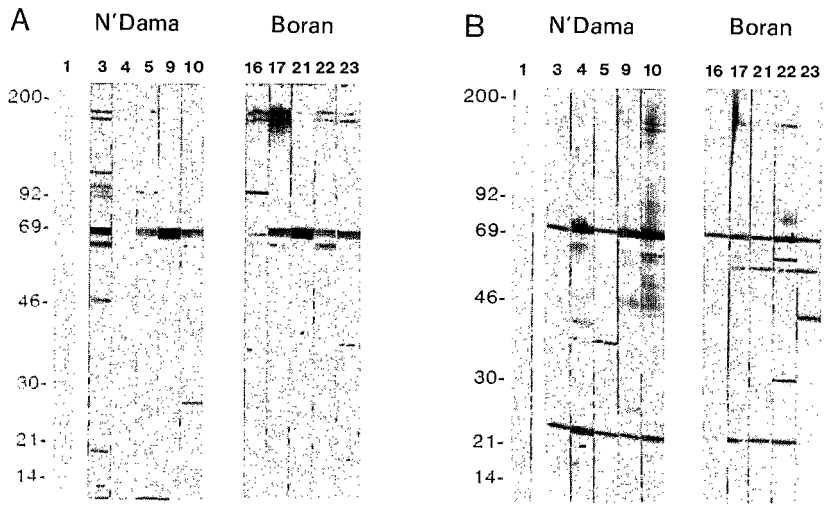

Figure 4. Immunoblotting analysis of IgM (A) and IgG1 (B) responses to invariant antigens of *T. congolense* 4–7 days post detection of parasitaemia. The nitrocellulose strips were incubated with sera from a naïve animal (no. 1) and from individual N'Dama (nos. 3 to 10) and Boran cattle (nos. 16 to 23). Molecular mass markers in kilodaltons are indicated on the left of each panel.

only detectable against a small number of antigens, mainly of 65 to 70 kD (data not shown).

2. Specific trypanosome antigens recognized during infection

Most of the specific antibodies were directed to proteins of Mr ranging from 20 to 70 kD (Figure 5). A weak response was observed in several animals in the range of expected Mr for the VSG of *T. congolense* (45–50 kD). This was probably due to crossreactions involving epitopes common to different VSGs, since the antigen used on Western blots was unrelated to any of the clones used for the infections.

Three invariant antigens were more consistently and

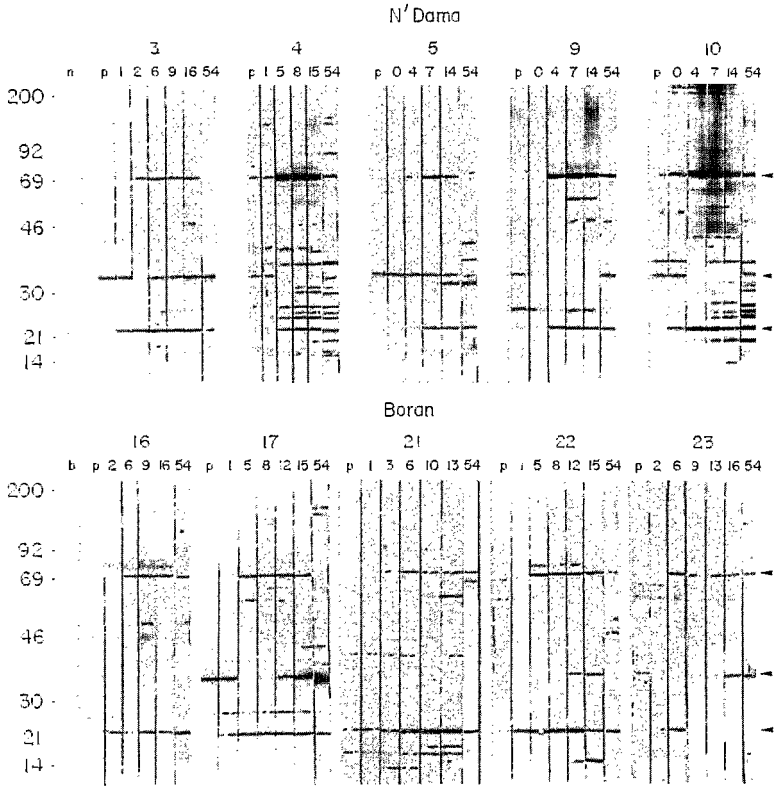

Figure 5. Kinetics of IgG1 responses to invariant antigens of *T. congolense* following rechallenge with IL 13-E3, as analysed by immunoblotting. The nitrocellulose membranes were incubated with sera from individual animals taken prior to re-infection (p) and at various days after detection of parasitaemia (indicated above corresponding strips). Sera from naïve N'Dama and Boran cattle were used to probe strips (n) and (b), respectively. Positions of the molecular mass markers in kilodaltons are indicated on the left. Arrows show the position of immunodominant antigens (23, 33 and 69 kD).

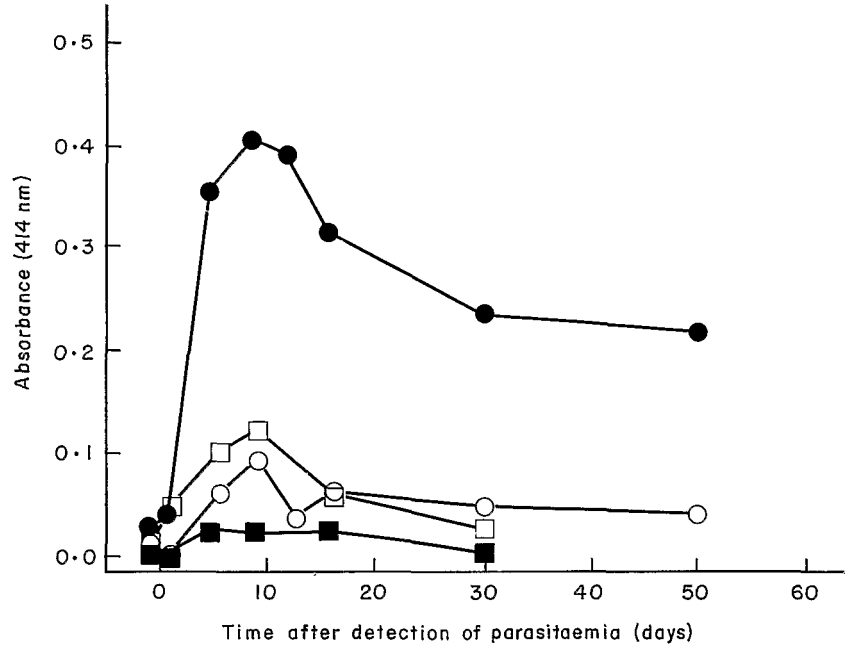

Figure 6. Kinetics of IgM (—■—, —□—) and IgG1 (—●—, —○—) responses to the 69 kD trypanosome antigen in N'Dama (solid symbols) and Boran cattle (open symbols) following rechallenge with *T. congolense* IL 13-E3. Each point represents the mean of five cattle.

strongly recognized. These antigens had Mr of 23, 33, and 69 kD (Figure 5). Low levels of antibodies against the 23 and 69 kD antigens were detected in the pre-rechallenge sera from some individuals in both breeds. In all animals these antigens elicited an antibody response 0 to 5 days after detection of parasitaemia. The 23 kD antigen elicited an IgG1 response only, which appeared to be equally strong in N'Dama and Boran cattle, as assessed by immunoblotting (Figure 5).

The 69 kD antigen was the major invariant antigen which stimulated antibodies of the IgM class (Figure 4a). This antigen also elicited IgG1 and IgG2 antibodies. Two N'Damas (nos. 3 and 5), which experienced low and transient parasitaemia, had lower IgG1 responses to the 69 kD antigen than the other three N'Damas. However, IgG1 specific for this antigen was detectable on immunoblots up to dilutions of sera ranging from 1:500 to 1:4000 in the N'Damas, but from 1:100 to 1:1000 in the Borans. The data from the ELISA using the eluted 69 kD protein as coating antigen confirmed these results. Of anti-69 kD

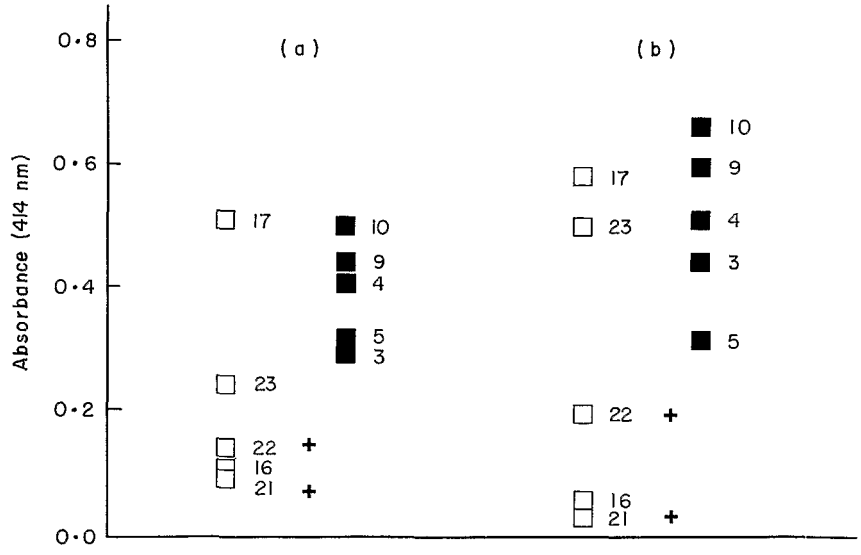

Figure 7. Levels of 33 kD-specific antibodies (IgG1) in individual N'Dama (solid symbols, nos. 3 to 10) and Boran cattle (open symbols, nos. 16 to 23). (a) prior to, and (b) during the course of re-infection (54 DPD) with *T. congolense* IL 13-E3. Plus signs indicate Boran cattle which required drug therapy.

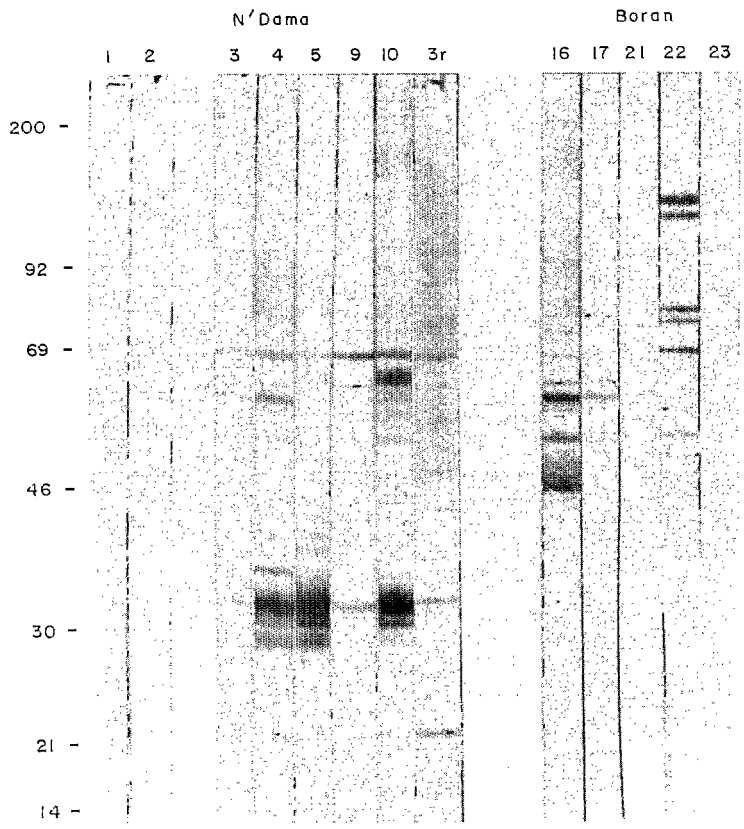

Figure 8. Pattern of recognition of *T. congolense* invariant antigens during primary infection with ILNat 3·1, as analysed by immunoblotting. The transfers were incubated with sera from individual N'Damas (nos. 3 to 10) and Borans (nos. 16 to 23) taken 130 days post infection, or at the time of treatment for Borans 16, 17 and 21. Strips (1) and (2) were probed with sera from naïve N'Dama and Boran cattle, respectively. Strip (3r), probed with the serum from N'Dama no. 3 in the rechallenge experiment (see Figure 5) indicates the position of the 33 kD antigen. Positions of the molecular mass markers in kilodaltons are indicated on the left.

antibodies, high levels of IgG1 but low levels of IgM were detected in the N'Dama group; in contrast low levels of IgG1 but high levels of IgM developed in the Borans (Figure 6).

The third major antigen identified was a 33 kD protein. It was recognized by IgG1, but not by IgM or IgG2. Antibodies specific for the 33 kD antigen were present in the pre-rechallenge sera from all five N'Damas and two of the Borans (nos. 17 and 23). Following the onset of parasitaemia, these antibodies were no longer detectable or reacted less strongly on immunoblots for a period of 5 to 10 days. Thereafter, they reappeared and persisted throughout infection, as shown by immunoblotting (Figure 5) and by ELISA using the electroeluted 33 kD protein (Figure 7). None of these animals (nos. 17 and 23) required drug therapy. The sera from Borans nos. 16, 21 and 22 did not react with the 33 kD antigen prior to the rechallenge. During infection, anti-33 kD antibodies were either not detectable, or transiently detected at low levels. All these three Borans became severely anaemic and two of them required drug therapy.

Analysis 2

To further assess the importance of antibody recognition of the 33 kD antigen, the responses to this antigen were examined in the same animals following primary infection. The kinetics of parasitaemia and anaemia in the ten primary infected Boran and N'Dama cattle have been described (Paling *et al.* 1991a). Briefly, Boran and N'Dama cattle were equally susceptible to infection since they experienced similar prepatent periods and similar levels of parasitaemia up to 26 DPI. From 26 DPI, lower numbers of trypanosomes were detected in the N'Damas compared to the Borans. In addition, mean PCV continued to decrease in the Borans whereas that of the N'Damas started to recover between 45 and 70 DPI. Of the five Borans, three animals (nos. 16, 17 and 21) required drug therapy between 28 and 40 DPI. The other two (nos. 22 and 23) remained chronically anaemic (PCV between 16% and 22%) for the duration of the experiment (6 months). None of the N'Damas required therapy.

Figure 8 shows the pattern of antigen recognition by sera collected 5 months after primary inoculation, or at the time of drug therapy for those Borans that were treated. Against the 33 kD antigen, only antibodies of the IgG1 class were detected. These antibodies were detected in all five N'Damas but in none of the five Borans during infection.

DISCUSSION

The present study investigated the humoral immune response to non-VSG parasite antigens in trypanotolerant and trypanosusceptible cattle following a rechallenge infection with *T. congolense*. Both IgM and IgG1 responses were elicited, but IgG1 antibodies were detected against more antigens than IgM. N'Dama cattle generated higher levels of specific IgG1 than the Boran. Three major invariant antigens were identified: a 69 kD antigen to which the IgM response was primarily directed in both breeds; a 23 kD antigen which was recognized equally by the two breeds; and a 33 kD antigen which elicited detectable antibodies only in cattle which resisted the disease.

Increases in total serum IgG levels in trypanosome-infected cattle have been reported (Clarkson & Penhale 1973) but these increases are moderate and do not appear to be a consistent feature of bovine trypanosomiasis. In contrast, an increase in the serum IgM level is characteristic (Bideau, Gidel & Moity 1966, Clarkson & Penhale 1973, Kobayashi & Tizard 1976, Luckins 1972, 1976, Nielsen *et al.* 1978). The data presented here suggest that antibodies against invariant parasite antigens do not significantly contribute to the high IgM level. It is likely that the increase in serum IgM is due either to repeated stimulation of the immune system by VSGs from different antigenic variants (Seed *et al.* 1969) or to polyclonal activation of the B-cell repertoire (Greenwood 1974, Hudson *et al.* 1976).

IgG2 was found to play a minor role in the recognition of invariant antigens in both breeds. This is consistent with the observation that serum IgG2 levels do not increase during infection (Kobayashi & Tizard 1976). Since IgG1 and IgG2 appear to have very similar functions in cattle (McGuire, Musoke & Kurtti 1979), it is unlikely that the failure to mount an IgG2 response has an effect on the control of infection.

One other study in cattle has been reported in which immmunodominant trypanosome antigens of Mr 110, 150, and 300 kD were identified (Shapiro & Murray 1982). Animals which recognized at least one of the three molecules were shown to control the disease more effectively than animals which failed to recognize any of them. Proteins of these Mr were not identified as major antigens in the present study. However, our study differed in three ways from that of Shapiro & Murray (1982), firstly in the species of trypanosomes used (we used *T. congolense*, instead of *T. brucei* followed by mixed infections), secondly in the route used to administer the infection (controlled fly challenge instead of sub-cutaneous injection followed by uncontrolled natural challenge) and thirdly in the techniques used to detect antibodies (immunoblotting instead of immunoprecipitation). These differences may account for the variation in results between the two studies.

In the present study, a 23 kD antigen was identified as immunodominant in cattle. Its pattern of recognition was the same in both groups of cattle and was not associated with levels of parasitaemia or anaemia.

A 69 kD antigen elicited both IgM and IgG in all animals. However, higher titres of anti-69 kD IgM were observed in the Borans than in the N'Damas, and conversely the N'Damas responded with higher levels of anti-69 kD IgG1. Analysis of the isotypic responses to whole trypanosome lysate also revealed that the Borans had higher IgM responses but weaker IgG1 responses compared to the N'Damas. These observations suggest that an impairment in the switch from IgM to IgG1 might occur in trypanosusceptible cattle during infection. In studies using *T. brucei* infected cattle, the failure to induce measurable IgG2 titres to VSG was also attributed to an impairment in the IgM to IgG2 switch (Musoke *et al.* 1981). Since isotype switch involves T-cell help (Kishimoto & Hirano 1988, Paul & Ohara 1987) the dysfunction suspected in trypanosusceptible cattle may be associated with low parasite-specific helper T-cell responses. The marked decrease in serum complement C3 levels during infection (Kobayashi & Tizard 1976, Nielsen *et al.* 1978) could also affect this phenomenon, since C3 deficiencies have been shown to result in switch impairment (Böttger & Bitter-Suermann 1987). A more efficient isotype switching in trypanotolerant cattle might be related to their superior ability to maintain complement levels during trypanosome infection (Authié & Pobel 1990).

Recognition of the third immunodominant antigen, a 33 kD protein, was associated with differences in susceptibility to the disease. Prior to the rechallenge experiment, antibodies specific for this antigen were detectable in the sera from all five N'Damas, and two out of five Borans. During the sixth infection, all of these animals recovered spontaneously, although the two Borans remained parasitaemic for longer and had lower PCVs than the N'Damas. The three Borans which had no detectable antibodies to the 33 kD antigen became severely anaemic and two of them required drug therapy to prevent possible death. Therefore, the presence of antibodies to the 33 kD antigen prior to the rechallenge appeared to associate with the capacity to control the disease. That anti-33 kD antibodies were detected in the five trypanotolerant N'Damas but in none of the susceptible Borans during the primary infection, reinforces this association.

In all but one animal (N'Dama no. 5) which had antibodies to the 33 kD antigen prior to the rechallenge,

there was an increase in these antibody titres during infection. This increase occurred after a transient disappearance of antibodies during the rising phase of parasitaemia, suggesting that the antibodies were being absorbed. This would indicate that the 33 kD antigen is either released from the parasite or accessible on living trypanosomes. In N'Dama no. 5, although anti-33 kD antibodies were detected prior to and during re-infection, there was no increase in titre. This result is consistent with the generally weak IgG responses observed in the two N'Damas (nos. 3 and 5) which experienced transient parasitaemia and thus low antigenic challenge. Since recognition of the 33 kD trypanosome antigen appears to correlate with higher resistance to disease, it is possible that this molecule is a pathogenic factor. The neutralization of its effects by pre-existing antibodies might mitigate early pathological events, and therefore may be as important as the production of anti-33 kD antibodies during infection.

In conclusion, following a *T. congolense* rechallenge, trypanotolerant N'Dama cattle mounted a higher IgG1 response to invariant trypanosome antigens than did the susceptible Boran. In addition, antibodies to a 33 kD antigen were detected in the N'Damas and in two of the three self-curing Borans, but not in Borans unable to control the disease. Thus, antibodies to the 33 kD antigen may be a marker or a direct mediator of a capacity to control the disease. Their presence might be of relevance to survival of cattle repeatedly exposed to natural trypanosome challenge.

ACKNOWLEDGEMENTS

We are very grateful to F.McOdimba for monitoring parasitaemia and PCV during the experiments, and to J.R.Scott for help with statistical analysis. This is ILRAD publication no. 947.

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