**Trypanosoma congolense:** A Comparison of T-Cell-Mediated Responses in Lymph Nodes of Trypanotolerant and Trypanosusceptible Cattle during Primary Infection

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*International Livestock Research Institute, P.O. Box 30709, Nairobi, Kenya; and †Department of Statistics and Modelling Science, University of Strathclyde, Livingstone Tower, Glasgow G1 1XH, Scotland

Lutje, V., Taylor, K. A., Kennedy, D., Authié, E., Boulangé, A., and Gettinby, G. 1996. Trypanosoma congolense: A comparison of T-cell-mediated responses in lymph nodes of trypanotolerant and susceptible Boran cattle during primary infection. Experimental Parasitology 84, 320–329. A comparison of T-cell-mediated immune responses in trypanotolerant N’Dama and susceptible Boran cattle during primary infection with tsetse-transmitted Trypanosoma congolense was conducted to assess whether different patterns of T-cell activation occurred during trypanosome infection. Proliferation and IFN-γ synthesis in response to trypanosome antigens and to the mitogen Con A were measured in LNC before infection and 10 and 35 days postinfection. Phenotypic analysis of LNC was also carried out. No significant differences in the in vitro proliferation of LNC to VSG, to hsp70/BiP, or to Con A were detected between the breeds. In contrast, IFN-γ production in response to Con A was higher in Boran cattle at 35 days p.i. A reduction in the number of CD2+ and CD4+ T-cells and an increase in the percentage of B-cells, CD8+ T-cells, and γδ T-cells during infection in both N’Dama and Boran was revealed by cytfluorimetric analysis of lymph node cells. © 1996 Academic Press, Inc.

**INDEX DESCRIPTORS AND ABBREVIATIONS:** Trypanosomiasis, bovine; Trypanosoma congolense; T-cell proliferative responses; IFN-γ synthesis; surface phenotype. PCV, packed cell volume; LN, lymph node; LNC, lymph node cells; p.i., postinfection; Con A, concanavalin A; CP, congopain; hsp70, T. congolense 69-kDa heat-shock protein; BiP, immunoglobulin heavy chain binding protein; 125IUDR, 5-[125I]iodo-2-deoxyuridine; cpm, counts per minute; SI, stimulation index; VSG, variant surface glycoprotein.

**INTRODUCTION**

African trypanosomes are tsetse-transmitted extracellular protozoan parasites responsible for inducing sleeping sickness in man and nagana in livestock. These diseases have greatly hampered human settlement and economic development in vast areas of sub-Saharan Africa. An effective way of controlling trypanosomiasis is still lacking; together with vector control and chemotherapy, the combined use of trypanotolerant livestock and immune intervention may prove effective in reducing the impact of the disease and its devastating economic consequences.

The plasma membrane of African trypanosomes is covered by homodimers of the VSG (Cross 1975). Antibodies directed against VSG are involved in clearance of parasites. However, following transcriptional switching among VSG genes and subsequent expression of variant antigenic types on the trypanosome surface coat, the parasites are able to escape the host’s immune response. This results in successive waves of parasitemia that characterize the disease (Borst and Cross 1982). Thus, sterile immunity is never achieved and the infection is usually chronic.

The role of T-cells during infection of natural hosts and experimental animals with African trypanosomes has been questioned for several reasons: (1) Because of the repetitive structure of surface-exposed epitopes of the VSG, antibodies directed against these epitopes, which are responsible for clearing each parasitic wave,
were considered to result from T-cell-independent B-cell responses (Mansfield 1990); (2) as trypanosomes are extracellular parasites, MHC-restricted cytotoxic T-cells are presumably not involved in their clearance; (3) more important, trypanosome antigen-specific T-cell responses have been difficult to detect in infected animals. In contrast, strong trypanosome-specific T-cell proliferation develops in cattle following infection and treatment with trypanocidal drugs (Lutje et al. 1995a, Emery et al. 1980).

Recent studies have reconsidered the role of T-cells in African trypanosomiasis. Mouse antibody responses to surface-exposed epitopes of the VSG of Trypanosoma brucei rhodesiense were shown to result from an aggregate of T-cell-independent and T-cell-dependent responses (Reinitz and Mansfield 1990). During trypanosome infection, murine peritoneal T-cells secreted IL-2 and IFN-γ in response to in vitro stimulation with VSG (Schleifer et al. 1993). In infected cattle, T. congolense VSG and the invariant antigens CP (Authié et al. 1992) and hsp70/BiP (Boulangé and Authié 1994) induced in vitro proliferation and interleukin synthesis by LNC (Lutje et al. 1995b). In vivo, CP and hsp70/BiP may have biological activities responsible for some aspects of the pathology associated with African trypanosomiasis in cattle. Because antigenic variation of the surface coat makes the prospect of a VSG-specific vaccine unlikely, anti-disease vaccines targeted at invariant antigens may be an alternative for the control of African trypanosomiasis. A similar strategy has been proposed for malaria (Playfair et al. 1990). Further, T-cell activation and cytokine production may also participate in disease pathogenesis.

A genetic basis for resistance to African trypanosomiasis has been well documented from studies in natural and experimental hosts. West Africa taurine (Bos taurus) breeds of cattle, such as the N'Dama, through their long coexistence with the parasites, have evolved a degree of resistance to the disease, which results in better control of parasitemia and less severe anemia than zebu-type (B. indicus) breeds such as the Boran (Murray et al. 1982). The mechanisms of trypanotolerance in cattle and other species are not known but might involve an effective immune response to trypanosome antigens and/or differential cytokine synthesis with distinct effects on immunological functions, as well as a more effective hemopoiesis.

We have demonstrated previously that T-cell-mediated responses to VSG and invariant antigens of T. congolense are detectable in regional LN of infected trypanosusceptible Boran cattle (Lutje et al. 1995b). Here we expand the analysis of T-cell-mediated immunity by comparing phenotype and proliferative and cytokine responses in LNC of Boran cattle with those of trypanotolerant N'Dama during primary experimental challenge with T. congolense. We demonstrate that although phenotype and blastogenic responses of LNC are similar in animals from the two breeds, T-cell-mediated IFN-γ synthesis differs between N'Dama and Boran.

**MATERIALS AND METHODS**

**Animals and experimental infection.** Six age- and sex-matched Boran and six N'Dama cattle were raised and maintained in an area of Kenya free from tsetse flies. Before experimental infection with T. congolense, the animals were treated twice with fenbendazole (Panacur, Hoechst, Germany) and with imidocarb dipropionate (Imizol, Coopers, England) at 2 mg/kg, at least 1 month before infection. Laboratory-raised tsetse flies (Glossina morsitans centralis) were fed on a goat infected with T. congolense clone IL1180, a derivative of stock STIB/212, originally isolated in Tanzania (Geigy and Kaufman 1973). Cattle were infected by 10 infected tsetse flies biting on the left flank and the left side of the neck; these areas drain into the left prefemoral and left prescapular LN, respectively. Parasitemia in blood was monitored throughout infection by the dark ground phase-contrast method (Paris et al. 1982). PCV was measured three times a week. The animals were euthanized 35 days p.i.

**Antigens and mitogens.** Swiss × Balb/c mice were irradiated with 800 rads from a cesium-137 source and inoculated intraperitoneally with T. congolense ILNat 3.1 stabilates. Mice were exsanguinated at the first peak of parasitemia and blood was collected in phosphate-buffered glucose, pH 8.0, containing 10 IU/ml heparin sodium (Novo Nordisk, Denmark). Trypanosomes were isolated by anion-exchange chromatography on DEAE-52 (Whatman Biosystems, UK) as described by Lanham and Godfrey (1970), washed and frozen at 10⁶/ml after addition of leupeptin at 10 µg/ml, 1 mM phenylmethylsulfonyl fluoride, and 0.2 mM N-tosyl-L-phenylalanine chloromethyl ketone. All the protease inhibitors were purchased from Sigma (St. Louis, MO).
Soluble VSG from *T. congolense* ILNat 3.1 was prepared from whole parasite lysates by electrophoresis from SDS-polyacrylamide gels according to the method of Hunkapiller et al. (1983). C63, a recombinant form of *T. congolense* hsp70/BiP, was prepared as described (Lutje et al. 1995b). N61 (Boulangé and Authié 1994), another recombinant protein of hsp70/BiP, comprising 560 N-terminal amino acids of the mature 69-kDa antigen (Boulangé and Authié 1994), was produced by directional cloning of an EcoRI–HindIII fragment of the complete open reading frame into the expression vector pMAL-cRI (Maina et al. 1988). The fusion protein was purified from bacterial lysate and cleaved by factor Xa as described (Duplay et al. 1984). The recombinant polypeptide was further purified by immunoaffinity chromatography using the mAb 1B2 (Authié, unpublished).

**Cell cultures for proliferative and IFN-γ assays.** The right and left prefemoral LN were surgically removed from the animals under sedation and local anesthesia, before infection and at 10 and 11 days post-infection, respectively. The left presupercapular LN was obtained at autopsy 35 days post-infection. LN tissue was finely minced in sterile RPMI 1640 supplemented with 10% heat-inactivated bovine serum product (FetalClone, Hyclone Labs, UT), 25 mM t-glutamine, 200 U/ml penicillin, 150 μg/ml streptomycin, and 5 × 10⁻⁵ M 2-mercaptoethanol (complete culture medium). LNC were washed twice in complete culture medium, counted, and resuspended at 5 × 10⁶/ml. Aliquots of 100 μl LNC were cultured in triplicate in round-bottom wells of 96-well culture trays (Costar, MA), with culture medium (control wells), Con A (Sigma), anti-bovine IFN-γ (Ciba-Geigy, Switzerland). Cultures maintained with medium alone or with Con A received 25 μl aliquots of 10⁶/ml VSG, C63, or N61 at 10 μg/ml. Cell cultures were maintained at 37°C in a humidified atmosphere of 5% CO₂ in air. After 5 days, 100 μl supernatant was collected from each well and stored at −80°C for detection of IFN-γ. Each culture then received 0.5 μCi ¹²⁵IUDR (Amersham, UK) and was incubated for a further 4–5 hr. Cells were harvested onto filter mats using a Titertek Cell Harvester (Flow Labs, Ayrshire, UK) and each sample was counted for 1 min in a gamma counter (Beckman Instruments, CA). Counts per minute obtained for each individual culture were transformed into SI by calculating the ratio with the mean cpm of six replicate cultures maintained with medium alone.

The concentration of IFN-γ in supernatants was measured using a commercial sandwich ELISA test (Commonwealth Serum Labs, Parkville Australia). Optical density values were obtained on a Titertek Multiscan ELISA plate reader (Labsystems, Finland) and related to a standard curve prepared with appropriate dilutions of recombinant bovine IFN-γ (Ciba-Geigy, Switzerland). Cultures maintained with medium alone produced little or no IFN-γ; these background values have been subtracted from the quantities of IFN-γ produced in cultures stimulated with Con A, VSG, C63, and N61.

**Monoclonal antibodies.** Bovine CD2⁺ T-cells were identified by mAb IL-A43 (Davis and Spliter 1991). Bovine CD4⁺ and CD8⁺ cells were identified with MAb IL-A11 and IL-A105, respectively (Baldwin et al. 1986; MacHugh and Sopp 1991). WC1⁺ γδ T-cells were identified with mAb IL-A29 (Morrison et al. 1988). B-cells were stained with mAb IL-A30 (Naessens et al. 1988), and MHC class II⁺ cells with mAb J11 (Teale and Kemp 1987).

**Immunofluorescent staining and FACS analysis.** LNC to be used for immunofluorescent stains were washed twice in ice-cold Leibovitz’s 15-medium, supplemented with 5% heat-inactivated bovine serum product and 0.2% Na azide (IFA buffer). Cells were resuspended at 10⁵/ml in IFA buffer and 25-μl aliquots were distributed in V-bottom microtitrator plates (Costar, MA). LNC then received 25 μl of the required mAb at a dilution that had previously been determined as optimal and were stained as described (Howard et al. 1992). Anti-mouse FITC (Southern Biotechnology, AL) was added at 1/30. Fluorescence was assayed on a FACStar II (Becton–Dickinson, CA) using a live gate and analyzed using in-house software. Five thousands cells were analyzed for each sample; results are expressed as percentages of the total cell number.

**Statistical analysis.** Data were analyzed using the two-factor analysis of variance repeated measures design (Winer 1971) with breed and time as main effects and breed × time as an interaction effect. This provided *F* tests for differences between breeds, differences between points in time, and for breed patterns over time. Probabilities of less than 0.05 were considered significant and calculations were carried out using Minitab V9.1 statistical software for Windows.

**RESULTS.**

**Clinical parameters.** Parasitemia and PCV were measured following infection to confirm that cattle under experiment behaved as resistant or susceptible to trypanosomiasis. Briefly, parasites were detected in the blood of all N’Dama cattle by Day 13 p.i. and in all Boran cattle by Day 15 p.i. PCV, an indicator of anemia, started declining in both breeds 20 days p.i., with a faster rate of decline in Boran cattle (data not shown).

**Changes in cell subsets after trypanosome challenge.** Changes in the percentages of CD2⁺, CD4⁺, CD8⁺, and γδ T-cells, B-cells, and MHC class II⁺ cells in LNC of Boran and N’Dama cattle were monitored before infection and at 10 and 35 day p.i. (Table I). A significant reduction in the percentage of CD2⁺ and CD4⁺ T-cells was detected in both breeds over time (*P* = 0.02 and *P* = 0.002, respectively); no difference was detected between the two breeds. The percentage of CD8⁺ T-cells increased between the preinfection time point and Day 10 p.i. and remained higher in Boran cattle, but
these values were at the limit of significance \( (P = 0.06) \). The CD4:CD8 ratio slightly decreased in both breeds during infection (data not shown). Significant increases in the percentages of WC1+ \( \gamma \delta \) T-cells \( (P < 0.001) \) and B-cells \( (P < 0.001) \) were detected in LNC of both breeds. A significant increase in the percentage of MHC class II+ cells \( (P < 0.001) \) was observed at 35 days p.i.

**Con A-induced proliferation and IFN-\( \gamma \) secretion.** Cultures of LNC from Boran and N’Dama cattle were stimulated with the T-cell mitogen Con A before infection and at 10 and 35 days p.i. Maximal in vitro proliferation was detected before infection with the parasite (Fig. 1a). In contrast, responses to Con A in draining lymph nodes were reduced 10 days p.i. and almost totally depressed at 35 days p.i. Comparable responses were detected in LNC from animals of the two breeds, without interbreed or time/breed differences. On Day 35 p.i., proliferation to Con A was also measured in cultures of contralateral LNC; responses in the contralateral LN were comparable to those of the draining LN.

Levels of IFN-\( \gamma \) present in the supernatants of the same cultures tested for proliferation were similar in Boran and N’Dama cattle before infection and at 10 days p.i. (Fig. 1b), but at 35 days p.i. they were significantly higher in Boran cattle \( (P = 0.009) \). On Day 35 p.i., we also measured IFN-\( \gamma \) secretion in LNC from the contralateral LN; responses to Con A were higher than in the draining LN in both breeds.

**Antigen-induced proliferation and IFN-\( \gamma \)-secretion.** Proliferative responses to *T. congolense* VSG, C63, and N61 (Figs. 2a, 2b, and 2c) were measured before infection and at 10 and 35 days p.i. Little or no response to these trypanosome antigens was detected prior to infec-

### TABLE I

Percentages of Cell Subsets in Regional Lymph Nodes of *T. congolense*-Infected Cattle

<table>
<thead>
<tr>
<th>Mab</th>
<th>Cell subsets</th>
<th>Preinfection</th>
<th>Day 10</th>
<th>Day 35</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N’Dama</td>
<td>Boran</td>
<td>N’Dama</td>
<td>Boran</td>
</tr>
<tr>
<td>ILA 43</td>
<td>CD2*</td>
<td>71.1 ± 7.7</td>
<td>66.8 ± 14.2</td>
<td>56.4 ± 12.9</td>
</tr>
<tr>
<td>ILA 11</td>
<td>CD4*</td>
<td>45.2 ± 4.4</td>
<td>47.2 ± 5.0</td>
<td>39.4 ± 7.6</td>
</tr>
<tr>
<td>ILA 105</td>
<td>CD8*</td>
<td>47.0 ± 6.5</td>
<td>44.2 ± 7.1</td>
<td>51.9 ± 8.7</td>
</tr>
<tr>
<td>ILA 29</td>
<td>( \gamma \delta )</td>
<td>3.26 ± 2.4</td>
<td>2.8 ± 1.6</td>
<td>14.7 ± 4.4</td>
</tr>
<tr>
<td>ILA 30</td>
<td>IgM*</td>
<td>19.2 ± 5.0</td>
<td>16.1 ± 5.1</td>
<td>30.1 ± 10.6</td>
</tr>
<tr>
<td>J 11</td>
<td>MHC class II*</td>
<td>36.5 ± 6.9</td>
<td>31.2 ± 6.4</td>
<td>36.0 ± 5.3</td>
</tr>
</tbody>
</table>

FIG. 1. Proliferative (a) and IFN-\( \gamma \) (b) responses of LNC from N’Dama (■) and Boran (□) cattle to Con A before infection and at 10 and 35 days p.i. Each graph represents the mean SI (a) or pg/ml (b) of LNC from six animals, ± the standard error. At 35 days p.i., responses from both draining and contralateral LN were measured.
LNC from both breeds responded poorly to VSG at Days 10 and 35 p.i. C63 and N61 induced proliferative responses in LNC from both breeds at Day 10 p.i., but these were hardly detectable by 35 days p.i. No proliferation to the irrelevant antigen, ovalbumin, was detected in LNC at any time point (data not shown). No significant differences were detected in the proliferation of LNC from the two breeds to any of these antigens.

The same trypanosome antigens induced in vitro secretion of IFN-γ in LNC obtained at 10 days p.i. and, at a lower level, at 35 days p.i. (Figs. 3a, 3b, and 3c). Levels of this cytokine were generally higher in supernatants of Boran LNC at 35 days p.i., but statistically significant differences with N’Dama LNC were not detected. IFN-γ secretion in response to VSG was slightly higher in cells from the contralateral LN at 35 days p.i.

**DISCUSSION**

The work presented here is part of an ongoing study aimed at characterizing the repertoire of bovine cellular immune responses to infection with African trypanosomes. We have previously described proliferative and cytokine responses elicited by *T. congolense* antigens in cells from the LN draining the site of an infected tsetse bite, in susceptible Boran cattle (Lutje et al. 1995b). In the present study, we compared T-cell-mediated responses to Con A and to trypanosome antigens in two age- and sex-matched groups of susceptible Boran and resistant N’Dama.

Our results did not show statistically significant differences in the intensity of mitogen- or antigen-induced proliferation of LNC from Boran and N’Dama cattle, thus suggesting that this type of response does not correlate with resistance or susceptibility to the disease. A previous study (Flynn et al. 1992) reports earlier and higher proliferative responses to *T. congolense* lysate in PBM of infected N’Dama than in PBM from Boran; no responses were detected in LNC. In contrast, in our hands, proliferative responses to defined trypanosome antigens were detectable in LNC from both breeds; these responses were highest at 10–11 days p.i. and greatly reduced at 35 days p.i. We did not detect trypanosome antigen-specific responses in PBM of infected cattle (Lutje et al. 1995b). The
short duration of trypanosome antigen-specific T-cell responses in LN has also been observed in T. brucei-infected mice (Gasbarre et al. 1980) and may be a consequence of the generalized immunosuppression seen in African trypanosomiases or may result from the migration of primed T-cell to other sites. To test this hypothesis, proliferation to VSG at 35 days p.i. was also measured in the contralateral LN but no significant differences with the draining LN were observed. Together with the lack of trypanosome antigen-specific responses in PBM (Lutje et al. 1995b), this suggests that T-cells primed to trypanosome antigens do not recirculate in the peripheral lymphatic compartment. We did not detect preferential recognition of any of the trypanosome antigens used in either Boran or N’Dama cattle. Further, responses to VSG and to C63 were lower than in previously reported experiments (Lutje et al. 1995b), possibly the result of using different antigen preparations. N61, a recombinant protein of hsp70/BiP, elicited high responses in LNC at Day 10 p.i., indicating that primed and functional T-cells were present in lymph nodes of both breeds. Another reason for variations between experiments is the intrinsic variability of cellular immune parameters measured in outbred animals; several factors such as age, sex, and circadian and circannual rhythms have been found to affect cell-mediated immune responses in farm animals (Kristensen et al. 1982).

Mitogen-induced proliferation of LNC was highest before infection, greatly reduced by Day 10, and almost totally suppressed at 35 days p.i. in both cattle breeds. Con A induces activation of all T-cell subsets by direct stimulation of the T-cell receptor/CD3 complex, and the reduction in LNC responsiveness at 10 and 35 days p.i. confirms the suppressive influence of trypanosome infection on the immune system that has been demonstrated in trypanosome-infected mice and in sleeping sickness patients, and is considered responsible for the high incidence of secondary infections in trypanosome-infected hosts (reviewed by Vickerman et al. 1993). In agreement with our studies, but with different kinetics, Flynn and Sileghem (1991) observed a transient reduction in Con A-induced proliferation of LNC in Boran cattle, between 7 and 28 days p.i., and in N’Dama cattle at 21 days p.i. (Flynn and Sileghem 1993). Further, in LNC
from another four *T. congolense*-infected Boran cattle, Con A-induced T-cell proliferation was transiently depressed, and was restored at 30 days p.i. (Taylor et al., unpublished). Our results indicate a longer duration of immune suppression in LNC; similarly, Vickerman et al. (1993) suggested that immunosuppression in bovine trypanosomiasis is more pronounced in late infections. An explanation for the differences in the kinetics of suppression observed in different experiments is still lacking, although it is possible that antigen load or intensity of initial parasitemia, which may vary between infections, play a significant role.

In contrast to proliferation, IFN-γ synthesis by LNC was higher in Boran than in N'Dama cattle in response to trypanosome antigens and to Con A, at 35 days p.i. IFN-γ synthesis was not correlated to the intensity of proliferative responses, which at this time point were depressed in both breeds, or to the distribution of cell subsets in Boran and N'Dama LNC. Indeed, in separate experiments, using FACS-sorted bovine cell subpopulations, we detected Con A-induced IFN-γ synthesis in both CD4+ and CD8+ cells, but not in γδ TCR+ cells (Lutje unpublished). IFN-γ is also produced by natural killer cells (Farrar and Schreiber 1993), as part of the innate immune response to pathogens; this cell type, however, is not well characterized in cattle. Furthermore, Con A-induced *in vitro* cytokine synthesis might not be directly related to *in vivo* production. Studies are in progress to determine the cytokine mRNA pattern of bovine LNC during trypanosomiasis infection, to allow us to correlate our *in vitro* observations with *in vivo* IFN-γ production. IFN-γ has been shown to regulate macrophage activation, antigen-presenting function, and Ig isotype switch (Farrar and Schreiber 1993), as well as to depress proliferation of Th2 cells (Mosmann and Coffman 1991); all of these functions might be relevant to the immunological response to trypanosomiasis. In cattle, IFN-γ has been shown to induce IgG2 production in PWM-stimulated B-cells (Estes et al. 1994), but its effect on the synthesis of other isotypes, or in different disease situations, is not known. IFN-γ has also been shown to inhibit proliferation of murine hematopoietic progenitors (Koike et al. 1992), and a similar mechanism, if confirmed in cattle, might have significant consequences on the development of anemia during trypanosomiasis.

Comparable changes in the distribution of cell subsets in LNC were detected during *T. congolense* infection in Boran and N’Dama cattle. A decrease in CD4+ cells and an increase in the CD8+ subset led to a change in the CD4/CD8 ratio. A similar reduction in CD4+ cells has been described in Chagas’ disease (Dos Reis et al. 1995) and in human HIV infection (Groux et al. 1992), and has been related to apoptosis. Similarly in bovine trypanosomiasis, an initial activation of CD4+ cells caused by the encounter with high doses of trypanosome antigens, not followed by appropriate costimulatory signals by antigen-presenting cells, could result in CD4+ T-cell unresponsiveness, expressed as a block of IL-2 production and T-cell proliferation, or in programmed cell death. Indeed, a suppression of IL-2 secretion is a feature of infected LNC in cattle (Sileghem and Flynn 1992). In contrast to the reduction in CD4+ cells, an increase in the percentage of B cells, WC1+ γδ T-cells, and MHC class II+ cells was observed in LNC of both breeds. Increases in the number of B-cells during trypanosome infection have been reported previously (Williams et al. 1991), and some authors relate this to an increase in CD5+ B-cells (Naessens and Williams 1992). Ruminants have a high number of γδ T-cells (Hein and MacKay 1991) but their precise function in immunity to infections is still unknown. An increase in the relative percentage of γδ T-cells has been reported in chronic malarial infections in man (Langhorne et al. 1992) and an immunoregulatory role for this cell’s subset has also been suggested in bovine infection with *Babesia bovis* (Brown and Rice-Ficht 1994). Also, bovine T-cell lines developed *in vitro* to a trypanosome antigen of MW 100,000 had a predominant γδ TCR phenotype (Flynn and Sileghem 1994). Altogether, the effect of γδ T-cells on the outcome of trypanosome infection remains unclear.
This study is part of a series, aimed at a better understanding of bovine immune responses during infection with African trypanosomes. A detailed analysis of B-cell responses was conducted during the same experiment and reported separately (Taylor et al. 1995). These studies demonstrated that isotypic differences in the antibody response of Boran and N’Dama cattle to *Trypanosoma congolense* antigens, previously detected in serum (Williams et al., in press; Authié et al. 1993), are also present at the cellular level (Taylor et al. 1995). As we observed an interbreed difference in IFN-γ levels in the same group of animals, we suggest that this interleukin may affect B-cell activation and isotype switch. However, we also must consider whether other immune parameters, not measured in the present study, might be responsible for differential isotypic expression and especially for the different clinical outcomes of the infection. Immune functions during early trypanosome infection, such as antigen presentation by afferent lymph veiled cells and T-cell responsiveness in afferent lymph, are currently being analyzed and will be reported separately.

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**References**


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