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Immune effector mechanisms involved in the control of parasitaemia in *Trypanosoma brucei*-infected wildebeest (*Connochaetes taurinus*)

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SUMMARY

The course of *Trypanosoma brucei* infection in the wildebeest (*Connochaetes taurinus*) was studied. A low but persistent parasitaemia developed in all five wildebeest following intravenous inoculation with 1×10^8 organisms of clone ILTat 2·1. Unlike cattle controls, however, the wildebeest did not develop anaemia. In both cattle and wildebeest, radioimmunoassay studies revealed a classical sequence of production of IgM, IgG1 and IgG2 antibodies which had the capacity to bind to the corresponding purified variable surface glycoprotein and to neutralize the infectivity of ILTat 2·1. Investigations into the interaction between post-infection sera, trypanosomes and freshly isolated peripheral blood leucocytes (PBL) of wildebeest and cattle showed that sera from the wildebeest had a higher capacity to induce adherence of trypanosomes to homologous PBL. The adherence and phagocytosis-inducing activity resided in the IgM fraction. Cross-testing of the antibodies and PBL revealed that wildebeest IgM antibodies induced high adherence indices when tested on cattle PBL. High adherence indices were also observed when cattle IgM antibodies were tested on PBL of wildebeest. It was concluded (i) that the phagocytic system of the wildebeest was superior to that of cattle, (ii) that freshly prepared wildebeest PBL bear receptors for wildebeest as well as cattle IgM, and (iii) that cattle PBL bear a receptor for wildebeest IgM that would appear to be different from that for cattle IgM.

INTRODUCTION

Some West and Central African breeds of cattle, particularly the N'dama and Baoule, are known to thrive in areas of high trypanosomal challenge where others like the large humped Zebu cannot (Murray, Trail & Grootenhuis, 1984). There is, however, some disparity of opinions about the degree of resistance exhibited by the 'trypanotolerant' breeds since they too do become clinically ill and often die from the disease (Murray *et al.*, 1977). It is also widely accepted that many wild Bovidae are almost completely refractory to the effects of trypanosomiasis. The mechanisms responsible for the resistance/tolerance in both the domesticated and wild Bovidae are unknown. An attempt to explain resistance in trypanotolerant cattle on the basis of superior antibody production was not convincing, since both the resistant and susceptible cattle made antibodies of equal potency (Pinder *et al.*, 1984). As wild Bovidae are thought to be refractory to trypanosomiasis, it was felt that an in-depth study of parasite clearance mechanisms in these animals could provide a better model to identify a factor or factors responsible for trypanotolerance.

The animal species selected for use in this investigation was the wildebeest (*Connochaetes taurinus*). These animals perhaps form the largest number of a single species of wild Bovidae found in the parks of Eastern Africa, and natural infections of the wildebeest with *Trypanosoma brucei* have been recorded (Baker, 1968; Dillmann & Townsend, 1979). In the studies reported here, we examined the persistence of parasitaemia in *T. brucei*-infected wildebeest, and the effect of the parasitaemia on the health of this animal species. In addition, the nature and function of humoral responses of the wildebeest to the infecting trypanosomes were investigated.

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MATERIALS AND METHODS

Cattle

Four steers (crosses between Zebu and Charolais breeds) were obtained from areas known to be free from trypanosomiasis and were screened before use for antibodies to *T. congolense*, *T. vivax* and *T. brucei* by indirect immunofluorescence (Wilson, 1969) and found to be negative.

Wildebeest

Three wildebeest calves, aged 3 months, were born in captivity at the Veterinary Research Laboratory, Muguga, Kenya, while

two adults were captured from the wild at Athi, Kapiti Plains, Kenya. The five wildebeest were screened before use for antibodies to the trypanosome population used in this study by indirect immunofluorescence (Wilson, 1969) and neutralization (Nantulya *et al.*, 1979). They were found to be negative.

Mice

BALB/c mice were obtained from the ILRAD stock.

Trypanosomes

T. brucei clone ILTat 2·1 was derived from STIB 247 (Geigy & Kauffmann, 1973); ILTat 1·3 was derived from stock 227 (Barbet & McGuire, 1978) and MITat 1·2 was derived from stock 427 (Cross, 1977). All parasites were grown in lethally irradiated (900 rads) mice and isolated from infected mouse blood following the method described by Lanham & Godrey (1970).

Infection

The five wildebeest and the four steers were each inoculated intravenously with 1×10^8 ILTat 2·1 trypanosomes. Each animal was bled on Days 0, 3, 7, 10, 21, 32, 46 and 55, and blood was collected into tubes containing ethylenediamine tetraacetate (EDTA). The level of parasitaemia was assessed by microscopic examination of the blood buffy coat (Murray, Clifford & McIntyre, 1979). Blood for serum was obtained, at the same time, from each animal and the serum separated and stored at $-20°$ until needed.

Neutralization assay

Pre- and post-infection sera from all the animals were assayed for neutralizing activity against the infecting clone (ILTat 2·1) and two other unrelated clones (ILTat 1·3 and MITat 1·2) as described by Nantulya *et al.* (1979).

Isolation of wildebeest IgG subclasses

Whole wildebeest serum was precipitated three times with 50% saturated ammonium sulphate. After dialysing exhaustively against 0·001 M sodium phosphate buffer, pH 8·0, to remove the sulphate ions, the Igs were separated on diethylaminoethyl cellulose (DE52) by step-wise elution using 0·001 M and 0·01 M sodium phosphate buffers, pH 8·0. The fractions under each peak were pooled, concentrated by ultrafiltration and tested against rabbit anti-whole wildebeest serum by immunoelectrophoresis (Leid & Williams, 1974) to identify the immunoglobulin class. The immunoglobulins eluting with 0·001 M buffer appeared pure and had electrophoretic mobilities similar to that of bovine IgG2. The 0·01 M buffer eluted immunoglobulins with electrophoretic mobility similar to bovine IgG2 and IgG1. Further purification to separate IgG1 from IgG2 was achieved on a protein A column which bound all the IgG1 but not the IgG2 immunoglobulins. The bound IgG1 was then eluted from the protein A column with glycine–HCl buffer, pH 2·8.

Isolation of wildebeest IgM

The wildebeest IgM was prepared following the method described by Fey *et al.* (1976) with a minor modification. Instead of filtration through Sephadex G-200, Sepharose 6B (Pharmacia Fine Chemicals, Piscataway, NJ), equilibrated with 0·1 M Tris–HCl, pH 8·6, containing 1 M sodium chloride and 0·2% sodium azide was used. Further purification was achieved by isoelectro-

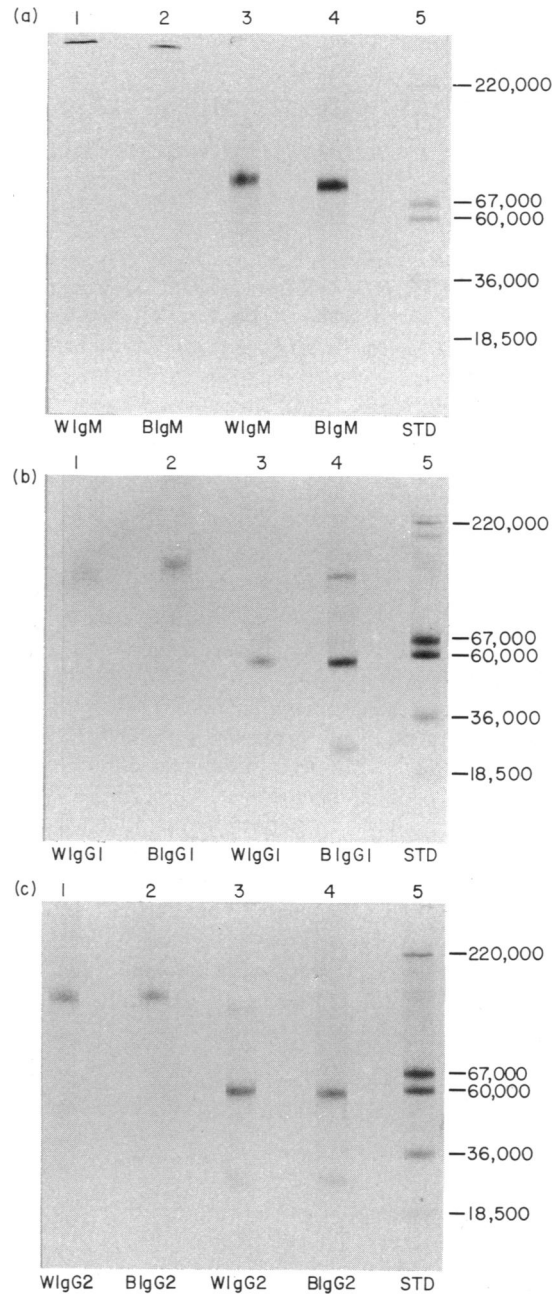

Figure 1. SDS–PAGE analysis of purified wildebeest and cattle immunoglobulins. The gels were run under unreduced (Lanes 1 and 2) and reducing (Lanes 3 and 4) conditions. Lane 5 is the Bio-Rad high molecular weight standards. W, wildebeest; B, bovine.

focusing, which was done following the instructions issued by the manufacturer (LKB Products, Bromma, Sweden), using 2% ampholine and a pH gradient of 3·5–10 for 18–24 hr.

Disc gel electrophoresis (SDS–PAGE)

The purity of the isolated immunoglobulins was assessed by 5% sodium dodecyl sulphate polyacrylamide gradient gel electrophoresis (SDS–PAGE) performed according to described methods (Weber & Osborne 1969). Bovine immunoglobulins were used for comparison. The isolated immunoglobulins gave

single bands and their relative molecular weights are shown in Fig. 1a, b and c.

Antisera to bovine and wildebeest immunoglobulins

Antisera to bovine and wildebeest immunoglobulins were prepared following the method described by McGuire, Musoke & Kurtti (1979).

Quantification of total serum immunoglobulins

IgM, IgG1 and IgG2 serum immunoglobulins of cattle and the wildebeest were quantified using the radial immunodiffusion technique as described by Mancini, Carbonara & Heremans (1965).

Radioimmunoassay

Sera from all cattle and wildebeest were tested for antibody activity against the variable surface glycoprotein (VSG) of the infecting trypanosome clone by radioimmunoassay as described by Musoke *et al.* (1981). The VSG of the clone was prepared as described by Cross (1975) and radiolabelled with ^{125}I (Hunter, 1978). The radioactivity in the precipitates was measured in a Packard 5360 Auto-Gamma Scintillation Spectrometer, and three times the background counts (pre-infection sera) was taken as the baseline.

Separation of cattle and wildebeest peripheral blood leucocytes (PBL)

Blood from both species (normal donors) was collected in heparin (50 units/ml of blood) and PBL separated by a modification of the method described by Aalund, Hoerlein & Adler (1970) as described in AG:DP/RAF/67/077 FAO Technical Report 3, 1978. The viability of the cells was determined by trypan blue exclusion.

Separation of adherent cells

The concentration of viable PBL was adjusted to give 1×10^7 cells per 0·2 ml. The cells (0·2 ml of the cell suspension per well) were transferred to 24-well culture plates (Cluster^6, Costar, Cambridge, MA). One ml of RPMI-1640 containing 10% fetal bovine serum (FBS) was added and the plates incubated at 37° in 5% CO_2/air mixture for 90 min. The plates were washed twice with Dulbecco's minimum essential medium (MEM) and the remaining cells in the wells were assumed to be adherent cells. The same PBL were similarly cultured on coverslips, stained with Giemesa and used for differential count of the cell population adhering to the plates.

Metabolic labelling of the trypanosomes with ^{35}S-methionine

After separation from blood elements by DE-52, the trypanosomes were washed twice in phosphate-buffered saline glucose (PSG), pH 8·0, and radiolabelled with ^{35}S-methionine according to the method described by Rovis & Dube (1981). The radiolabelled trypanosomes were washed three times with PSG and resuspended at a concentration of 1×10^8 trypanosomes/ml of PSG. The radiolabelled trypanosomes were checked for the presence of the surface coat by immunofluorescent staining of live parasites and/or by agglutination using rabbit antiserum to the VSG of the infecting clone (ILTat 2·1).

Assessment of the effect of post-infection serum on the interaction of adherent cells and the infecting trypanosomes

Two hundred microlitres of pre- or post-infection serum, diluted in RPMI-1640, were added to each well containing adherent cells from uninfected cattle or uninfected wildebeest, followed by an equal volume of the radiolabelled trypanosomes containing 2×10^7 trypanosomes. Each serum dilution was tested in duplicate. Controls included two wells with trypanosomes alone and two with cells alone. The plates were shaken and incubated for 10 min at 37° in the presence of 5% CO_2/air mixture. Sera from each species were tested on adherent cells of both cattle and the wildebeest. The plates were washed three times with MEM. The adherent trypanosomes were harvested by trypsinization (FAO Technical Report 3, 1978). The suspended trypanosomes and some adherent cells were filtered through glass-fibre filters (Whatman GF/A), washed twice with PSG and transferred to vials to dry. The radioactivity on the dried filters was counted in a liquid scintillation spectrometer with 10 ml Aquasol-2 (New England Nuclear, Boston, MA) as scintillation cocktail. The adherence index (AI) was calculated as follows:

$$AI = \frac{\begin{array}{c}\text{c.p.m. in the presence of} \\ \text{post-infection serum} \\ \text{and adherent cells}\end{array} - \begin{array}{c}\text{c.p.m. in the presence of} \\ \text{post-infection serum} \\ \text{without adherent cells}\end{array}}{\begin{array}{c}\text{c.p.m. in the presence of} \\ \text{pre-infection serum} \\ \text{and adherent cells}\end{array} - \begin{array}{c}\text{c.p.m. in the presence of} \\ \text{pre-infection serum} \\ \text{without adherent cells}\end{array}}.$$

Assessment of the effect of IgG- and IgM-enriched fractions from infection serum on the interaction of adherent cells and the infecting trypanosomes

IgG and IgM were isolated from infection serum which had a high AI by affinity chromatography as described by Musoke *et al.* (1981). The specific IgM and IgG fractions were tested on the adherent cells as described for whole serum.

RESULTS

Parasitaemia

The wildebeest had detectable parasitaemia 7 days post-infection (Fig. 2a), as compared to cattle which had demonstrable parasitaemia 3 days post-infection (Fig. 2b). The level of parasitaemia was lower in the wildebeest. Although the wildebeest were still parasitaemic 7 months post-infection, they had no signs of anaemia as judged from the packed red cell volumes.

Antibody responses

Quantification of the various wildebeest immunoglobulin classes revealed increases in the total IgM following infection. The average quantity of IgM in the five wildebeest was 0·77 mg/ml before infection and had increased to 3·22 mg/ml in two of the wildebeest and 2·5 mg/ml in the remaining three by Day 12 post-infection. This constituted a three- to four-fold increase in IgM levels. An average increase in the total serum IgM of 12-fold was observed in the wildebeest between 21 and 32 days post-infection. There was only a 0·5- to one-fold increase in the levels of total IgG1 and IgG2 in the wildebeest. In cattle, total immunoglobulin concentrations ranged from 3·8 mg/ml (pre-infection) to 22 mg/ml (post-infection) for IgM, 9·0 mg/ml to 30·9 mg/ml for IgG1 and 12·0 mg/ml to 20 mg/ml for IgG2.

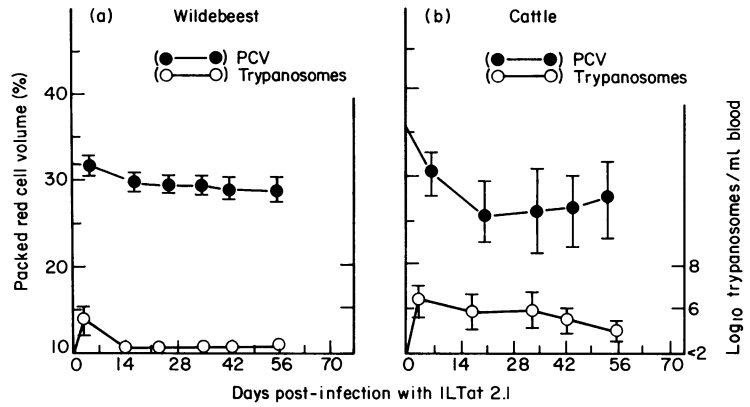

Figure 2. Comparison of mean parasitaemia (log_{10}) and packed red cell volume (%) in (a) wildebeest and (b) cattle infected with *T. brucei*.

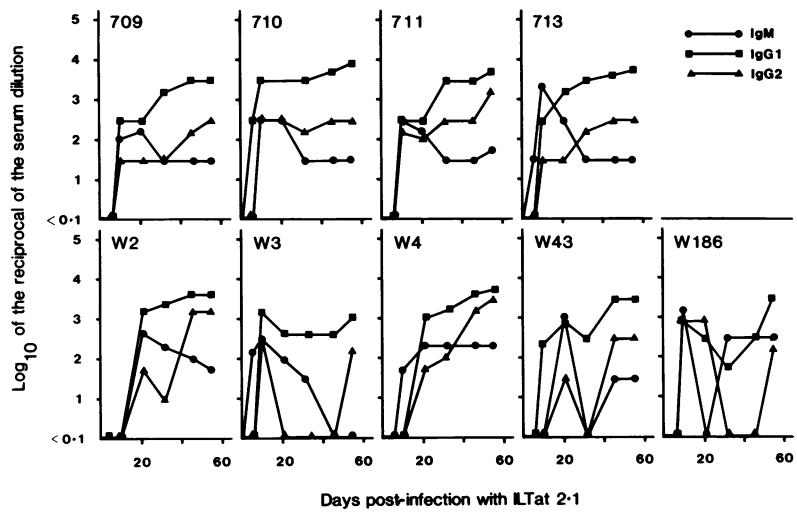

Figure 3. Class-specific antibodies to ILTat 2·1 in cattle (top) and wildebeest (bottom) infected with this trypanosome clone.

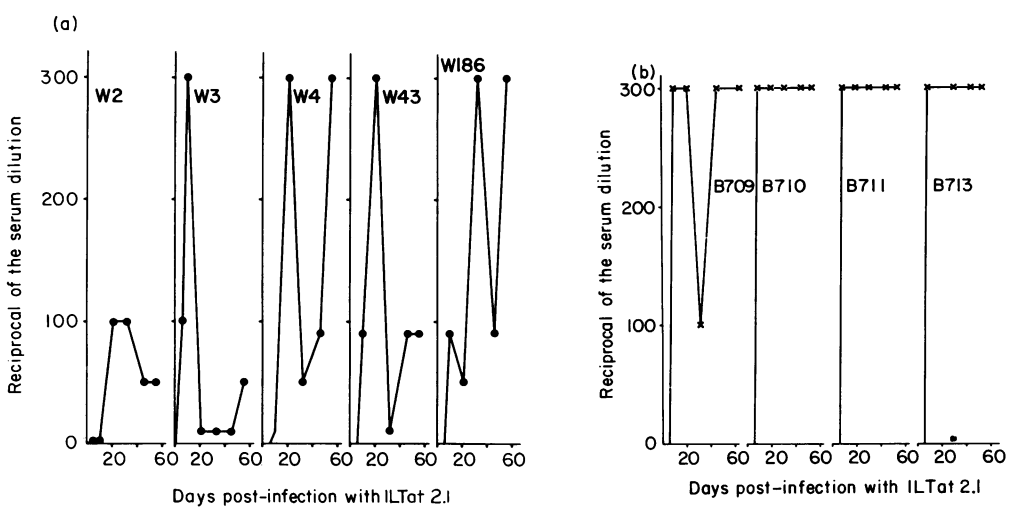

Figure 4. Neutralizing antibody titres to ILTat 2·1 in (a) wildebeest and (b) cattle infected with this trypanosome clone.

Radioimmunoassay studies revealed a classical sequence of production of IgM, IgG1 and IgG2 antibodies (Fig. 3) against the VSG of the infecting trypanosomes in both cattle and wildebeest post-infection sera. A higher frequency of recurrence of antibody peaks was observed in the wildebeest (Fig. 3).

Neutralization of ILTat 2·1

Neutralizing capacities of the sera from the wildebeest and cattle against the infecting trypanosomes are shown in Fig. 4a and b. As with the radioimmunoassay, multiple peaks of neutralizing antibody activity against the infecting trypanosomes were noted in four out of five wildebeest compared to one out of four cattle (Fig. 4a and b). The time duration of the antibody peaks was, however, much shorter in wildebeest than in cattle. The infection serum had no neutralizing effect on two unrelated VATs (ILTat 1·3 and MITat 1·2). A quantitative comparison of the neutralizing efficacy of affinity-purified IgM antibodies from the wildebeest and cattle revealed that 9·5 mg of wildebeest IgM and 10 mg of cattle IgM were needed to neutralize 1×10^4 ILTat 2·1 trypanosomes.

Adherent cells

Differential counts (Table 1) of the adherent cells on coverslips after incubation for 90 min revealed the presence of monocytes, neutrophils, small lymphocytes and eosinophils in both species of donor animals. In both cases monocytes were predominant, followed by neutrophils. The wildebeest had more adherent neutrophils (42%) than cattle (31%). A few small lymphocytes and eosinophils were also observed.

Effect of infection serum on attachment of trypanosomes to both wildebeest and cattle adherent cells

The trypanosomal adherence indices (AIs) are shown in Fig. 5. The wildebeest and cattle infection sera induced high AIs on wildebeest adherent cells (Fig. 5a). Similarly, high AIs were induced by the wildebeest infection serum on cattle adherent cells (Fig. 5b). In contrast, infection serum from cattle did not induce significant adherence of trypanosomes to homologous (cattle) adherent cells (Fig. 5b).

Effect of IgM- and IgG-enriched fractions of infection serum on adherence of trypanosomes to both wildebeest and cattle adherent cells

In order to identify the class of antibody responsible for the adherence of the trypanosomes, both IgM and IgG fractions of antibody were isolated and used to sensitize the trypanosomes.

Table 1. Differential counts of cells that adhered to the tissue culture plates after *in vitro* culture for 2 hr*

Cell donor	Lymphocytes	Neutrophils	Monocytes	Eosinophils
Cow	13·00±5·43	31·4±6·47	48·60±8·79	7·00±4·85
Wildebeest	6·2±3·700	42·00±4·00	50·20±2·86	1·6±1·34

* The cells were stained with Giemsa.

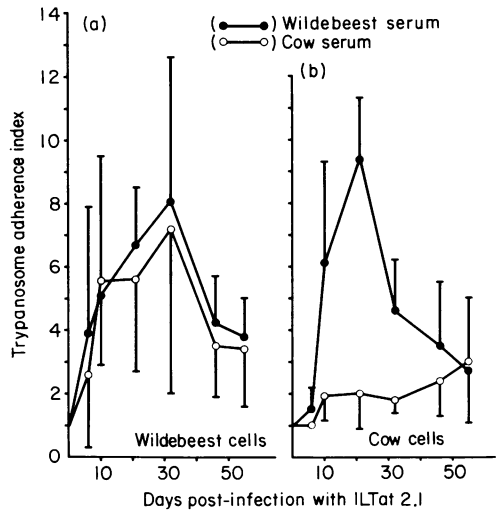

Figure 5. Indices of adherence of trypanosomes to wildebeest or cattle PBL in the presence of homologous or heterologous serum.

Only IgM fractions induced adherence of trypanosomes. There was no increase in adherence of trypanosomes in the presence of IgG1 or IgG2 fractions. Like whole serum, the IgM fractions from both wildebeest and cattle induced high AIs on wildebeest adherent cells, while only the IgM fraction from the wildebeest induced high AIs on cattle adherent cells.

DISCUSSION

The results of the work reported here have demonstrated that wildebeest infected with *T. brucei* can harbour the parasite for longer than 7 months. Unlike the situation in cattle, however, anaemia did not develop in the wildebeest. It had been hypothesized on the basis of detection of trypanosomes in blood samples collected during field surveys that the wild mammalian hosts suffer from trypanosomiasis and that the survivors acquire some immunity (Cunningham, 1968; Wells & Lumsden, 1968). From the present results and others (unpublished observations), however, it is apparent that wild Bovidae do not readily suffer the pathogenic effects of trypanosomal infections despite harbouring the parasites for a long time.

This work has further shown that the wildebeest mounts a classical immune response to the infecting trypanosomes that is similar to that in cattle. Like cattle, wildebeest produced neutralizing antibodies against the infecting clone, and the majority (four out of five) displayed at least two peaks of antibody activity. This frequency of recurrent antibody peaks was higher than in cattle, in that only one out of four cattle showed this response over a similar post-infection time interval. The time duration of the antibody peaks was, however, much shorter in the wildebeest than in cattle. Explanation for these differences was not apparent.

The significance of the phenomenon of multiple antibody peaks in the survival of the trypanosomes in the hostile environment is not yet clear. Musoke *et al.* (1981) have demonstrated that the antibodies in the recurrent peaks of immune responses of cattle to *T. brucei* are more efficient in the elimination of the parasites than the antibodies in the first peak. If recurrence of antibody peaks to the infecting VAT also

applies to all other VATs that arise during the infection as the work on *T. congolense* would suggest (Masake, Musoke & Nantulya, 1983), then this would imply that the animal with more frequent antibody recurrences would eliminate reappearing or cross-reacting VATS faster and more efficiently. This phenomenon, however, would appear to have no bearing on the ultimate elimination of parasitaemia since the wildebeest still had parasitaemia 7 months post-infection.

Since in cattle the PBL acting in concert with anti-trypanosome antibodies may play an important role in the control of parasitaemia (Ngaira *et al.*, 1983), a comparative study of the phagocytic efficiency of both the wildebeest and cattle PBL was undertaken. It was found that serum from infected wildebeest had a higher capacity to induce adherence of trypanosomes to homologous PBL than cattle serum on cattle PBL. In the absence of infection serum there was no significant adherence of trypanosomes to the PBL of either animal species. In an *in vivo* context, this was interpreted to mean that phagocytosis of antibody-sensitized trypanosomes is more efficient in the wildebeest than in cattle. Indeed, Young, Kanhai & Stagg (1975) have reported that circulating peripheral blood monocytes from infected buffalo are often engorged with trypanosomes—a feature not commonly seen in cattle. Thus, an efficient phagocytic system would explain, at least in part, the very low parasitaemia observed in the wildebeest and other wild Bovidae (Grootenhuis *et al.*, 1982).

We wondered whether the high adherence indices of the wildebeest system was due to the PBL being more 'active' than those of cattle or whether the wildebeest antibodies were more efficient at binding to the wildebeest PBL than cattle antibodies on cattle PBL. Wildebeest infection sera were, therefore, tested against cattle PBL and *vice versa*. The results of this experiment were intriguing, in that whereas the wildebeest infection serum induced high AIs on both wildebeest and cattle PBL, serum from cattle induced high AIs only when tested on wildebeest PBL.

Failure of antibody from cattle to induce adherence of trypanosomes to freshly isolated cattle monocytes has been reported before and was attributed to a lack of receptors for bovine IgM and IgG1 on freshly isolated cattle monocytes (Ngaira *et al.*, 1983). This however, does not seem to apply to the wildebeest, since their fresh PBLs were able to bind trypanosomes sensitized by either cattle or wildebeest infection serum or IgM, indicating that fresh PBL from the wildebeest possess a receptor(s) for both cattle and wildebeest IgM. Furthermore, these results suggest that freshly isolated cattle PBL possess a receptor(s) for wildebeest IgM since wildebeest infection sera and IgM induced adherence of trypanosomes to cattle PBL. The receptor(s) on cattle PBLs for wildebeest IgM, however, would appear to be different from that for cattle IgM because cattle sera or IgM did not induce adherence of trypanosomes to cattle PBL.

It has been reported that the number of neutrophils per mm^3 blood is higher in trypanotolerant N'dama cattle than in susceptible Zebu (Kissling, Karbe & Freitas, 1982). These authors subsequently suggested that this may partially account for the trypanotolerance observed in the N'dama. Although the wildebeest had more adherent neutrophils than cattle, and if AI is taken as an index for clearance of the parasites, wildebeest serum was just as efficient on cattle leucocytes as it was on wildebeest leucocytes. It would seem, therefore, that it might not be the cells *per se* responsible for the low parasitaemia observed in wildebeest, but also the efficiency of the antibody to induce adherence/phagocytosis.

Thus, elaboration of high levels of neutralizing and phagocytosis-promoting antibodies in the wildebeest, coupled with the readily available receptor for IgM on PBL of the wildebeest, would facilitate a faster and more efficient clearance of trypanosomes from the infected animals. The rapid clearance of each trypanosome VAT that emerges would lead to a reduced sum total of parasites, thereby giving rise to the low levels of parasitaemia observed in the wildebeest and hence reduced pathogenic effects. It is suggested, therefore, that this mechanism could, in part, be responsible for the trypanoresistance reported in the wildebeest and other wild animals as well as trypanotolerant cattle.

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