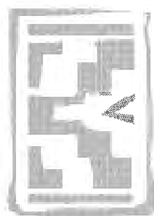

Differential expression of surface membrane antigens on bovine monocytes activated with recombinant cytokines and during *Trypanosoma congolense* infection

V.O. TAIWO, V.O. ANOSA and J.O. OLUWANIYI

Department of Veterinary Pathology, University of Ibadan, Ibadan, Nigeria

ABSTRACT

TAIWO, V.O., ANOSA, V.O. & OLUWANIYI, J.O. 2000. Differential expression of surface membrane antigens on bovine monocytes activated with recombinant cytokines and during *Trypanosoma congolense* infection. *Onderstepoort Journal of Veterinary Research,* 67:289–296

The expression of surface membrane antigens on peripheral blood monocytes (PBM) of cattle of the Boran and N'Dama breeds activated with recombinant cytokines (TNF-α and IFN-γ) and during experimental infection with *Trypanosoma congolense* was investigated using monoclonal antibodies (MoAbs) and fluorescein-activated cell sorter (FACS). The surface antigens investigated were C3bi receptor, major histocompartibility (MHC) II complex (Ia antigen) and two monocyte/macrophage (Mϕ) differentiation antigens.

The study revealed that both cytokines caused the enhancement of the expression of all the PBM surface antigens studied. rBoIFN-γ at low concentrations was more efficient in causing the activation of PBM. While the PBM of Boran cattle were more significantly activated to express the C3bi receptor *vis-à-vis* the Ia antigen than N'Dama cattle, the reverse was the case with the PBM of N'Dama cattle which expressed more Ia antigens than Boran PBM. Similar results were observed during *T. congolense* infection in the two breeds of cattle.

The significantly higher expression of C3bi receptor and correspondingly lower Ia antigen expression by the PBM of Boran cattle, both during trypanosomosis and *in vitro* may be responsible for the higher rate of erythrocyte phagocytosis, hence the development of more severe anaemia by Boran cattle during trypanosomosis than N'Dama. In addition, the expression of significantly higher numbers of Ia antigen by N'Dama Mϕ, hence are more able to process, present and initiate better trypanosome antigen-specific immune response than Boran cattle during infection. These two attributes are known genetic characteristics of trypanotolerance in cattle.

Keywords: Anaemia, Boran, cattle, cytokines, N'Dama, *Trypanosoma congolense*, trypanotolerance

INTRODUCTION

Cells of the mononuclear phagocytic system (MPS) are found in different body compartments with considerable variations in maturity and functions (Gordon, Starkey, Hume, Ezekowitz, Hirsch & Austyn 1986). The central cell of this system, the macrophage (Mϕ), is derived from the peripheral blood monocyte (PBM) which itself arose from precursors in the bone marrow (Lasser 1983). Tissue Mϕ remain relatively inactive and non-dividing except when stimulated to proliferate and show increases in size and activities when stimulated by various types of antigens, mitogens and cytokines in their microenvironment (Gordon 1986). This phenomenon is termed "activation" (Adams & Hamilton 1984). Once activated, Mϕ undergo a series of morphological, biochemical and functional changes which culminate in varying increases (and decreases in some cases) in phagocytic and secretory activities as well as surface antigen/receptor expression (Adams & Hamilton 1984, 1987; Nathan 1987). Among more than 30 specific antigens/receptors expressed by resting or activated Mϕ are the major histocompartibility (MCH) class II, also known as immune-associated (Ia) antigen (Unanue & Allen 1987), and the receptor for the third component of complement (C3bi receptor) (Gordon *et al.* 1986; Ross 1989).

Accepted for publication 5 October 2000—Editor

Mononuclear phagocytes participate in host defense against cancer and many infections by acting independently (direct cytotoxicity) and in association with lymphocytes (Douglas & Musson 1986). While phagocytosis is mediated through complement (C3bi) and immunoglobulin Fc receptors on Mϕ (Ross 1989), specific immune responsiveness is mediated through cooperation with T and B lymphocytes by virtue of their ability to process and present antigens in conjunction with Ia antigens on their surface membrane (Unanue & Allen 1987). Both of these Mϕ characteristics are reportedly enhanced during human and animal trypanosomosis (Nathan, Nogueira, Juangbhanich, Ellis & Cohn 1979; Grosskinsky, Ezekowitz, Berton, Gordon & Askonas 1983).

Trypanosomosis is a devastating disease of livestock in more than a third of the African continent where profitable livestock production is still a problem due to it (Murray & Gray 1984; Ikede 1989). However, there exist in some parts of this region breeds of cattle that are relatively more tolerant to the disease than are others (Chandler 1952). A typical example is the N'Dama cattle which are known to develop less severe anaemia and remain productive in areas where the zebu cattle usually do not (Roberts & Gray 1973a, b; Njogu, Dolan, Wilson & Sayer 1985; Paling, Moloo, Scott, Getinby, McOdimba & Murray 1991). Excessive erythrocyte destruction with attendant complications of severe anaemia and the development of less effective trypanosome-specific immune response by zebu *vis-à-vis* N'Dama cattle during trypanosomosis have been reported by various workers (Murray, Morrison & Whitelaw 1982; Pinder, Ribeau, Tamboura, Hauck-Bauer & Roelants 1984; Paling *et al.* 1991).

PBM and Mϕ of both Boran (an East African zebu) and N'Dama cattle have been reported to be activated both *in vitro* and during trypanosome infections (Anosa & Kaneko 1983; Bancroft, Sutton, Morris & Askonas 1983; Grosskinsky *et al.* 1983; Anosa, Logan-Henfrey & Wells 1997; Taiwo & Anosa 2000). The objective of this study was to determine the dynamics of the expression of Ia antigens and C3bi receptors on Boran and N'Dama PBM activated *in vitro* with recombinant cytokines (rBoIFN-γ and rHuTNF-α) and during *Trypanosoma congolense* infection.

MATERIALS AND METHODS

Isolation of PBM from animals

Two groups of animals were used for this study. The first group consisted of 16 cattle, eight Boran and eight N'Dama cattle which had been used in the study of the effects of extraneous factors on erythrophagocytic activity of cultured PBM (Taiwo & Anosa 2000). The second group consisted of ten cattle, five Boran and five N'Dama, which had been used for the determination of *in vitro* erythrophagocytosis by cultured PBM during *Trypanosoma congolense* infection (Taiwo & Anosa 2000).

PBM was isolated from the anti-coagulated jugular vein blood of each animal as previously described (Taiwo & Anosa 2000). Two separate experiments were carried out. In the first experiment, recombinant BoIFN-γ and HuTNF-α at 0, 10, 100 and 1 000 U/mℓ concentrations were used to stimulate isolated PBM in culture medium for 18 h (Taiwo & Anosa 2000). Thereafter, the cultured cells were gently removed from tissue culture plates using the Titertek Cell Harvester (Flow Laboratories, USA). This experiment was carried out thrice at weekly intervals using the same animals. In the second experiment, PBM isolated directly from the blood of each *T. congolense*-infected animal on 0, 14, 28, 42 and 56 days post-infection (DPI) were used.

Staining of PBM with monoclonal antibodies

Four monoclonal antibodies (MoAbs)—IL-A15 (IgG_1), IL-A21 (IgG_{2a}), IL-A24 (IgG_1) and IL-A109 (IgM), recognizing respectively CD11b (anti-Mac-1, C3bi receptor; Ellis, Davis, Machugh, Emery, Kaushall & Morrison 1988), MHC class II antigen (Baldwin, Teale, Naessens, Goddeeris, Machugh & Morrison 1986), and macrophage differentiation antigens (Ellis *et al.* 1988; Naessens 1991) were used for this study. All the MoAbs were prepared at the International Livestock Research Institute (formerly ILRAD), Nairobi, Kenya as ascitic fluids in mice against peripheral blood leukocytes. Each PBM sample consisted of 10^6 cells in 25 μℓ of culture medium. A combination of two MoAbs per staining protocol, i.e. IL-A15 and IL-A21, and IL-A24 and IL-A109 was carried out in both experiments. IL-A15 and IL-A24 were conjugated with fluorescein isothiocyanate (FITC) while IL-A21 and IL-A109 were conjugated with *trans*-rhodamine isothiocyanate (TRITC). Immunofluorescent staining of the cells was carried out following the procedures described by Lalor, Morrison, Goddeeris, Jack & Black (1986).

Briefly, a mixture of an equal volume of the fluorochrome-conjugated combination MoAbs were used. Twenty-five microlitres of the prescribed working dilutions of the MoAbs (containing 0,2 % sodium azide) were added to triplicate PBM samples in 96-well V-bottomed sero-cluster plates (Costar, Cambridge, MA 02140, USA). The plates were vortexed gently on a rotamixer and then incubated for 40 min at 4 °C. Thereafter, the cells were washed twice in 200 μℓ of cold IFA buffer (L15 medium) by centrifugation at 200 *g* at 4 °C for 5 min. The cells were fixed with 2 % formaldehyde and dispensed homogenously into sample cuvettes containing 5 mℓ of phosphate-buffered saline (PBS).

Sorting of 5 x 10^5 stained PBM from each sample was carried out on a fluorescein-activated cell sorter (FACStar-Plus, Bectin Dickinson, USA) with the argon ion laser (Model 2025, Spectra Physics, USA) set at 488 nm and 500 mW. Dual fluorescein channels were used for green (FITC; 530 nm) and red (TRITC; 600 nm) for fluorescent light detection. The photomultiplier was set at 500 V. The parameters used for sorting are narrow angle forward light scatter properties (size) of the cells, and the amount of bound fluorochrome (Fl.1 for FITC and Fl.2 for TRITC) were jointly assessed on an attached computer and recorded in numbers and in 1- or 2-parameter histograms.

RESULTS

Assays using recombinant cytokines

The patterns of the expression of the C3bi receptor (IL-A15), MHC class II (Ia) antigen (IL-A21) and two monocyte/Mϕ differentiation antigens (IL-A24 and IL-A109) on bovine PBM stimulated *in vitro* with different concentrations of rBoIFN-γ and rHu-TNF-α are shown on Tables 1 and 2, respectively, and on Fig. 1. The results showed that both rBoIFN-γ and rHu-TNF-α are potent activators of bovine PBM for increased expression of the C3bi receptor, Ia antigen and the antigen recognized by IL-A24 on PBM. rBoIFN-γ appeared to be a more potent stimulant of bovine PBM than rHu-TNF-α (Tables 1 and 2). Increasing concentrations of both cytokines caused significant enhancement in the expression of the C3bi receptor on Boran PBM than those of N'Dama cattle. Conversely, the expression of the Ia antigen was more pronounced on the stimulated PBM of N'Dama than those of the Boran cattle. These enhancements were expressed as both increases in PBM population and mean fluorescence expressed per cell (an indicator of number of epitopes per cell). Fig. 1 shows the differential graphical expression of the C3bi receptor on stimulated PBM of one N'Dama steer (ND 15) and one Boran cow (F 81) using different concentrations of both cytokines. The results also showed that 10 U/mℓ of rBoIFN-γ and 100 U/mℓ of rHuTNF-α were optimal for effective stimulation of bovine PBM for the expression of these surface antigens. A 10 U/mℓ concentration of rBoIFN-γ specifically caused a 2–3 fold expression of the C3bi receptor on the PBM of F 81 (Fig. 1).

Assays during *T. congolense* infection

Freshly isolated PBM were used for these assays on two-weekly basis during the infection. The results are as shown on Table 3 and Fig. 2. Progressive increases were observed in the expression of the C3bi

TABLE 1 Surface membrane antigen expression by cultured bovine PBM stimulated with rBoIFN-γ

MoAb (stimulant conc.; U/mℓ)	N'Dama PBM		Boran PBM	
	% positive	Mean fluorescence[a]	% positive	Mean fluorescence
IL-A15 (C3bi receptor)				
0	51,9 ± 2,6[b]	521	69,2 ± 2,2	499
10	73,6 ± 1,8	650	83,1 ± 3,1	1 092
100	76,2 ± 3,3	722	88,9 ± 3,6	1 301
1 000	81,5 ± 2,4	900	93,3 ± 2,7	1 242
IL-A21 (Ia antigen)				
0	53,2 ± 2,0	499	35,6 ± 1,8	487
10	83,6 ± 4,8	1 325	68,2 ± 4,1	666
100	90,3 ± 2,7	1 498	70,8 ± 3,6	739
1 000	86,8 ± 2,5	1 612	81,3 ± 2,3	882
IL-A24				
0	42,2 ± 2,6	161	38,6 ± 3,0	182
10	72,9 ± 3,9	209	76,2 ± 2,8	336
100	82,1 ± 4,3	435	92,8 ± 2,1	582
1 000	93,6 ± 8,1	507	89,9 ± 3,3	661
IL-A109				
0	49,2 ± 1,9	67	43,8 ± 1,3	73
10	53,1 ± 3,2	120	59,2 ± 3,2	132
100	72,0 ± 3,7	128	73,1 ± 1,9	129
1 000	68,9 ± 2,6	159	74,3 ± 2,8	163

[a] An indicator of approximate number of epitopes of the antigen per PBM surface
[b] Data presented as mean ± standard error of three separate experiments
IL-A24 & IL-A109 recognize monocyte/Mϕ surface differentiation antigens

TABLE 2 Surface membrane antigen expression by cultured bovine PBM stimulated with rHuTNF-α

MoAb (stimulant conc.; U/mℓ)	N'Dama PBM		Boran PBM	
	% Positive	Mean fluorescence[a]	% Positive	Mean fluorescence
IL-A15 (C3bi receptor)				
0	52,1 ± 3,3[b]	509	68,6 ± 3,1	524
10	56,6 ± 2,2	615	79,3 ± 5,0	993
100	62,1 ± 0,8	667	86,1 ± 3,2	806
1 000	44,8 ± 2,9	825	76,2 ± 2,5	1 099
IL-A21 (Ia antigen)				
0	50,4 ± 2,6	515	38,5 ± 2,3	482
10	82,7 ± 8,1	809	58,1 ± 3,9	649
100	93,1 ± 3,9	920	67,3 ± 2,0	732
1 000	50,5 ± 2,8	1 019	62,8 ± 3,7	602
IL-A24				
0	39,2 ± 1,5	145	43,1 ± 3,9	211
10	64,3 ± 2,6	252	63,6 ± 2,2	282
100	78,1 ± 1,9	333	89,3 ± 1,6	486
1 000	72,0 ± 2,4	269	76,1 ± 2,1	461
IL-A109				
0	43,4 ± 3,8	88	41,1 ± 3,2	92
10	52,2 ± 8,3	111	49,3 ± 1,8	108
100	59,0 ± 2,7	143	57,1 ± 2,2	155
1 000	40,2 ± 6,6	108	39,9 ± 3,1	127

[a] An indicator of approximate number of epitopes of the antigen per PBM surface
[b] Data presented as mean ± standard error of three separate experiments
IL-A24 & IL-A109 recognize monocyte/Mϕ surface differentiation antigens

TABLE 3 Surface membrane antigen expression by PBM isolated from *T. congolense*-infected cattle

MoAb (cattle breed)	Days post-infection				
	0	14	28	42	56
IL-A15 (C3bi receptor)					
N'DAMA PBM	43,2 ± 2,1 (522)[a]	52,4 ± 2,6 (608)	59,3 ± 3,4 (653)	57,8 ± 2,2 (721)	62,1 ± 1,1 (622)
BORAN PBM	41,6 ± 3,4 (601)	63,6 ± 1,2 (722)	72,4 ± 2,8 (802)	78,1 ± 1,9 (904)	82,3 ± 3,9 (832)
IL-A21 (Ia antigen)					
N'DAMA PBM	48,5 ± 1,5 (438)	92,1 ± 4,1 (625)	89,8 ± 3,8 (806)	97,2 ± 2,6 (798)	89,8 ± 2,5 (932)
BORAN PBM	43,2 ± 5,1 (471)	65,5 ± 3,8 (502)	72,4 ± 1,8 (622)	80,8 ± 4,8 (603)	81,2 ± 3,1 (728)
IL-A24					
N'DAMA PBM	39,1 ± 1,4 (159)	50,3 ± 2,1 (225)	68,1 ± 2,2 (328)	71,1 ± 2,8 (355)	68,0 ± 3,8 (421)
BORAN PBM	41,8 ± 2,2 (161)	63,2 ± 1,8 (380)	69,3 ± 3,8 (422)	79,3 ± 4,2 (438)	72,5 ± 2,7 (528)
IL-A109					
N'DAMA PBM	32,8 ± 2,6 (90)	42,4 ± 2,1 (108)	52,2 ± 1,5 (121)	38,2 ± 2,6 (68)	43,9 ± 3,2 (124)
BORAN PBM	37,2 ± 1,8 (75)	43,1 ± 1,7 (88)	48,1 ± 2,2 (130)	40,1 ± 3,2 (80)	45,8 ± 2,9 (132)

[a] Mean ± standard error (mean fluorescence per PBM); $n = 5$
IL-A24 & IL-A109 recognize monocyte/Mϕ surface differentiation antigens

receptor, Ia antigen and the antigen recognized by IL-A24 on the PBM of both breeds of cattle during infection. However, while the PBM of Boran cattle showed more significant increases in the expression of the C3bi receptor with increasing days post-infection than N'Dama cattle, a highly significant increase

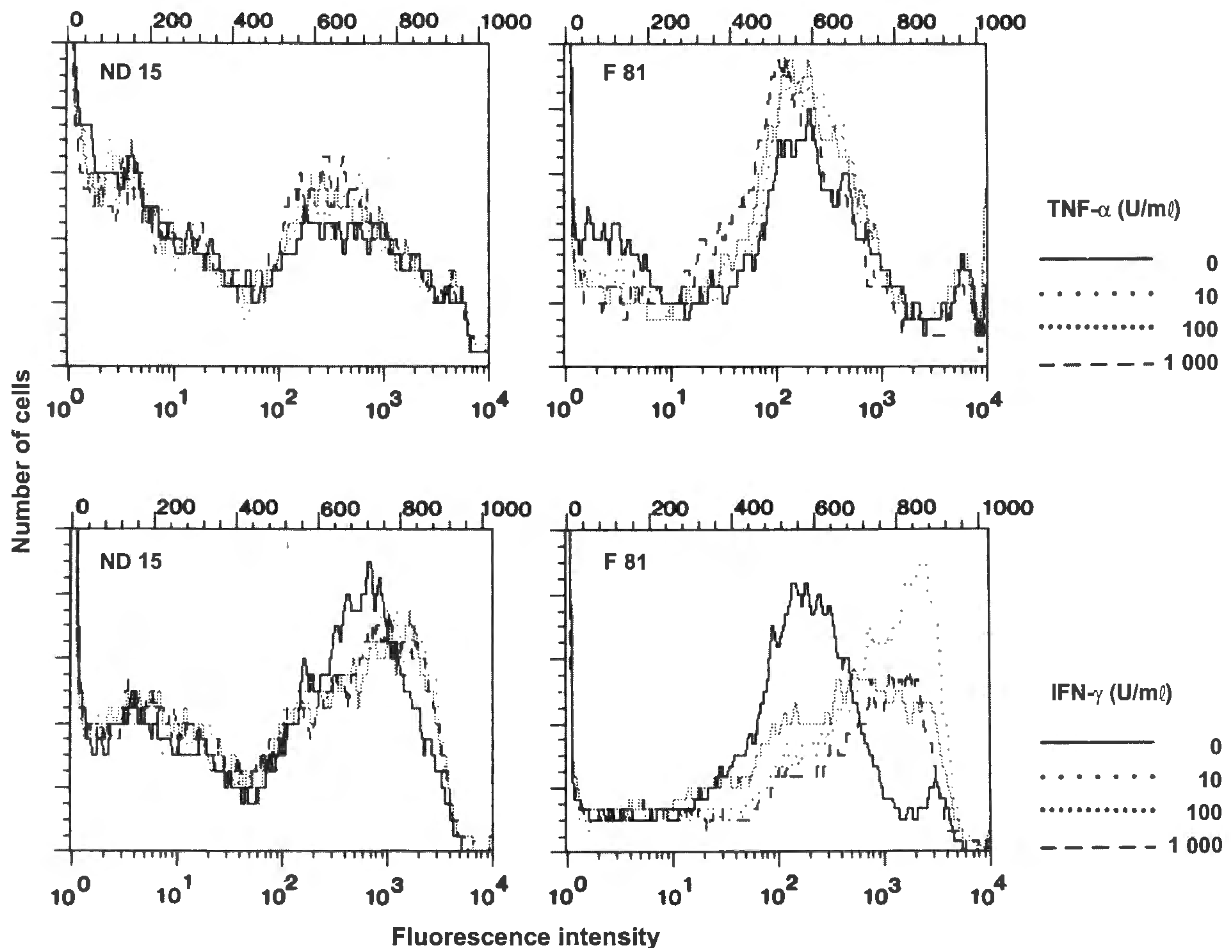

FIG. 1 FACS profile of bovine PBM stimulated with different concentrations of rHuTNF-α and rBoIFN-γ for the expression of the C3bi receptor (IL-A15). ND 15 and F 81 are N'Dama steer & Boran cow, respectively

in Ia antigen expression was observed to be more on the PBM of N'Dama cattle than those of the Boran. As in the *in vitro* stimulation assays, the increases were expressed as both increases in the number of PBM positive for the antigens and mean fluorescence expressed per cell.

Fig. 2 shows the FACS profile of PBM isolated from one N'Dama bull (ND 28) and one Boran cow (BH 40) stained with the four MoAbs on 14 and 28 days post-infection (DPI). This figure reveals an earlier (14 DPI) and more intense expression of the Ia antigen on a majority (94,4 %) of the PBM of ND 28 than on those of BH 40 (76,4 %). Conversely, BH 40 showed a consistently more intense expression of the C3bi receptor as revealed by a more forward horizontal placement of the cells than those of ND 28 on both days. This pattern was more or less the same for all the individual animals of both breeds used in this experiment.

The expression of the antigens recognized by IL-A24 and IL-A109 were similar in both breeds of cattle throughout the course of infection. However, Boran cattle showed a consistently higher level of expression of the antigen recognized by IL-A24 than N'Dama cattle (Table 3; Fig. 2).

DISCUSSION

This study has shown that the PBM of both Boran and N'Dama cattle could be activated for enhanced C3bi receptor and MCH class II (Ia antigen) expression both *in vitro* (using cytokines) and during *T. congolense* infection. IFN-γ is a lymphokine (Trinchieri & Perussia 1985) and a potent activator of macrophages (Steeg, Johnson & Oppenheim 1982; Keller, Joller, Keist, Binz & van der Meide 1988). It is reported to cause enhanced phagocytosis of opsonized sheep erythrocytes (Pontzer & Russell, 1989), destruction of many parasitic protozoa (Nathan *et al.* 1979; Bancroft *et al.* 1983; Kelly 1986; Pentreath 1991) and antigen presentation to immune T cells as a result of enhanced expression of Ia antigens (Koerner, Hamilton & Adams 1987) by Mϕ. It is not surpris-

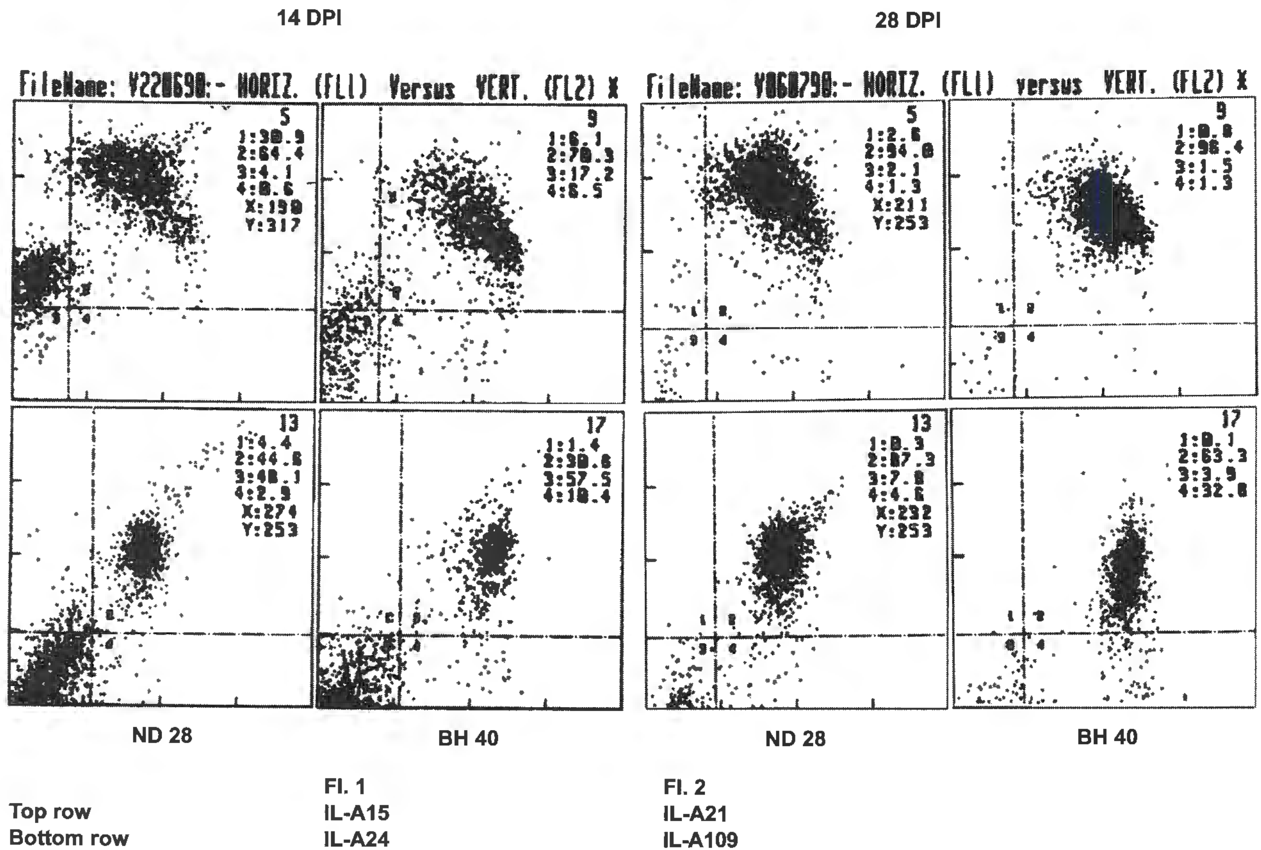

FIG. 2 FACS profile of the expression of surface antigens on PBM of *T. congolense*-infected Boran and N'Dama cattle on 14 and 28 days post-infection. ND 28 and BH 40 are N'Dama bull and Boran cow, respectively. Each dot represents the placement of one stained cell on each axis

ing that rHuTNF-α activated bovine Mϕ for enhanced *in vitro* erythrophagocytosis (Taiwo & Anosa 2000) and surface membrane antigen expression in this study because rHuTNF-α is reported to have about 80% protein homology with rBoTNF-α (Marmenout. Franson, Tavernier, Van den Heyden, Tizard, Kawashima, Shaw, Johnson, Semon, Muller, Ruysschaert, Van Vliet & Fiers 1985; Pennica, Hayklick, Bringman, Palladion & Goeddel 1985). TNF-α is a very potent autocrine stimulator of Mϕ (Beutler & Cerami 1986). Its production by Mϕ is known to be induced by particulate and soluble antigens, tumour cells and cytokines such as interleukin (IL) 1, IL6 and IFN-γ (Le & Vilcek 1987; Bate, Taverne & Playfair 1988; Titus, Sherry & Cerami 1991). While TNF-α is reported to activate Mϕ for increased phagocytosis of latex beads (Shalaby, Aggarwal, Rinderknecht, Svedersky, Finkle & Palladino 1985), it was reported to suppress IFN-induced Ia antigen expression on Mϕ (Steeg *et al.* 1982).

In this study, a significantly higher number of the PBM of Boran cattle were activated by the two cytokines and during *T. congolense* infection to express more epitopes of the C3bi receptor than N'Dama PBM. Conversely, there was a much higher expression of the Ia antigen by a larger population of N'Dama PBM than those of Boran. The implications of these findings are firstly, with the expression of significantly higher number of C3bi receptors, activated Boran PBM will have a higher phagocytic potential than equally activated N'Dama PBM. This implies that Boran Mϕ will destroy more erythrocytes at any given time during trypanosome infection or *in vitro* erythrophagocytosis assays than N'Dama Mϕ. Secondly, N'Dama Mϕ will be more proficient in processing and presenting antigens to T and B cells for a more antigen-specific cell and antibody-mediated immune response by virtue of the higher expression of the Ia antigens on their surface than Boran Mϕ. The corollary then will be that Boran cattle will suffer a more severe anaemia and be less able to produce specific anti-parasite immune response (with subsequent higher parasitaemia) during trypanosomosis. N'Dama cattle, on the other hand, will suffer from less severe anaemia and will be better able to mount a more specific anti-parasite immune response during trypanosomosis. In previous studies (Dargie, Murray, Murray, Grimshaw & McIntyre 1979a, b; Paling *et al.* 1991; Pinder, Bauer, Melick & Fumoux 1988; Authie, Muteti & Williams 1993), N'Dama cattle were re-

ported to have been able to effectively control trypanosome parasitaemia, produced more trypanosome antigen-specific antibodies (of the IgG class) and developed less severe anaemia than correspondingly infected West African and East African zebu cattle. These attributes have been reported to be under heritable polygenic control mechanisms that characterize the N'Dama cattle as being trypanotolerant (Dolan 1987).

The results of the present study have shown that cytokine production, especially of IFN-γ and TNF-α, and responsiveness to such cytokines may modulate activation of bovine Mϕ in different breeds of cattle for the enhancement (or suppression) different Mϕ functions during an infection. The differential response to cytokine activation by the PBM of cattle of the N'Dama and Boran breeds used in this study may be related to genetic influences determining the level of tolerance or susceptibility to trypanosome infection in these animals.

ACKNOWLEDGEMENTS

The research fellowship award by the International Livestock Research Institute (ILRI), Nairobi, Kenya, where a major part of this research was carried out, is gratefully acknowledged. We thank Messrs Joseph Nthale and James Magondu of ILRI for excellent technical assistance and Miss Olabowale Ogundugba for typing the manuscript.

REFERENCES

ADAMS, D.O. & HAMILTON, T.A. 1984. The cell biology of macrophage activation. *Annual Review of Immunology,* 2:283–318.

ADAMS, D.O. & HAMILTON, T.A. 1987. Molecular transductional mechanisms by which IFN-gamma and other signals regulate macrophage development. *Immunological Review,* 97:5–27.

ANOSA, V.O. & KANEKO, J.J. 1984. Pathogenesis of *Trypanosoma brucei* infection in deer mice (*P. maniculatus*). Ultrastructural pathology of the spleen, liver, heart and kidney. *Veterinary Pathology,* 21:229–237.

ANOSA, V.O., LOGAN-HENFREY, L.L. & WELLS, C.W. 1997. The haematology of *Trypanosoma congolense* infection in cattle. I. Sequential cytomorphological changes in the blood and bone marrow of Boran cattle. *Comparative Haematology International,* 7:14–22.

AUTHIE, E., MUTETI, D.K. & WILLIAMS, D.J.L 1993. Antibody responses to invariant antigens of *Trypanosoma congolense* in cattle of differing susceptibility to trypanosomiasis. *Parasire Immunology,* 15:101–111.

BANCROFT, G.J., SUTTON, C.J., MORRIS, A.G. & ASKONAS, B.A. 1983. Production of interferons during experimental African trypanosomiasis. *Clinical and Experimental Immunology,* 52:135–143.

BALDWIN, C.L., TEALE, A.J, NAESSENS, J.G, GODDEERIS, B.M., MACHUGH, N.D. & MORRISON, W.I. 1986. Characterization of a subset of bovine T lymphcytes that express BoT4 by monoclonal antibodies and function: similarity to lymphocytes defined by human T4 and murine L3T4. *Journal of Immunology,* 136:4385–4391.

BATE, C.A.W, TAVERNE, J. & PLAYFAIR, J.H.L. 1988. Malaria parasites induce TNF production by macrophages. *Immunology,* 64:227–231.

BEUTLER, B. & CERAMI, A. 1986. Cachectin and tumour necrosis factor as two sides of the same biological coin. *Nature,* 320: 584–588.

CHANDLER, R.L. 1952. Comparative tolerance of West African cattle to trypanosomiasis. *Annals of Tropical Medicine and Parasitology,* 46:127–134.

DARGIE, J.D., MURRAY, P.K., MURRAY, M., GRIMSHAW, W.M.T. & MCINTYRE, W.I.M. 1979a. Bovine trypanosomiasis: red cell kinetics of N'Dama and Zebu cattle infected with *Trypanosoma congolense. Parasitology,* 78:271–286.

DARGIE, J.D., MURRAY, P.K., MURRAY, M. & MCINTYRE, W.I.M. 1979b. Blood values and erythrokinetics of N'Dama and Zebu cattle experimentally infected with *Trypanosoma brucei. Research in Veterinary Science,* 26:245–247.

DOLAN, R.B. 1987. Genetics of trypanotolerance. *Parasitology Today,* 39:137–143.

DOUGLAS, S.D. & MUSSON, R.A. 1986. Phagocytic defects—monocytes/macrophages. *Clinical Immunology and Immunopathology,* 40:62–68.

ELLIS, J.A., DAVIS, W.C., MACHUGH, N.D., EMERY, D.L., KAUSHAL, A & MORRISON, W.I. 1988. Differentiation antigens on bovine mononuclear phgacytes identified by monoclonal antibodies. *Veterinary Immunology and Immunopathology,* 19:325–340.

GORDON, S. 1986. Biology of the macrophage. *Journal of Cell Science* (Suppl.), 4:267–286.

GORDON, S., STARKEY, P.M., HUME, D., EZEKOWITZ, R.A.B., HIRSCH, S. & AUSTYN, J. 1986. Plasma membrane markers to study differentiation, activation and localization of murine macrophages. Ag F4/80 and the mannosyl, fucosyl receptor, in *Handbook of Experimental Immunology,* edited by D.M. Weir. Oxford: Blackwell Scientific Publishers.

GROSSKINSKY, C.M., EZEKOWITZ, R.A.B., BERTON, G., GORDON, S. & ASKONAS, B.A. (1983). Macrophage activation in murine African trypanosomiasis. *Infection and Immunity,* 39:1080–1086.

IKEDE, B.O. 1989. The Nigerian Livestock Industry: Assets, Liabilities and Potentials. 1987 University Lectures, Ibadan University Press.

KELLER, R., JOLLER, P., KEIST, R., BINZ, H. & VAN DER MEIDE, P.H. 1988. Modulation of major histocompartibility complex (MCH) expression by interferons and microbialagents. Independent regulation of MHC class II expression and induction of tumoricidal activity in bone marrow-derived mononuclear phagocytes. *Scandinavian Journal of Immunology,* 28: 113–121.

KELLY, J.M. 1986. The role of interferons in parasitic protozoan infection. *Parasitololgy Today,* 2:173–174.

KOERNER, T.J., HAMILTON, D.A. & ADAMS, D.O. 1987. Suppressed expression of surface Ia on macrophages by lipopolysaccharide: evidence for regulation at the level of accumulation of mRNA. *Journal of Immunology,* 139:239–243.

LALOR, P.A., MORRISON, W.I., GODDEERIS, B.M., JACK, R.J. & BLACK, S.J. 1986. Monoclonal antibodies identify phenotypically and functionally distinct cell types in the bovine lymphoid system. *Veterinary Immunology and Immunopathology,* 13:121–140.

LASSER, A. 1983. The mononuclear phagocytic system. A Review. *Progress in Pathology,* 14:108–126.

LE, J. & VILCEK, J. 1987. Biology of disease. Tumour necrosis factor and interleukin-1: Cytokines with multiple overlapping biological activities. *Laboratory Investigation,* 56:234–248.

MARMENOUT, A., FRANSON, L., TAVERNIER, J., VAN DEN HEYDEN, J., TIZARD, R., KAWASHIMA, E., SHAW, A., JOHNSON, M.-J. SEMON, D., MULLER, R., RUYSSCHAERT, M.-R., VAN VLIET, A. & FIERS, W. 1985. Molecular cloning and expression of human tumour necrosis factor and comparison with mouse tumour necrosis factor. *European Journal of Biochemistry,* 152:515–522.

MURRAY, M. & GRAY, A.R. 1984. The current situation on animal trypanosomiasis in Africa. *Preventive Veterinary Medicine,* 2:23–30.

MURRAY, M., MORRISON, W.I.& WHITELAW, D.D. 1982. Host susceptibility to African trypanosomiasis. *Advances in Parasitology,* 21:1–68.

NAESSENS, J. 1991. Characterisation of lymphocyte populations in African buffalo (*Syncerus caffer*) and waterbuck (*Kobus defassa*) with workshop monoclonal antibodies. *Veterinary Immunology and Immunopathology,* 27:153–162.

NATHAN, C.F. 1987. Secretory products of macrophages. *Journal of Clinical Investigation,* 79:319–326.

NATHAN, C., NOGUEIRA, N., JUANGBHANICH, C., ELLIS, J. & COHN, Z. 1979. Activation of macrophages *in vitro* and *in vivo:* Correlation between hydrogen peroxide release and killing of *Trypanosoma cruzi. Journal of Experimental Medicine,* 149:1056–1068.

NJOGU, A.R., DOLAN, R.B., WILSON, A.J. & SAYER, P.D. 1985. Trypanotolerance in East African Orma Boran cattle. *Veterinary Record,* 117:632–636.

PALING, R.W., MOLOO, S.K., SCOTT, J.R., GETTINBY, G., MCODIMBA, F.A. & MURRAY, M. 1991. Susceptibility of N'Dama and Boran cattle to sequentila challenges with tsetse-transmitted clones of *Trypanosoma congolense. Parasite Immunology,* 13:427–439.

PENNICA, D., HAYKLICK, J.S., BRINGMAN, T.S., PALLADINO, M.A. & GOEDDEL, D.U. 1985. Cloning and expression in *Escherichia coli* of the cDNA of murine tumour necrosis factor. *Proceedings of the National Academy of Sciences USA,* 82:6060–6064.

PENTREATH, V.W. 1991. The search for primary events causing the pathology of African sleeping sickness. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 85:145–147.

PINDER, M., LIBEAU, G, HIRSCH, W., TAMBOURA, I., HAUCKBAUER, R. & ROELANTS, G.E. 1984. Anti-trypanosome immune responses in bovids of differing susceptibility to African trypanosomiasis. *Immunology,* 51:247.

PINDER, M., BAUER, J., MELICK, A. & FUMOUX, F. 1988. Immune responses of trypanoresistant and trypanosusceptible cattle after cyclical infection with *Trypanosoma congolense. Veterinary Immunology and Immunopathology,* 18:245

PONTZER, C.H. & RUSSELL, S.W. 1989. Culture of macrophages from bovine bone marrow. *Veterinary Immunology and Immunopathology,* 21:351–362.

ROBERTS, C.J. & GRAY, A.R. 1973a. Studies on trypanosome-resistant cattle. I. The breeding and growth performance of N'Dama, Muturu and Zebu cattle maintained under the same conditions of husbandry. *Tropical Animal Health and Production,* 5:211–219.

ROBERTS, C.J. & GRAY, A.R. 1973b. Studies on trypanosome-resistant cattle. II. The effects of trypanosomiasis on N'Dama, Muturu and Zebu cattle. *Tropical Animal Health and Production,* 5:220–233.

ROSS, G.D. 1989. Complement and complement receptors. *Current Opinion in Immunology,* 2:50–62.

SHALABY, M.R., AGGARWAL, B.B., RINDERKNECHT, E., SVEDERSKY, L.P., FINKLE, B.S. & PALLADINO, M.A. 1985. Activation of human polymorphonuclear neutrophil functions by interferon-g and tumour necrosis factors. *Journal of Immunology,* 135:2069–2073.

STEEG, P.S., JOHNSON, H.M. & OPPENHEIM, J.J. 1982. Regulation of murine macrophage Ia antigen expression by immune interferon-like lymphokine: inhibitory effect of endotoxin. *Journal of Immunology,* 129:2042.

TAIWO, V.O. & ANOSA, V.O. 2000. *In vitro* erythrophagocytosis by cultured macrophages stimulated with extraneous substances and those isolated from the blood, spleen and bone marrow of Boran and N'Dama cattle infected with *Trypanosoma congolense* and *Trypanosoma vivax. Onderstepoort Journal of Veterinary Research,* 67:273–287.

TITUS, R.G., SHERRY, B. & CERAMI, A. 1991. The involvement of TNF, IL-1 and Il-6 in the immune response to protozoan parasites. *Parasitology Today,* A13–A16.

TRINCHIERI, G. & PERUSSIA, B. 1985. Immune interferon: a pleiotropic lymphokine with multiple effects. *Immunology Today,* 6:131–136.

UNANUE, E.R. & ALLEN, P.M. 1987. The basis for the immunoregulatory control of macrophages and other accessory cells. *Science,* 236:551–557.