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The role of the macrophage in induction of immunosuppression in *Trypanosoma congolense*-infected cattle

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SUMMARY

Impairment of T-cell function in Boran (*Bos indicus*) cattle during primary infection with *Trypanosoma congolense* ILNat 3.1 was found to occur in peripheral blood, spleen and, in particular, the lymph nodes. Lymph node cells from infected cattle failed to proliferate in response to mitogenic stimulus and suppressed proliferation of both normal peripheral blood mononuclear cells and lymph node cells in co-culture assays. The addition of indomethacin, to inhibit prostaglandin synthesis, had no effect on the ability of lymph node cells from infected cattle to suppress the proliferative response of responder cells from uninfected cattle. The supplementation of the culture media with catalase, which degrades hydrogen peroxide, either alone or in combination with indomethacin, also did not result in restoration of proliferation. This suggested the presence of suppressor cells in lymph nodes of infected cattle which exert their effects via a prostaglandin-independent mechanism. By depleting lymph node cells from infected cattle of the monocyte–macrophage population using a cell sorter it was possible to abrogate the previously observed immunosuppression, thus indicating a key role for these macrophages in the induction of trypanosome-associated immunosuppression.

INTRODUCTION

The ability of experimental infection with African trypanosomes to induce a profound impairment of immune function in laboratory animals has been well documented. This impairment may be manifested as reduced proliferative responses of mononuclear cells to mitogens such as pokeweed mitogen (PWM), concanavalin A (Con A) and lipopolysaccharide (LPS);[1–6] as depression of the mixed leucocyte reaction;[4] as decreased ability to reject allogeneic skin grafts;[4] or as depression of humoral responses to heterologous test antigens.[1]

These experimental models have also been used to study the underlying mechanisms involved in the induction of the observed immunodepression. The studies have revealed that exhaustion of antigen-reactive B lymphocytes which have been driven into differentiation,[2,7,8] generation of suppressor T cells,[3,9,10] and generation of a suppressor macrophage population[2,11–14] all play a role.

However, the situation in species of economic importance, such as cattle, is poorly understood and the importance of immunosuppression in the pathogenesis of bovine trypanosomiasis is not known. Certainly infection of cattle with trypanosomes has been shown to cause a suppression of humoral responses to bacterial antigens such as *Mycoplasma mycoides* and *Brucella abortus*,[15,16] although investigation of the responses of mononuclear cells to various mitogenic stimuli throughout infection failed to demonstrate any profound impairment of cellular function.[17–19] These studies concentrated on the effect of infection on cells of the peripheral blood, presumably due to its ready availability in the field, and thus they may have overlooked the effects of trypanosome infection on other lymphoid organs.

To assess the impact of primary tsetse-transmitted infection with *Trypanosoma congolense* on the bovine immune system, we have monitored the responses of mononuclear cells isolated from various lymphoid compartments to mitogenic stimulus throughout infection. We found that cells isolated from lymph node tissue, but not from peripheral blood or spleen, show profoundly depressed responses to mitogenic stimulation following infection. We then attempted to investigate further the mechanisms involved in this trypanosome-associated immunosuppression and to phenotypically characterize the cell responsible for the induction of this phenomenon in infected cattle.

Abbreviations: Con A, concanavalin A; FACS, fluorescence-activated cell sorter; IL-2, interleukin-2; LNC_{inf}, lymph node cells from an infected animal; LNC_{norm}, lymph node cells from a normal animal; MHC, major histocompatibility complex; PBMC, peripheral blood mononuclear cells.

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MATERIALS AND METHODS

Animals and parasites

Boran (*Bos indicus*) cattle, aged 6–8 months, were obtained from the ILRAD breeding herd kept at Kapiti Plains, Kenya, an area known to be free of trypanosomiasis. Primary infections were performed in all animals with *Trypanosoma congolense* ILNat

3.1.[20] ILNat 3.1 is a double cloned derivative of STIB 212, a stock isolated from a lion in the Serengeti region of Tanzania in 1971.[21] Cattle were infected by five bites on the left flank from infected *Glossina morsitans centralis*, which had been fed previously on a goat infected with *T. congolense* ILNat 3.1.[22]

Lymph node biopsy

Lymph node tissue from either the node draining the tsetse bite sites of infected cattle or from normal infected control cattle was removed surgically under local anaesthesia. Cattle were first sedated by intravenous injection of 0·2 mg/kg xylazine (Chanazine; Chanelle Pharmaceuticals Ltd, Co. Calway, Ireland) and the area around the node was then infiltrated with 10 ml of 3% lignocaine hydrochloride (Chanelle Veterinary Ltd, Co. Galway, Ireland) before removal of a piece of lymph node tissue approximately 1 cm in diameter.

Cell cultures

Bovine peripheral blood mononuclear cells (PBMC) were prepared from defibrinated peripheral venous blood by centrifugation over Ficoll–Paque (Pharmacia LKB, Biotechnology Inc., Piscataway, NJ). A single-cell suspension was prepared by removing a piece of the spleen approximately 1 cm in diameter from each animal at the time of slaughter. The tissue was gently forced through a sterile nylon gauze filter using the barrel from a 10-ml syringe. Splenic mononuclear cells were then prepared by centrifugation over Ficoll–Paque. Lymph node cells (LNC) were prepared in a similar manner. All cells were washed three times in RPMI before use in the assays.

The cells were cultured in 96-well flat-bottomed microculture plates (Nunclon, Nunc AS, Roskilde, Denmark) in a final volume of 200 μl RPMI-1640 medium (Gibco Biocult, Uxbridge, Middlesex, U.K.) supplemented with 10% foetal calf serum (Flow Laboratories, Irvine, Ayrshire, U.K.), 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol and 100 U penicillin/streptomycin per ml to give final cell concentrations of 5×10^4 or 1×10^5 cells per well ($2{\cdot}5 \times 10^5$ or 5×10^5 cells/ml). The cells were stimulated with 6·25 μg/ml concanavalin A (Con A; Sigma, St Louis, MO). Indomethacin (10 μg/ml) and catalase (7500 units/ml), added to the cultures in some experiments, were purchased from Sigma. Cultures were incubated at 37° in a humidified atmosphere containing 5% CO_2, for 5 days.

Cells were then pulsed with 0·5 μCi 5-[^{125}I] Iodo-2-deoxyuridine (IUdR) per well (Amersham International plc, Amersham, Bucks, U.K.) and 5 hr later collected onto glass filter paper (Skatron AS, Lier, Norway) using a semi-automated cell harvester (Flow Laboratories, Skatron AS). Incorporation of IUdR was assessed directly using a gamma-counter (Gamma 5500; Beckman, Fullerton, CA). The data are expressed as the mean of triplicate cultures.

Cell sorting

Infected lymph node cell populations were depleted of the monocyte–macrophage fraction using the mouse monoclonal antibody IL-A24. This monoclonal antibody recognizes cells of the mononuclear phagocyte series and granulocytes.[23] The IL-A24-coated LNC were stained with a fluorescein-labelled goat anti-mouse Ig antiserum and removed by sorting on a FACStar Plus (Becton-Dickinson, Mountain View, CA). The flow rate never exceeded 2000 sorted cells/seconds and the viability of the sorted cells was always >99% as assessed by trypan blue exclusion.

MHC typing

Animals used were MHC typed using the automated lymphocytoxicity test.[24] Briefly, PBMC were stained with carboxyfluorescein diacetate (Calbiochem, La Jolla, CA) and resuspended at a concentration of 3×10^6/ml. One microlitre of this suspension was then added to each well of a Terasaki plate (Greiner, Nürtingen, Germany) with 1 μl of typing sera. A panel of sera was used to define the bovine MHC class I antigens.[25] After 30 min at room temperature, 5 μl of rabbit complement (Cedarlane, Hornby, Ontario, Canada) were added. After a further 60 min the reaction was stopped and counter-stained with propidium iodide (Sigma) and the extracellular fluorescence quenched with ink (Ernst Leitz, Wetzlar, Germany). After a minimum of 20 min at 4°, the test was read on an automated fluorescence microscope. The cattle used were either identical twins (Exp. 3) or full siblings paired on the basis of MHC class I antigens at the A locus (Exps 1 and 2).

RESULTS

Suppression of mitogenic responses

A group of eight Boran cattle were infected with *T. congolense* ILNat 3.1 and one animal was slaughtered at weekly intervals post-infection. A further two animals were used as uninfected control animals. Mononuclear cells were prepared from peripheral blood, spleen, the left prefemoral lymph node (which drained the tsetse bite site) and the contralateral lymph node. The responses of mononuclear cells derived from these sites to

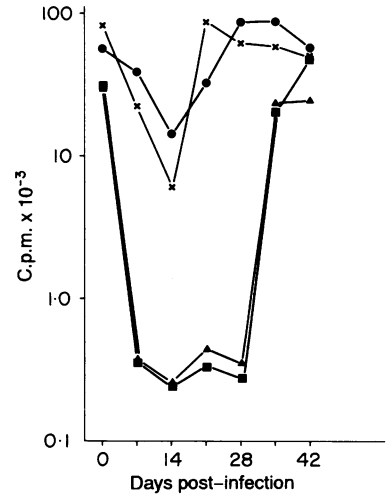

Figure 1. Impairment of bovine T-cell function following infection with *Trypanosoma congolense*. Mononuclear cells were isolated from the peripheral blood (●), spleen (×), the lymph node draining the tsetse bite (▲) and the contralateral lymph node (■), of Boran (*Bos indicus*) cattle throughout the course of tsetse-transmitted primary infection with *T. congolense* ILNat 3.1. The cells were stimulated *in vitro* with Con A (6·25 μg/ml final concentration) and the proliferation was assessed by the uptake of ^{125}IUdR after 5 days of culture. Each value represents the mean of triplicate cultures from which the background proliferation has been subtracted. The standard deviation was always within 10% of the mean.

mitogenic stimulation with Con A was assessed throughout the course of the infection.

Prior to infection, cells derived from all of the lymphoid tissues examined showed marked proliferative responses when stimulated with Con A *in vitro* (Fig. 1). Following infection, the responses of cells derived from both peripheral blood and spleen were similar, with a transient depression of cellular proliferation in response to mitogenic stimulus. This was particularly evident at 14 days post-infection, following which the cellular responses returned to levels similar to those found in normal uninfected control animals. In contrast, cells derived from the draining and contralateral lymph nodes were profoundly suppressed from as early as 7 days post-infection. This suppression was last observed at 28 days post-infection and a restoration of proliferation was observed by 35 days post-infection.

At day 14 post-infection, when the depression observed in peripheral blood and spleen was at its peak, these cells were still capable of a modest degree of proliferation, whereas the ability of lymph node cells to respond to mitogenic stimulus was completely abrogated. This differential tissue susceptibility to the effects of trypanosome-induced immunosuppression is not simply due to an increased rate of cell death in lymph node tissues, since cells from the lymphoid compartments examined all had comparable viabilities throughout the experiment.

Table 1. LNC from infected cattle suppress the potential of lymph node cells from normal cattle to respond to mitogenic stimulation

No. of cells in culture*	Expected c.p.m.†	Actual c.p.m.‡	% inhibitions§
Exp. 1¶			
5×10^4 LNC_{norm}	—	25,930	—
1×10^5 LNC_{norm}	51,860	57,121	None
5×10^4 LNC_{inf}	—	779	97
5×10^4 LNC_{norm} + 5×10^4 LNC_{inf}	26,709	856	97
Exp. 2			
5×10^4 LNC_{norm}	—	41,307	—
1×10^5 LNC_{norm}	82,614	79,371	4
5×10^4 LNC_{inf}	—	586	99
5×10^4 LNC_{norm} + 5×10^4 LNC_{inf}	41,893	652	98
Exp. 3			
5×10^4 LNC_{norm}	—	15,510	—
1×10^5 LNC_{norm}	31,020	33,399	None
5×10^4 LNC_{inf}	—	213	99
5×10^4 LNC_{norm} + 5×10^4 LNC_{inf}	15,723	244	98

* LNC from normal cattle (LNC_{norm}) or lymph node cells from infected cattle (LNC_{inf}) were cultured either alone or co-cultured together, in the presence of the mitogen Con A (6·25 μg/ml final concentration).

† The 'expected c.p.m.' is the proliferative response expected in the absence of suppressor cells and is based on the additive proliferative responses seen in LNC_{norm} and LNC_{inf}.

‡ The cellular proliferation was assessed after 5 days in culture by the uptake of ^{125} IUdR. Values given represent the mean of triplicate cultures. The standard deviation was always within 10% of the mean value.

§ The percentage inhibition refers to the decreased proliferation observed when compared to that expected from mitogen-stimulated responder cells.

¶ Each experiment refers to a separate pair of MHC-matched cattle.

Suppression is cell-associated

In an attempt to investigate the mechanisms responsible for the previously described lymph node immunosuppression, the potential of lymph node cells from infected cattle (LNC_{inf}) to suppress the functional activity of lymph node cells from normal uninfected cattle (LNC_{norm}) was studied. In this experiment pairs of MHC-matched Boran cattle were used, one animal was infected whilst its MHC-matched partner served as a normal control. Lymph node biopsies were performed on both animals between 18 and 20 days post-infection and co-cultures were performed in which 5×10^5 LNC_{norm}/ml were co-cultured with 5×10^5 LNC_{inf}/ml and the cellular proliferation measured after 5 days in culture. To assess the influence of increased cell density in the co-cultures (1×10^6 total cells/ml) on the functional activity of LNC_{norm}, a control was included in which LNC_{norm} were cultured at 1×10^6 cells/ml rather than 5×10^5 cells/ml.

In Table 1 the results of experiments performed on different pairs of MHC-matched cattle are shown. The results demonstrate that the increased cell concentration had a negligible effect on the capacity of LNC_{norm} to proliferate in response to a mitogenic stimulus. Thus, the proliferative response observed in the co-culture should correspond with the sum of the responses measured in LNC_{norm} and LNC_{inf}, i.e. the 'expected c.p.m.' in Table 1. However, our results clearly demonstrate the marked

Table 2. Mitogen-induced proliferation of PBMC from normal cattle is inhibited by LNC from infected cattle*

Normal	Infected	Proliferation (c.p.m.)
Exp. 1†		
5×10^4 $PBMC_{norm}$	None	14,538
1×10^5 $PBMC_{norm}$	None	30,747
None	5×10^4 LNC_{inf}	408
5×10^4 $PBMC_{norm}$	5×10^4 LNC_{inf}	252
Exp. 2		
5×10^4 $PBMC_{norm}$	None	13,797
1×10^5 $PBMC_{norm}$	None	29,479
None	5×10^4 LNC_{inf}	416
5×10^4 $PBMC_{norm}$	5×10^4 LNC_{inf}	559
Exp. 3		
5×10^4 $PBMC_{norm}$	None	17,060
1×10^5 $PBMC_{norm}$	None	20,976
None	5×10^4 LNC_{inf}	397
5×10^4 $PBMC_{norm}$	5×10^4 LNC_{inf}	421

* PBMC from normal uninfected cattle ($PBMC_{norm}$) or LNC from infected cattle (LNC_{inf}) were cultured either alone or co-cultured together, in the presence of the mitogen Con A (6·25 μg/ml final concentration). The cellular proliferation was assessed after 5 days in culture by the uptake of ^{125}IUdR. Values given represent the mean of triplicate cultures. The standard deviation was always within 10% of the mean value.

† Each experiment refers to a separate pair of MHC-matched cattle.

Table 3. Indomethacin and catalase fail to restore the ability of LNC from infected cattle to respond to mitogenic stimulus*

	LNC_{norm}	LNC_{inf}	Co-culture	Expected c.p.m.† in co-culture	LNC_{norm} double conc.
Exp. 1‡					
Medium only	25,930	779	856	26,709	57,121
Indomethacin	24,151	837	746	24,988	49,280
Catalase	77,343	636	725	77,979	86,822
Indomethacin and catalase	74,403	658	1200	75,612	78,708
Exp. 2					
Medium only	41,307	586	652	41,893	82,614
Indomethacin	36,322	613	687	36,935	49,375
Catalase	78,110	425	581	78,691	105,686
Indomethacin and catalase	64,591	692	1089	65,283	89,642

* LNC from normal (LNC_{norm}) or infected (LNC_{inf}) cattle were cultured either alone or co-cultured together, in the presence of Con A (6·25 μg/ml final concentration). Indomethacin (10 μg/ml) or catalase (7500 U/ml), which inhibit prostaglandin synthesis or degrade hydrogen peroxide, respectively, were added to the culture medium either alone or in combination. A control in which LNC_{norm} were cultured at 1×10^6/ml instead of 5×10^5/ml was included to assess the effect of cell crowding on proliferation. Cellular proliferation was assessed after 5 days of culture by the uptake of ^{125}IUdR. Values given represent the mean of triplicate cultures. The standard deviation was always within 10% of the mean value.

† The expected c.p.m. is the proliferative response expected in the absence of suppressor cells and is based on the additive proliferative responses seen in LNC_{norm} and LNC_{inf}.

‡ Each experiment refers to a separate pair of MHC-matched cattle.

Table 4. Restoration of mitogenic responsiveness is associated with macrophage depletion*

Normal cells	Infected cells	Proliferation (c.p.m.)†
Exp. 1‡		
5×10^4 LNC_{norm}	None	25,930
1×10^5 LNC_{norm}	None	57,121
None	5×10^4 LNC_{inf}	779
5×10^4 LNC_{norm}	5×10^4 LNC_{inf}	856
None	5×10^4 LNC_{inf}-macrophage depleted	1164
5×10^4 LNC_{norm}	5×10^4 LNC_{inf}-macrophage depleted	51,440
Exp. 2		
5×10^4 LNC_{norm}	None	41,307
1×10^5 LNC_{norm}	None	79,371
None	5×10^4 LNC_{inf}	586
5×10^4 LNC_{norm}	5×10^4 LNC_{inf}	652
None	5×10^4 LNC_{inf}-macrophage depleted	805
5×10^4 LNC_{norm}	5×10^4 LNC_{inf}-macrophage depleted	58,471
Exp. 3		
5×10^4 LNC_{norm}	None	9460
1×10^5 LNC_{norm}	None	15,773
None	5×10^4 LNC_{inf}	213
5×10^4 LNC_{norm}	5×10^4 LNC_{inf}	244
None	5×10^4 LNC_{inf}-macrophage depleted	698
5×10^4 LNC_{norm}	5×10^4 LNC_{inf}-macrophage depleted	3825

* LNC from normal (LNC_{norm}) or infected (LNC_{inf}) cattle were cultured either alone or co-cultured together, in the presence of the mitogen Con A (6·25 μg/ml final concentration). The macrophage population, as defined by the monoclonal antibody IL-A24, was depleted from LNC_{inf} where indicated using a fluorescence-activated cell sorter.

† The cellular proliferation was assessed after 5 days in culture by the uptake of ^{125}IUdR. Values given represent the mean of triplicate cultures. The standard deviation was always within 10% of the mean value.

‡ Each experiment refers to a separate pair of MHC-matched cattle.

immunosuppressive effect of LNC_{inf}, which were capable of inhibiting the expected cellular proliferation by virtually 100%.

We further demonstrated that the suppressor ability of LNC_{inf} was not limited to LNC_{norm}. The results in Table 2 show that LNC_{inf} were capable of inhibiting the mitogen-induced proliferative response of PBMC derived from normal uninfected MHC-matched cattle, suggesting a possible role for LNC_{inf} in the depressed proliferative responses observed in peripheral blood and spleen during infection. $PBMC_{inf}$ were incapable of suppressing the proliferative responses of either LNC_{norm} or $PBMC_{norm}$ (data not shown).

T. congolense-induced immunosuppression is prostaglandin independent

To evaluate the influence of non-specific suppressive messengers in the trypanosome-induced immunosuppression, the experiments were repeated in the presence of indomethacin, which blocks prostaglandin synthesis or catalase which degrades hydrogen peroxide. Results in Table 3 show that the addition of either indomethacin or catalase did not restore the cellular proliferation in the co-culture. Interestingly, the addition of catalase appeared to increase the proliferative responses observed in LNC_{norm}, suggesting the endogenous release of hydrogen peroxide by these cells.

It has been reported previously that the capacity of *Corynebacterium parvum*-activated macrophages to suppress Con A-induced T-cell proliferation is only slightly affected by either indomethacin or catalase, but can be totally eliminated by both agents acting simultaneously.[26] However in our experiments, when indomethacin-treated co-cultures were supplemented with catalase there was no significant restoration of proliferation, thus confirming the prostaglandin-independent mechanism of immunosuppression.

Macrophage depletion restores mitogenic responsiveness

Having established that LNC_{inf} were capable of suppressing the proliferative responses to mitogenic stimulus of both LNC_{norm} and $PBMC_{norm}$ in a prostaglandin-independent fashion, it was then important to phenotypically characterize the infected cell-type responsible. To assess the involvement of the macrophage in this phenomenon, this population was identified by the monoclonal antibody IL-A24[23] and depleted from LNC_{inf} using a FACS. The macrophage-depleted LNC_{inf} were then co-cultured with LNC_{norm}. The results are shown in Table 4.

Removal of the macrophages from LNC_{inf} resulted in restoration of the proliferative response in the co-culture with LNC_{norm} in all animals examined. In some cases the proliferative values observed were similar to those obtained with equivalent numbers of LNC_{norm}. In others, whilst the restoration was not quite as marked, it was still significant when compared to the results obtained using the whole LNC_{inf} population. Thus it would appear that a cell capable of inducing the immunosuppression co-purifies with the mononuclear phagocyte fraction.

DISCUSSION

In this report we have attempted to evaluate the effect of tsetse-transmitted *T. congolense* infection on immune function in cattle. Many studies of experimental infection with African trypanosomes in rodents have demonstrated a profound impairment of T-cell function in these animals.[3,6,27] However, the relevance of these findings to the pathogenic mechanisms occurring in a bovine host is unknown. The present study employs the ability of T cells to respond to mitogenic stimuli to measure any alterations in the immuno-competence of cattle during primary infection with trypanosomiasis.

Our initial experiments demonstrated that there was indeed a profound impairment of T-cell function in lymph nodes draining the tsetse bite site and also in other peripheral lymph nodes. Such an impairment was not observed in the peripheral blood or spleen, although a transient depression in the responsiveness of cells isolated from these sources to mitogenic stimuli was observed. This latter result agrees with the previous studies conducted in cattle,[17,18] which failed to demonstrate any impairment of T-cell function following infection. The abrogation of mitogenic responsiveness observed in the lymph node tissue was relatively short-lived, with a return to normal observed by 35 days post-challenge. It is possible that the parasite may have evolved a mechanism to induce transient immunosuppression in the host merely to allow itself to establish infection in the host animal. Following this, and the completion of the first wave of parasitaemia, the lymphoid cells regain their ability to proliferate.

This impairment of T-cell responsiveness observed in the lymph nodes of infected cattle is very similar to that previously described in the lymph nodes and spleens of experimentally infected mice.

It was important to demonstrate that the observed immunosuppression in these cattle was due to the generation of a suppressor cell population and not simply due to a toxic depression of cellular proliferation associated with the presence of parasite antigens. By using related MHC-matched pairs of cattle it was possible to co-culture lymph node cells from an infected animal with those from its normal uninfected MHC-matched partner without the complications of alloreactivity. These experiments demonstrated that cells from the infected animals could completely abrogate the mitogen-induced proliferation of the normal responder cells. Furthermore, this phenomenon was not limited to one particular MHC haplotype, as similar results were obtained using different MHC-matched pairs of cattle. LNC from infected cattle were also capable of suppressing the proliferative responses of normal PBMC, thus suggesting that, *in vivo*, suppressor cells may travel via the efferent lymphatics to the peripheral blood and spleen, resulting in the transient immunodepression observed in these tissues.

The results from these co-culture experiments are very similar to others,[2,9,11] in which spleen cells from *T. brucei*- or *T. rhodesiense*-infected mice profoundly depressed the ability of normal spleen cells to respond to mitogens. Although our results demonstrate the involvement of suppressor cells in the lymph nodes of infected cattle, this does not preclude the existence of other immuno-suppressive mechanisms, which may be important in the depression of heterophile antibody production observed during the infection.[15,16] Such depression of antibody responses could be due to absorption by cross-reacting antigens released by disintegrating trypanosomes[28] or due to antigenic competition between trypanosomal antigens from the ongoing infection and heterologous test antigens.[29] This antigenic competition could take place at the macrophage level, possibly because macrophages that are already involved in the process-

ing of trypanosomal antigens may be unable to appropriately process other antigens. Alternatively, such macrophages could elaborate factors that inhibit the normal lymphocyte responses of the infected host to other antigens.

In experimental infections with *T. brucei*,[12] *T. cruzi*[30] and *Leishmania major*,[31] non-specific suppressive agents such as prostaglandins have been shown to be involved. However, in cattle we have been unable to demonstrate a role for prostaglandins in the suppression.

The suppressor cell involved in splenic immunosuppression associated with trypanosome infection in mice has been identified by some researchers as a suppressor T cell,[9,10] whereas others have shown it to be associated with an adherent macrophage-like population.[11] It has also been observed that the immunosuppression may be due to interactions between these two cell types.[2,27] We have previously shown that Mac-1$^+$ Thy-1$^-$ cells play a key role in the induction of immunosuppression in the lymph nodes of *T. brucei*-infected mice[13] and have been unable to demonstrate a role for T cells in this phenomenon. To assess the involvement of suppressor macrophages in the induction of immunosuppression in *T. congolense*-infected cattle, this population was removed from the infected lymph node cell population using a cell sorter. Co-culture experiments with these macrophage-depleted infected LNC resulted in a significant restoration of mitogen-induced proliferation, thus demonstrating the importance of this cell type in the induction of immunosuppression.

At present it is not possible to say whether the suppressor macrophages originated in the lymph node or travelled there from the tsetse bite site via the afferent lymphatic vessels. Certainly, an increase in the number of macrophages present in the chancre has been observed from Day 7 following challenge.[32] We intend to investigate the molecular mechanisms by which suppressor macrophages exert their immunosuppressive effects and compare these mechanisms with those in the murine model.

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