before and during their first tsetse-transmitted infection with *Trypanosoma congolense.* The researchers then studied the responses of these T-cell subpopulations to purified trypanosome antigens.

Populations of CD8+, CD4+ and gamma/delta T cells were isolated from *T. congolense*-infected cattle by staining the cells with monoclonal antibodies specific for these T-cell subpopulations and sorting the cells using a fluorescence-activated cell sorter. The scientists then assessed the ability of the purified N'Dama and Boran cell populations to respond to two types of trypanosome antigens: one was VSG and the other a group of proteins varying in size between 100 and 140 kilodaltons that in the earlier T-cell Western blotting studies appeared to elicit the greatest response from the immune system. Such molecules are known as immunodominant antigens.

In line with the results of the T-cell Western blotting experiments, the VSG antigen caused little detectable stimulation of the three T-cell subpopulations from both breeds of cattle. The nature of the T-cell responses to the other trypanosome antigens, however, was unexpected. Twenty-eight days after infection, the trypanosome immunodominant antigens caused Boran CD8+ T cells to proliferate slightly. A CD8+ response was also observed with N'Dama cells.

THE PROLIFERATION of CD8+ T cells in the presence of trypanosome antigens is of great interest. As mentioned, expression of the CD8 molecule has generally been associated with cytotoxic T-cell function, and in some cases with the suppression of antibody responses. Because CD8+ cytotoxic T-cell function is restricted to tumour cells and host cells infected with intracellular pathogens, it is unlikely to be involved in responses to trypanosomes, which occur outside cells.

The ILRAD scientists involved in this work speculate that the antigen specific activation of CD8+ T cells in the N'Dama, and the consequent release of cytokines from these activated cells, may contribute in some way to the N'Dama's ability to remain healthy when infected with trypanosomes.

In addition to the response by the CD8+ T cells, there was a marked response from gamma/delta T cells in the N'Dama. Some recent results have suggested that these cells play a regulatory rather than stimulatory role in immune response.

Tests are now being developed at ILRAD to measure the amount and type of regulatory cytokines released by T cells when they become activated. This information will enable scientists to analyse in greater detail the involvement of T cells in the development of immunity to trypanosomiasis in infected cattle.

Suppression of the Immune System in Trypanosomiasis

A STRIKING FEATURE of African trypanosomiasis, known as sleeping sickness when it occurs in people, is a profound suppression of the immune system of the infected mammalian host. It was reported as early as 1903 that a high incidence of bacterial infections occurred in people suffering from sleeping sickness. The increased susceptibility of trypanosome-infected patients to opportunistic infections has since been studied in detail and is now generally attributed to a suppression of the patients' immune responsiveness. The immune system of cattle that are susceptible to trypanosomiasis is also suppressed during infection. Indeed, under natural conditions, it is often opportunistic infection(s) rather than trypanosomiasis itself that kills trypanosome-infected animals.

Scientists at ILRAD are studying this phenomenon, known as immuno-suppression, under experimental conditions to gain a better understanding of its impact in trypanosomiasis and to find the mechanism or mechanisms that cause the suppression.

To study immunosuppression in trypanosomiasis, scientists have made extensive use of a mouse model. Experimental trypanosome infections in mice are much easier to manipulate and monitor than tsetse-transmitted infections in cattle. Furthermore, use of different and immunologically well characterized inbred mouse strains gives scientists a better chance of determining the mechanism(s) underlying immunosuppression.

Results of studies using trypanosome-infected mice generated two major hypotheses to account for immune suppression: (1) a polyclonal activation of B cells leading to a suppression of antibody-mediated immunity and (2) generation of macrophages that suppress both antibody-mediated immunity (B cells) and cellular immunity (T cells). Research at ILRAD has shown that

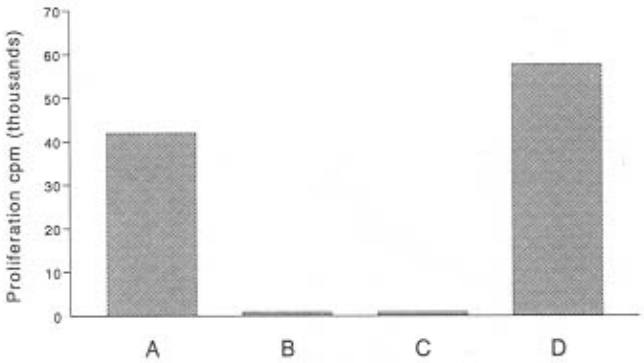
immunosuppression in cattle is not related to a polyclonal activation of B cells. Experiments were then made to determine the validity of the second hypothesis for trypanosomiasis in cattle.

The detection of suppressor cells in mice relies mainly on experiments in which cells from an infected mouse are mixed with cells from an uninfected mouse. Such co-culture experiments can be done only with cells from animals that are genetically identical. ILRAD scientists thus designed a co-culture experiment that would make use of cells obtained from identical twin cattle.

Suppressor macrophages profoundly suppress the proliferation of T cells. Thus, one would expect trypanosome infected cattle to have a marked inhibition of T-cell proliferation if suppressor macrophages were present. However, as reported in the main article of this issue, peripheral blood cells from infected cattle are still able to proliferate *in vitro* in response to trypanosome antigens. This fact suggests that generation of suppressor macrophages in trypanosome infections is, like the polyclonal activation of B cells in mice, a peculiarity of mouse infections. The cattle experiments, however, were all conducted with cells from the blood whereas the mouse studies used cells from the spleen and lymph nodes. To determine whether the discrepancy in T-cell proliferation between mice and cattle was due to a difference in species or to a difference in cell types, proliferations of blood cells, spleen cells and lymphnode cells in Boran cattle were monitored throughout an infection with *Trypanosoma congolense* parasites.

Both blood cells and spleen cells were still able to respond to a T-cell activator during infection, whereas the lymph node cells were suppressed 100%. It thus appeared that T-cell proliferation is profoundly suppressed in cattle suffering from African trypanosomiasis but that this suppression occurs mainly in the lymph nodes.

Experiments were then set up using genetically identical twin cattle produced at ILRAD by flushing a Boran embryo from its mother's uterus, splitting the embryo into two and implanting the two embryos in a foster mother, which subsequently produce the twins. When the two animals were old enough, one was infected with *T. congolense* while the other remained uninfected and was used as a control. Lymph node cells obtained from the control animal were cultured with a T-cell activator, which caused their proliferation. However, when lymph node cells from the infected animal were mixed *in vitro* with the uninfected cells, the T cells in the co-culture were suppressed 100%, demonstrating the presence of suppressor cells in the lymph nodes of infected cattle (see the figure below).



Bovine lymph node cells were obtained from identical twin animals: an uninfected control (LNC-norm) and a trypanosome-infected animal (LNC-inf). The cells were cultured with a T-cell activator (T-cell mitogen Concanavalin A). Proliferation of lymph node cells, which is an indicator of T-cell activation, was measured by incorporating a radioactive DNA precursor and expressed as counts per minute.

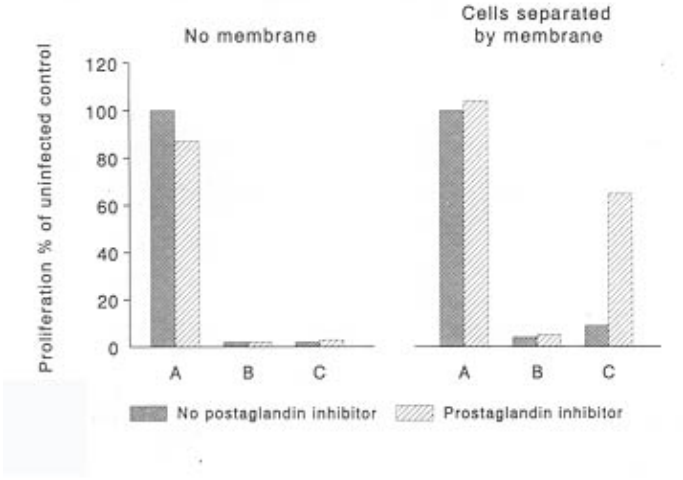
A = LNC-norm, B = LNC-inf, C = cp-culture with LNC-norm and LNC-inf, D = co-culture with LNC-norm and macrophage-depleted LNC-inf.

To investigate the role of macrophages in this suppression, a fluorescence-labelled monoclonal antibody that specifically binds to macrophages was added to lymph node cells from the infected animal and the fluorescent cells were removed by flow cytofluorometry. The macrophage-

depleted lymph node cells were then again mixed with lymph node cells from the control animal. In this co-culture, the proliferation of T cells from the control animal was not suppressed. This demonstrated that the macrophages were involved in T-cell suppression in cattle as well as mice.

TO DISCOVER how suppressor macrophages stop T cells proliferating, another co-culture was set up in which cells from the infected animal were physically separated from cells from the control animal by a semi-permeable membrane so that no cell contact could occur between the suppressor cells and the target cells whereas soluble factors secreted by the cells could pass through the membrane. In this culture, cells from the infected animal were still able to suppress the proliferation of cells from the uninfected control, indicating that suppression was mediated by soluble factors released by the suppressor macrophages.

It is well known that macrophages can suppress T-cell function by releasing prostaglandins, small lipid-like molecules that have a strong immunosuppressive activity. The role of such factors in trypanosome-mediated suppression was investigated by adding to the co-culture a drug that switches off prostaglandin synthesis. The addition of this drug markedly restored T-cell proliferation in the co-cultures separated by the membrane. However, in co-cultures where cells from the infected and uninfected animals were allowed to mix, no restoration occurred from the addition of this drug.



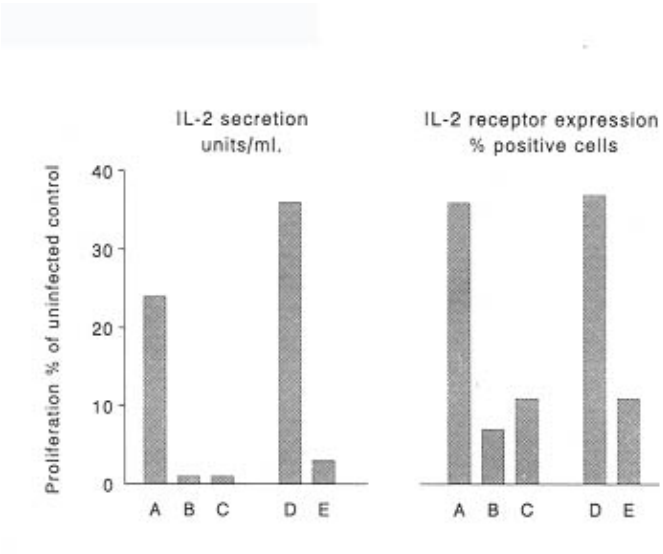
Bovine lymph node cells from an uninfected control animal (LNC-norm) and from a trypanosome-infected animal (LNC-inf) were cultured together in the presence of a T-cell activator. Two sets of cultures were set up. In the first, the cells were mixed in the same culture well; in the second, the cells were cultured in the same culture well but separated by a porous membrane to allow only soluble factors to pass through. Each set of cultures was supplemented by a drug to prevent prostaglandin secretion. Proliferative responses of the LNC, which is an indicator of T-cell activation, is expressed as a percentage of the uninfected control.

A = LNC-norm, B = LNC-inf, C = co-cultures with LNC-norm and LNC-inf.

It was concluded that two suppressor mechanisms were operating simultaneously. The first is a mechanism that does not require cell-to-cell contact and is mediated by prostaglandins (prostaglandin-mediated); the second is a mechanism that requires cell-to-cell contact and occurs independently of prostaglandin (macrophage-mediated). Prostaglandin-mediated suppression has been thoroughly studied and can easily be prevented by administering commercially available prostaglandin inhibitors. Therefore, further study focused on characterizing the macrophage-mediated (prostaglandin independent) suppression.

Induction of a proliferation of T cells is a complex phenomenon involving T-cell growth factors such as interleukin 2. Following activation, T cells both secrete interleukin 2 and express specific receptors for the growth factor on their surface. When secreted interleukin 2 binds to its receptor, the T cells are triggered to proliferate. During trypanosomiasis, the reduced proliferation of lymph node cells was found to be associated with a reduced secretion of interleukin 2. Results of further

co-culture experiments showed that suppressor cells were responsible for this reduction in interleukin 2 secretion. Since removal of the macrophages abrogated the suppression in the co-cultures whereas the addition of prostaglandin synthesis inhibitors had no effect, it followed that this aspect of suppression was macrophage- rather than prostaglandin-mediated.



Secretion of interleukin 2 (IL-2) is measured by a bio-assay using bovine blast cells and is expressed as functional units per ml. The expression of receptors for IL-2 is measured by fluorescence using an antiserum specific for the bovine IL-2 receptor. The results are expressed as a percentage of IL-2receptor positive cells.

A = lymph node cells from an uninfected control animal (LNC-norm). B = lymph node cells from a trypanosome-infected animal (LNC-inf). C = co-culture with LNC-norm and non-purified LNC-inf. D = co-culture with LNC-norm and macrophage-depleted LNC-inf. E = co-culture with LNC-norm and non-purified LNC-inf in the presence of a drug that prevents synthesis of prostaglandin.

IN SUMMARY, generation of suppressor cells during infection with trypanosomes occurs in both mice and cattle. In both species, macrophages play a central role in immunosuppression, which is achieved in two ways—by T cell contact with macrophage-secreted prostaglandin and by contact with macrophages themselves.

Suppression of T-cell activity is not, however, identical in both species. In cattle, macrophage-mediated suppression blocks both the expression of receptors for interleukin 2 on the surface of T cells and T-cell secretion of interleukin 2. In mice, this suppressor pathway blocks the expression of receptors for interleukin 2 on T cells but does not stop T-cell secretion of interleukin 2. Hence, the mechanisms of suppression in mice and cattle are similar but the tropism of these mechanisms probably differs. That suppression in cattle appears to be mediated by mechanisms that resemble suppressor mechanisms in mice suggests that it is possible to rely on the knowledge acquired from mouse infections to design new models to control immune suppression in cattle.

This article on immunosuppression in trypanosomiasis is based on a report by ILRAD scientist Maarten Sileghem. The article on T-cell responses (pp. 1–3) is based on a report by Norman Flynn, a former ILRAD scientist now working at the MRC Retrovirus Research Laboratory, University of Glasgow. The Editor thanks Ross Gray, Alan Teale and Diana Williams for critiquing these articles, and Declan McKeever, particularly, for his improvements of each of many drafts and several impromptu mini-seminars on the highly fluid nature of the 'facts' of immunology today.

ILRAD SCIENTIST AWARDED

On 24 April, an ILRAD senior scientist, Dr. Tony Musoke, was awarded the 1992 Kenya Veterinary Association Veterinarian of the Year and Ciba-Geigy Prize for his contribution to