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Do T Cells help animals develop immunity to trypanosomiasis?

TRYPANOSOMIASIS is a widespread disease of African livestock. It is caused by infection with protozoan parasites that are transmitted to the animals by the bite of an infected tsetse fly. Cattle infected with trypanosomes become chronically sick and unproductive; left untreated, most eventually die.

When mammals are infected with most types of pathogens, their immune system quickly generates large numbers of white blood cells, which are highly competent in clearing the infectious agents from the body. Most cattle in Africa, however, are unable to clear all trypanosomes from their circulation and to stop the development of disease.

This inability is partly due to a parasite mechanism known as antigenic variation, which helps trypanosomes evade destruction by the immune system of their animal host. The parasites repeatedly change the kind of antigenic protein displayed on their surface membrane to which the immune cells are directed, and in this way prolong their survival and finally exhaust the host's immune responses.

In animals infected with trypanosomes, there is a proliferation of a major type of white blood cell called B lymphocytes and a dramatic increase in the antibody molecules that B cells secrete. Bovine antibodies recognize and bind to molecules that make up the surface coat of trypanosomes, known as variable surface glycoproteins, or VSGs.

The binding of host antibody to parasites initiates a cascade of reactions, mediated by serum proteins of the host animal, which cause the rupture (lysis) of the parasites. The antibodies also help scavenger cells such as macrophages to clear antibody-coated trypanosomes from the circulation.

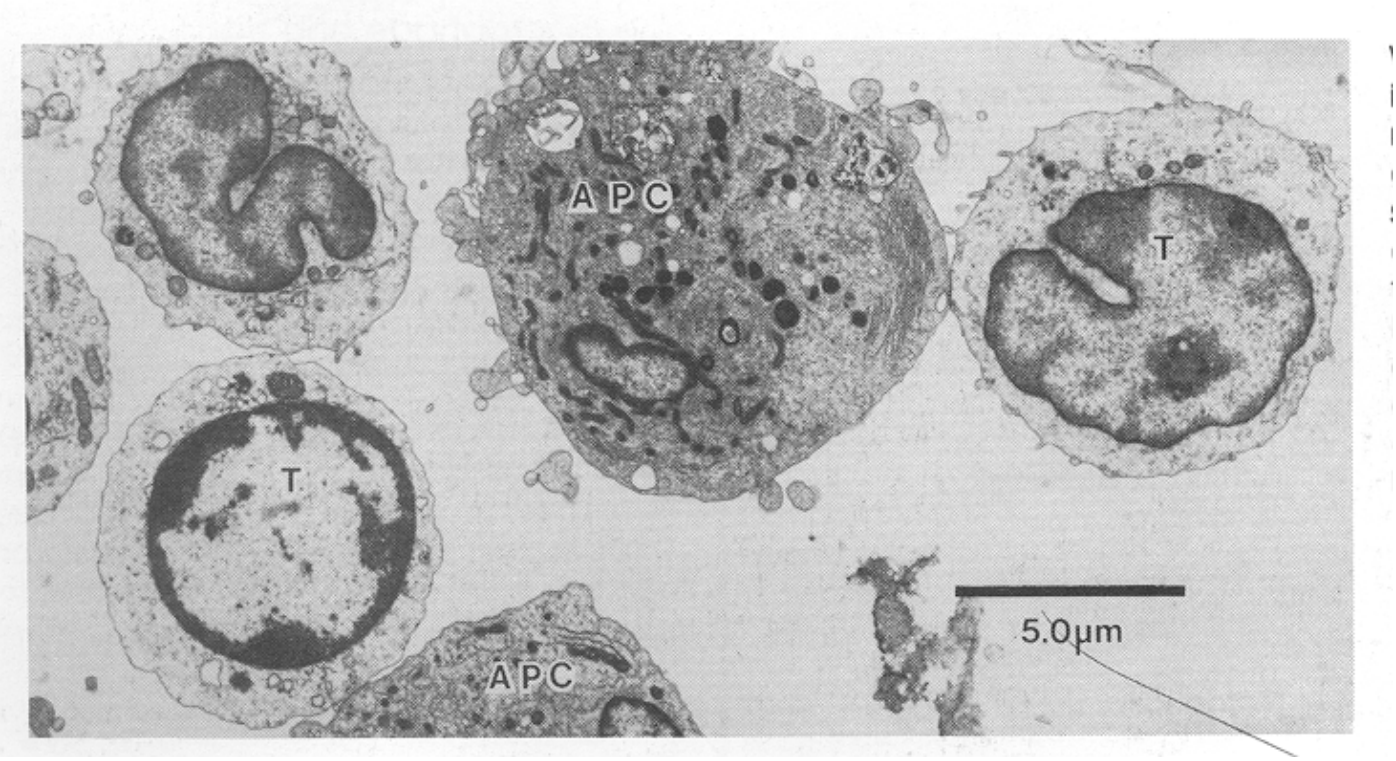
Due to the periodic variation of the protein surface coat of trypanosomes, however, not all parasites in an infected animal have antibody bound to their surface. These parasites multiply and create new waves of parasitaemia.

Recent studies at ILRAD have examined the role played by another major class of lymphocytes—the T cells—in protecting cattle against infection with trypanosomes.

B cells produce antibodies that bind to foreign organisms circulating freely. A major type of T lymphocyte known as cytotoxic T cells kill cells of the host animal that are infected with intracellular pathogens, such as the protozoan parasite *Theileria parva,* which causes East Coast fever in livestock, and the virus that causes influenza in people. Because African trypanosomes do not invade the cells of the host animal but rather remain free in the circulation and in lymphoid

and other tissues, it is unlikely that T cells have a direct cytotoxic effect against these extracellular parasites. ILRAD research reported in this issue, however, suggests that a related population of T cells may be involved in protecting cattle against trypanosomiasis.

A second type of T cell is known as a helper T cell. When helper T cells are stimulated by the presence of a foreign molecule, called an antigen, they proliferate and secrete chemical messengers called cytokines. These protein molecules are believed to regulate the majority of antibody and T-cell immune mechanisms.



White blood cells of the mammalian immune system appear in this electron micrograph. The large macrophage-like antigen presenting cells (APC) scavenge and degrade foreign matter and present the foreign molecules on their surface to T lymphocytes (T). Helper T cells secrete chemical messengers that help to fight the infection; cytotoxic T cells directly attack and kill cells of the host animal that have become infected with pathogens.

Different T and B cells are difficult to distinguish under light and electron microscopes, but they can be identified by applying immunological tests that detect specific proteins that each cell type carries on its surface.

B AND T CELLS recognize antigens of infectious agents in very different ways. Antibodies located both on the surface of B cells and free in the circulation recognize and bind to antigens directly. T cells, on the other hand, recognize conventional protein antigens only after they have been partially degraded by macrophages or other antigen-presenting cells of the host animal.

Antigen-presenting cells engulf antigens and chop them into small chains of amino acids called peptides. Each foreign peptide moves to the surface of the host cell in combination with a host protein known as an MHC molecule. (MHC is an abbreviation of the 'major histocompatibility complex', an area of the mammalian genome that encodes proteins that help to regulate immune responses.)

A peptide fits into a structural cleft of an MHC molecule. When the foreign peptide is displayed on the surface of the host cell in this cleft, helper T cells in the body that bear receptors for that particular foreign peptide bind to it.

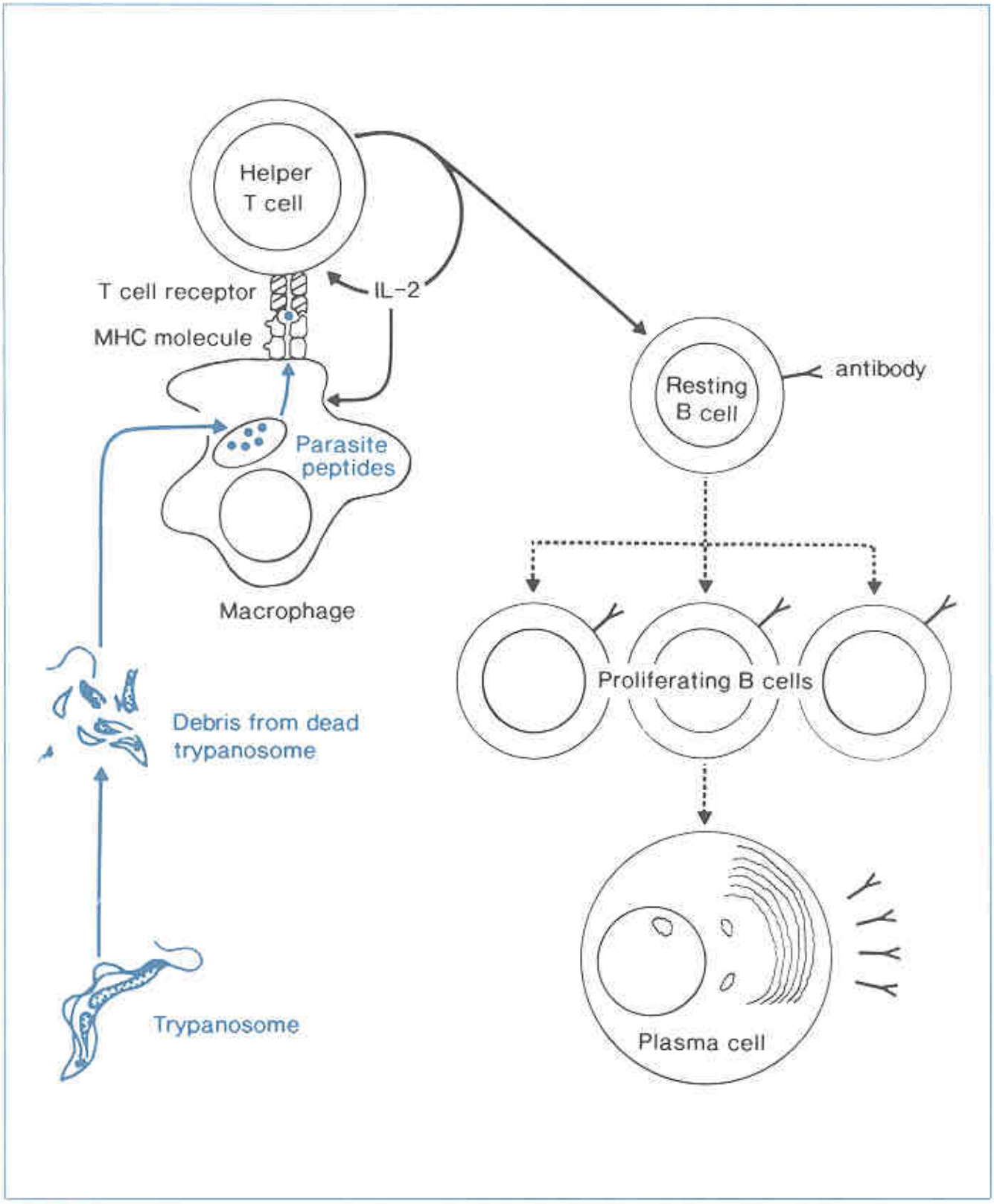
It is this event that stimulates the helper T cell to secrete cytokines, which help B cells to proliferate, to differentiate and to secrete antibodies. B cells are important in immunity to trypanosomiasis because the antibodies they secrete can act to clear a majority of trypanosomes from the circulation, as discussed above. This is particularly true in a few breeds of cattle indigenous in Africa, such as the relatively small, humpless N'Dama *(Bos taurus),* which are able

to remain productive and apparently healthy when infected with trypanosome parasites.

Past ILRAD research has shown that these 'trypanotolerant' animals—unlike the African Zebu and European dairy cattle, which are susceptible to trypanosomiasis—are able both to control the numbers of parasites in their blood and to stop the development of anaemia, a main characteristic of trypanosomiasis.

Recent ILRAD studies have shown that the antibody responses to trypanosome infection made by trypanotolerant N'Dama cattle are superior to those made by the relatively large, humped Boran (*Bos indicus)* cattle, which are susceptible to trypanosomiasis. (For details of the antibody studies, see the April 1991 issue of *ILRAD Reports.)*

Because T cells play a critical role in helping B cells to mount an immune response against infection, ILRAD scientists are comparing the activity of T cells in the trypanotolerant N'Dama and the susceptible Boran when these animals are infected with trypanosomes. Results of these experiments suggest that the N'Dama make a superior trypanosome-antigen-specific T-cell response as detected with cells from their peripheral blood. —Ed.



Helper T cells do not attack parasites and other pathogens themselves, but they direct much of an animal's immune response against infectious agents.

In this simplified diagram of immune events, a professional scavenger cell of the body called a macrophage has engulfed several protein molecules released from trypanosome parasites that have died and ruptured. These parasite proteins are broken down by the macrophage into small peptide molecules (blue dots). One of the parasite peptides has been inserted into a binding cleft of an MHC molecule produced by the macrophage and transported to the surface of the host cell.

The parasite peptide-MHC molecule complex displayed on the macrophage's surface membrane is recognized by one of a few helper T cells in the body that bear a surface receptor molecule specific for this particular antigen-MHC complex. The helper T-cell receptor docks with the complex. This binding stimulates the helper T cell to secrete chemical messengers, such as interleukin-2, which cause T cells, B cells and macrophages to proliferate to help fight the infection.

IN THE ILRAD STUDIES, N'Dama T cells responded to trypanosome antigens earlier during infection than Boran T cells and the level of proliferation of N'Dama T cells *in vitro* when stimulated by antigen was higher than that in the Boran. Furthermore, the N'Dama maintained their T-cell responsiveness throughout the period of study, whereas the T-cell response in Boran cattle was a transient phenomenon, occurring only between weeks three and five following an experimental infection.

The trypanosome antigens used to stimulate the T cells *in vitro* in these studies were derived from the same parasites as those used to infect the cattle. The experiments were repeated to determine if T cells of cattle infected with one clone of trypanosomes recognize antigens from an antigenically unrelated clone.

A striking difference was demonstrated in the immune responses of Boran and N'Dama cattle. T cells from Boran cattle failed to recognize antigens from the different serodeme, whereas T cells from N'Dama proliferated in the presence of antigens derived from both clones. These results suggest that infected trypanotolerant cattle recognize a broader spectrum of trypanosome antigens than susceptible cattle.

TO DISCOVER MORE about these differences in antigen recognition, scientists employed a technique known as T-cell Western blotting. This technique is used to identify dominant antigens in complex mixtures of proteins such as suspensions of ruptured bacteria or parasites. The technique involves separating the proteins according to their size using sodium-dodecyl-sulphate polyacrylamide gel. After separation, the parasite proteins are transferred by electrophoresis onto nitrocellulose membranes and stained so that the transferred proteins may be seen.

The major protein bands can then be cut out and used directly to test which parasite proteins stimulate the greatest T-cell proliferation. Antigen presenting cells, such as macrophages, present in the peripheral blood are capable of scavenging the protein antigens off the nitrocellulose, degrading the antigens and presenting their antigenic peptides to T cells.

In this experiment, T cells obtained from both trypanotolerant N'Dama and susceptible Boran cattle recognized virtually all of the nitro-cellulose-bound trypanosome antigens used; there were no clear-cut differences in the antigen-recognition profiles of these two breeds. That both breeds responded equally well to fractionated nitrocellulose bound parasite antigens, whereas in the other ILRAD experiments the N'Dama had a higher response to total parasite antigen than the Boran, may be due to an exclusion in the nitrocellulose assays of minor parasite antigens lost during preparation of the nitrocellulose membranes.

Interestingly, there was negligible T-cell recognition of trypanosome VSG—the major antigen of the parasite surface coat—by both breeds of cattle, despite exposure to this antigen during infection. This suggests that the strong antibody responses of cattle to VSG antigens may not depend on T-cell activity.

IT WAS NOT POSSIBLE in these experiments to determine what type of T cells were responding to the trypanosome antigens. The experiments also told the scientists little about the cytokines released from the T cells when they became activated by antigen.

Broadly speaking, T cells can be divided into two major populations, those that express a CD8 molecule on their surface (CD8+) and those that express a CD4 molecule (CD4+). Classical cytotoxic T-cell function is found predominantly in the CD8+ fraction and helper T-cell function in the CD4+.

A third T-cell population, the gamma/delta T cell, is unusually prominent in young ruminants. (This cell is named after the gamma and delta gene products that form the antigen receptors located on its surface.) The function of this population is still unknown.

To learn more about the T-cell responses in these experiments, populations of CD8+, CD4+ and gamma/delta T cells were prepared from the peripheral blood of N'Dama and Boran cattle both

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

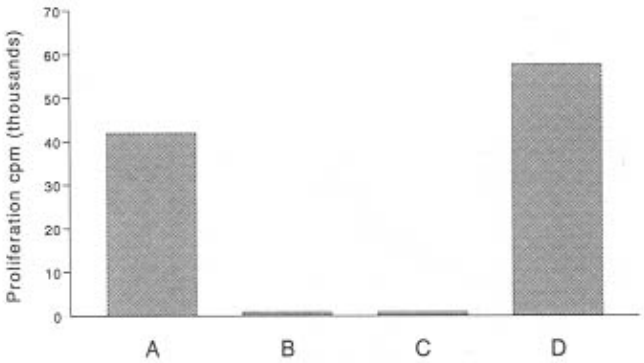
immunosuppression in cattle is not related to a polyclonal activation of B cells. Experiments were then made to determine the validity of the second hypothesis for trypanosomiasis in cattle.

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.

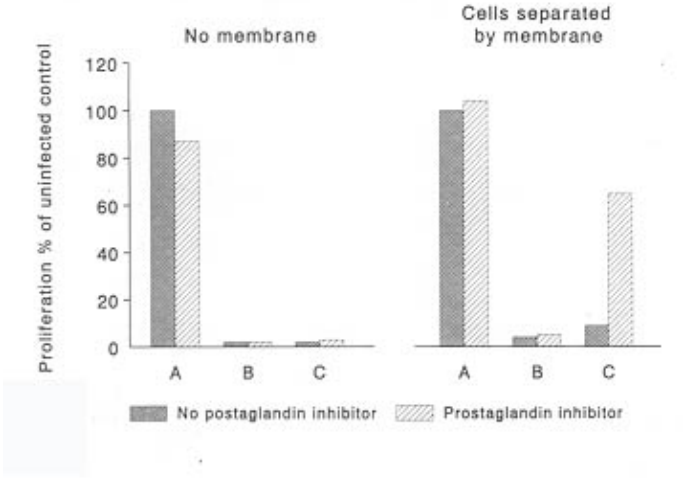
A = LNC-norm, B = LNC-inf, C = cp-culture with LNC-norm and LNC-inf, D = co-culture with LNC-norm and macrophage-depleted LNC-inf.

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.



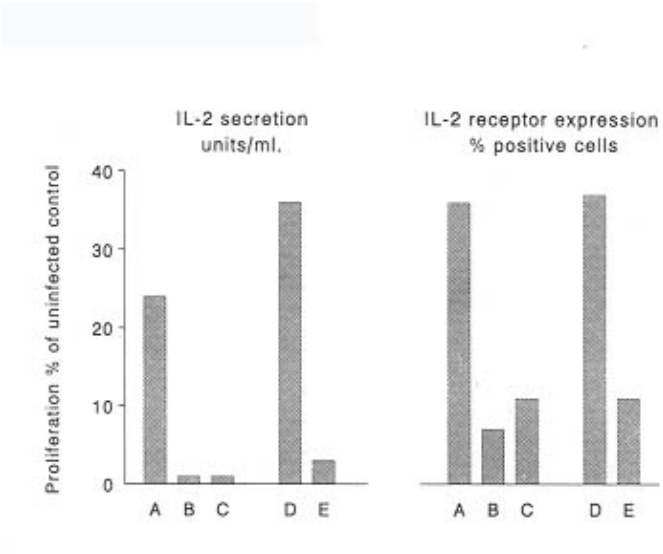
Bovine lymph node cells from an uninfected control animal (LNC-norm) and from a trypanosome-infected animal (LNC-inf) were cultured together in the presence of a T-cell activator. Two sets of cultures were set up. In the first, the cells were mixed in the same culture well; in the second, the cells were 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

veterinary research in Kenya. The citation identified Dr. Musoke's recent research towards the development of a novel vaccine for East Coast fever. In accepting the prize, presented at the KVA Silver Jubilee celebration, Dr. Musoke acknowledged the contribution over the years of his many colleagues and collaborators at ILRAD. This is the first time the prize has been awarded for contributions in research.

Multi-Drug-Resistant Trypanosome Populations in Cattle (M.Phil. Thesis)

The administration of chemotherapeutic drugs to treat trypanosomiasis in African domestic livestock is currently dependent on the salts of six compounds: diminazene, homidium, isometamidium, quinapyramine, suramin and cymelarsan. The first three compounds are used in cattle, sheep and goats, the latter three in camels.

Parasite resistance to these commonly used trypanocidal drugs is a problem in many parts of Africa and is an important constraint to the control of trypanosomiasis. In the field, resistance to trypanocides may occur when trypanosome parasites are exposed to subcurative doses of trypanocides. Resistance to one drug may also occur as a result of induction of parasite resistance to other related compounds. Such cross-resistance to diminazene, isometamidium and homidium, for example, is thought to develop as a result of resistance to quinapyramine.

From 1985 to 1989, the International Livestock Centre for Africa, based in Addis Ababa, Ethiopia, was involved in a project in the Ghibe Valley, in southwest Ethiopia, whose aim was first to determine the constraints to livestock production in that area and then to help improve production. Throughout the experimental period, 840 cattle were examined on a monthly basis for the presence of trypanosomes. Animals that were detected parasitaemic and had a packed red cell volume of less than 26% were treated with diminazene aceturate (Berenil, Hoechst, Germany) at a dose of 3.5 mg/kg body weight.

The monthly trypanosome prevalence data indicated that the majority of infections were *Trypanosoma congolense* and that the percentage of infections that relapsed following treatment increased approximately from 21% in 1987 to 39% in 1989. It therefore appeared that there was a high prevalence of trypanosome infections resistant to diminazene.

To confirm this hypothesis, field isolates were collected from cattle in different herds at Ghibe in 1989 and transported to ILRAD. Twelve of the stabilates were inoculated into individual Boran (*Bos indicus*) calves and characterized for their sensitivity to diminazene aceturate, isometamidium chloride (Samorin, Rhone Merieux, France) and homidium chloride (Novidium, Rhone Merieux, France).

All infections were resistant to treatment with diminazene aceturate at a dose of 7.0 mg/kg body weight. Eleven of the infections were also resistant to isometamidium chloride at a dose of 0.5 mg/kg body weight and homidium chloride at a dose of 1.0 mg/kg body weight. Studies in goats repeated and confirmed these observations. Thus, at the time the isolates were collected, there appeared to be a very high prevalence of trypanosome infections in cattle at Ghibe that were resistant to diminazene, isometamidium and homidium. All 12 stabilates produced infections that, on the basis of morphology, were *T. congolense*. Furthermore, hybridization of parasite DNA from these stabilates with repetitive DNA probes indicated that all populations contained savannah-type *T. congolense*.

Five clones were derived from one of the multi-drug-resistant populations and characterized in mice for their sensitivity to the trypanocides mentioned above. All five clones had diminazene aceturate, isometamidium chloride and homidium chloride 50% curative dose value in excess of 30, 10 and 20 mg/kg body weight, respectively. Compared to drug-sensitive populations of *T. congolense*, all five clones expressed high levels of resistance to all three trypanocides and the multi-drug-resistance phenotype of the parental population was expressed at the clonal level. If such resistance at the clonal level were highly prevalent at Ghibe, this would indicate that chemotherapy *per se* would not control trypanosomiasis at the site.

In other work, molecular karyotypes of 7 of the 12 isolates were determined. Eight populations were also characterized on the basis of electrophoretic variants of six enzymes. Six of the seven

populations examined had different karyotypes and all eight of the isolates belonged to different zymodemes.

The results of this work indicated that the multi-drug-resistance phenotype expressed by populations of *T. congolense* from Ghibe was associated with many genetically distinct populations rather than with a single genotype. The data therefore strongly indicated that an integrated chemotherapy and tsetse fly control program was the optimum method for controlling trypanosomiasis in livestock at Ghibe. Such a control program was begun in 1990 and is now under evaluation.

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M. Phil, Thesis abstract submitted in 1992
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ILRAD was founded in 1973 to conduct research into better ways of controlling livestock diseases. The current primary goal of the Laboratory is to develop safe, effective and economical methods to control two parasitic diseases that severely constrain animal production in Africa: trypanosomiais, transmitted to animals by the bite of a tsetse fly, and East Coast fever, a virulent form of theileriosis, transmitted to cattle by ticks. An international staff of about 50 scientists conducts basic research, much of it aimed at the development of vaccines, in the fields of biochemistry, cell biology, electron microscopy, epidemiology, genetics, immunology, molecular biology, pathology, parasitology and the socio-economics of animal disease control.

ILRAD is one of 17 international agricultural research centres sponsored by the Consultative Group on International Agricultural Research (CGIAR). The secretariat of the CGIAR is located in the World Bank headquarters, in Washington, D.C. The CGIAR is an informal umbrella organization of 40 national governments, international organizations and private foundations that together provide about US$300 million annually to the 17 centres for research, training and advisory services. The CGIAR aims to help farmers in developing countries increase their production of staple food crops, livestock, fish and trees in ways that improve the nutrition and well-being of low-income peoples and the management of natural resources.