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Why do livestock infected with trypanosomes develop anaemia?

Trypanosomiasis has been studied for almost a century, but scientists only now are beginning to determine what causes the severe anaemia, a striking feature of this disease

In tropical Africa, protozoan parasites cause several diseases of social and economic importance. One of the most devastating, trypanosomiasis, is caused by infection with trypanosomes, which are transmitted by tsetse flies to people, domestic livestock and wildlife. The disease constrains agricultural development on over a third of the African continent by causing livestock production losses due to poor weight gains, stunted growth, poor milk production, reproductive failure and finally death.

One of ILRAD's research objectives is to gain a better understanding of the diseases caused by trypanosomes. Despite recent advances at ILRAD and elsewhere in understanding the processes that occur during trypanosome infections, one central enigma remains: how do trypanosomes cause disease and tissue damage seen in acute and chronically ill animals? Evidence is accumulating that some infectious disease syndromes are not directly caused by the invading bacteria, parasites or their secretions, but by polypeptides (cytokines) and other proteins secreted by the white blood cells of the host animal in response to the invading organisms. The list of cytokines involved in disease processes is growing rapidly and now includes tumour necrosis factor (TNF), the interleukins, the interferons and the colony-stimulating factors. In a healthy animal these molecules function as immunomodulators, but they may have toxic effects on cells if they are produced in excess. The development of many disease processes follow similar pathophysiological pathways and are the result

of a cascade of events. By gaining a better understanding of how disease syndromes develop, we may be able to block the adverse effects and stimulate the beneficial effects of disease responses in living animals.

The clinical diseases caused by trypanosome infections in livestock have been recognized and studied by scientists for almost a century, but the mechanisms leading to anaemia, one of the most important disease manifestations, remain poorly understood and controversial. In order to readdress this important disease syndrome, ILRAD has assembled a multidisciplinary team of scientists to study the anaemia of trypanosomiasis. Scientists conducting these studies are using both cattle and laboratory bone marrow culture systems to better understand this prominent disease syndrome.

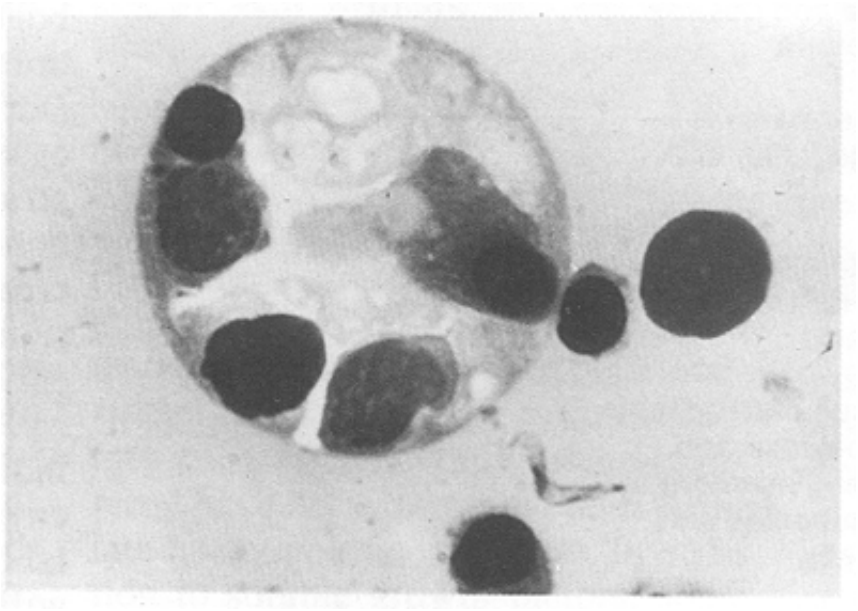

FIGURE 1. *Light micrograph of a large macrophage from the bone marrow of a calf infected with* Trypanosoma vivax, *a parasite that cages animal trypanosomiasis. Mature and immature red blood cells have been engulfed by the macrophage. A single trypanosome lies adjacent to the macrophage.*

Signs of trypanosomiasis

The clinical disease manifested in African animal trypanosomiasis varies in severity from mild to fatal depending on the susceptibility of the host animal and on the species, stock and virulence of the infecting parasite. Shortly after a natural trypanosome infection, an acute disease usually develops, lasting for several weeks. It may end in death, chronic disease or, occasionally, recovery. The chronic disease lasts for months or years aid ends in death, a persistent carrier state or self-cure.

Animals become infected with trypanosomes when they are bitten by tsetse flies. In the process of taking a blood meal from an animal, an infected fly deposits saliva laden with trypanosomes in the connective tissue of the animal's skin. Here the parasite differentiates from a form that develops in the fly to a 'bloodstream form', which is specially adapted to live in mammalian blood. The blood-stream form multiplies by binary fission and enter the animal's lymphatic and blood circulation.

For several days following infection, animals show no signs of disease. One to two weeks later, with the appearance of parasites in the blood, susceptible animals develop intermittent fever and anaemia. In experimentally infected, well-fed cattle, the disease tends to stabilize about four to six weeks following infection, after which the cattle may

become chronically anaemic. In endemic areas, however, where cattle are repeatedly bitten by tsetse flies carrying different types of trypanosomes, and where cattle are often forced to forage for food and to walk long distances for water, infected animals often continue to deteriorate until death. Cattle chronically infected with trypanosomes die of heart failure due to anaemia and damage to the heart muscle itself, or of secondary bacterial or viral infections. These secondary infections are believed to develop because the immune defence mechanisms are compromised in trypanosome-infected animals.

The biology of blood and bone marrow

The blood vascular system serves as highway for different types of cells to circulate throughout the body. Red blood cells (erythrocytes) remain within the circulatory system and transport oxygen and carbon dioxide bound to haemoglobin. White blood cells (leucocytes) combat infection and dispose of cell debris and foreign cells. White blood cells migrate across the walls of small blood vessels into tissues to perform their biological functions. The blood also contains specialized cell fragments called platelets, which help blood to clot (The main types of blood cells are listed in Table 1.)

TABLE 1. *The main types of mammalian blood cells and their functions*

Cell type	**Main functions**	**Typical concentration in bovine blood (cells/litre**
RED CELLS (ERYTHROCYTES)	Transport oxygen and carbon dioxide	5-10 x 10^{12}
WHITE CELLS (LEUGOCYTES)		4-12 x 10^{9}
Granulocytes Neutrophils	Phagocytose and destroy invading bacteria	2 x 10^{9}
Eosinophils	Destroy larger parasites and modulate inflammatory responses	7 x 10^{8}
Basophils	Release histamine and serotonin in certain immune reactions	5 x 10^{7}
Monocytes	Become tissue macrophages, which phagocytose and digest invading microorganisms, foreign bodies and senescent cells	4 x 10^{8}
Lymphocytes		4.5 x 10^{9}
B cells	Make antibodies	0.1–2.3 x 10^{9}
T cells	Kill-infected cells and regulate activities of other leucocytes	1.8–3.2 x 10^{9}
PLATELETS (cell fragments)	Initiate blood clotting	5 x 10^{11}

There are three broad classes of white blood cells—granulocytes, lymphocytes and monocytes. The most common type of *granulocyte* are the neutrophils, which engulf

and destroy microorganisms and are involved in early inflammatory responses. Eosinophils are granulocyte that modulate allergic inflammatory responses and help destroy parasite: *Lymphocytes* are cells involved in immune responses: B lymphocytes make antibodies; T lymphocytes kill virus-infected cells and regulate the activities of other white blood cells by secreting cytokines and by cell-to-cell contact. *Monocytes* circulate in the blood and pass through blood vessel walls to enter tissues, where they become macrophages. Macrophages (Figure 1), along with neutrophils, are scavengers in the body. These cells dispose of debris from dead cells as well as bacteria and other foreign cells by engulfing these in a process called phagocytosis.

Blood cell formation (haemopoiesis) takes place in the bone marrow. All blood cells originate from a common stem cell called a pluripotent stem cell (see Figure 2), most of which are found in the bone marrow. The pluripotent stem cell divides to produce more stem cells (self-renewal) or to produce committed progenitor cells, each of which is able to give rise only to one or a few types of blood cells. Committed progenitor cells are irreversibly destined to follow a particular developmental pathway, as shown in Figure 2. They divide profusely under the influence of colony-stimulating factors. A series of divisions allows the committed progenitor cells to amplify their numbers until terminally differentiated cells are produced, which no longer divide but cross from the bone marrow into a thin-walled vessel called a blood sinus.

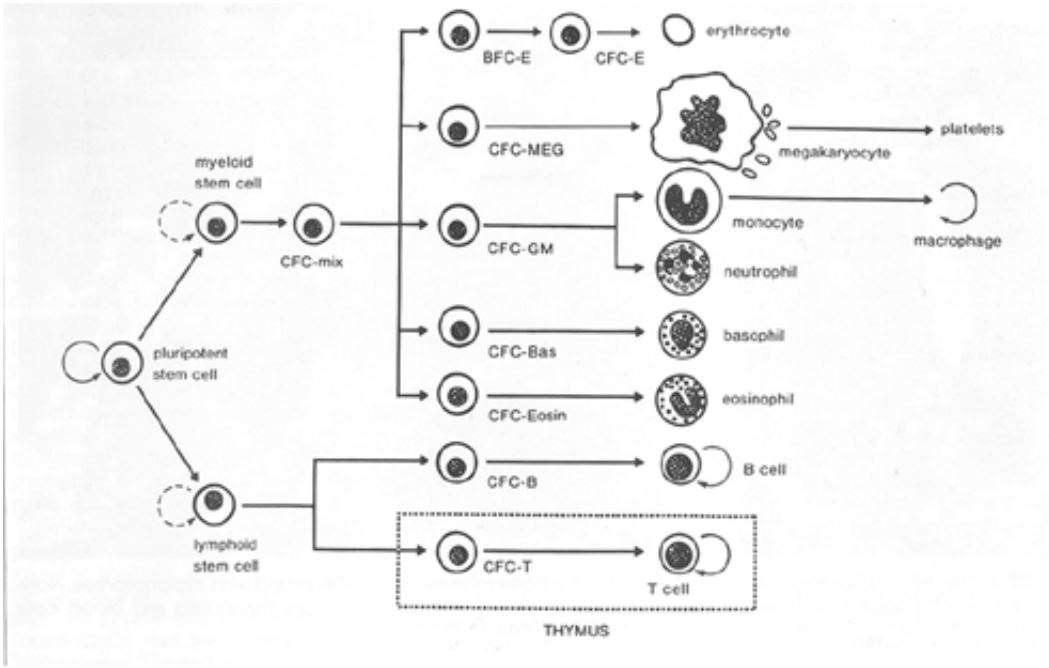

FIGURE 2. *A scheme showing how blood cells develop. The pluripotent stem cell normally divides to generate either more pluripotent stem cells (self-renewal) or committed progenitor cells, labelled CFC (for colony-forming cells), which are irreversibly committed to produce only one or a few types of blood cells. The progenitor cells are stimulated to proliferate by specific growth factors but progressively lose their capacity for division and develop into terminally-differentiated mature blood cells.*

Most blood cells have a short life span lasting a few hours to a few weeks, with the exception of red blood cells which may survive for over 100 days. In healthy animals, the bone marrow replenishes and maintains blood cell levels throughout life. During infection, most notably in diseases where severe and prolonged destruction of blood cells occur, the bone marrow normally reacts by increasing its rate of blood cell production. Bone marrow in this state is called 'responsive'.

Proliferation and differentiation of individual blood cells and their progeny can be

observed and analysed in laboratory culture. Bone marrow cells will survive, proliferate, and differentiate in culture if they are provided with specific growth factors or accompanied by cells that can produce these factors. Long-term proliferation of pluripotent haemopoietic stem cells has been achieved by overlaying bone-marrow cells onto layers of bone marrow stromal cells, presumably mimicking the environment in intact bone marrow. Alternatively, dispersed bone marrow cells can be cultured on a semisolid matrix such as methyl cellulose, and growth factors can be added artificially to the medium. In the methyl cellulose matrix the progeny of each isolated precursor cell remain together as a single colony. Bone marrow culture systems provide a way to assay for the factors that support or suppress haemopoiesis.

From studies of cultured haemopoietic cells, it is apparent that much of the control of haemopoiesis is mediated by a group of glycoprotein molecules, loosely termed haemopoietic growth factors. Eleven of these have been purified, their genes cloned and active recombinant molecules produced. Many of these haemopoietic growth factors are, however, species specific and therefore are limited in application to bone-marrow culture systems from one species of animal. The best understood of these growth factors is erythropoietin, a factor which regulates erythropoiesis (the formation of red blood cells). Another glycoprotein, interleukin 3 (IL-3), promotes the survival and proliferation of pluripotent stem cells and of most types of committed stem progenitor cells. Four distinct growth factors (IL-3, GM-CSF, G-CSF and M-CSF) are known to stimulate neutrophil and macrophage colony formation in culture. This group of growth factors is synthesized by several cell types. They are released into the blood in response to infections, thereby increasing the number of phagocytic cells released from the bone marrow into the bloodstream. Likewise, other humoral factors such as TNF and gamma-interferon have been shown to down-regulate haemopoiesis in culture. In addition to soluble growth factors, some of which are secreted locally by stromal cells in the bone marrow, there is evidence that cell-bound and extracellular-matrix-bound signals in the marrow also regulate haemopoiesis. How these and other growth factors regulate bovine bone marrow growth is unknown. What role one or more of these growth factors may have in regulating the bone marrow response of cattle infected with trypanosomes is now being investigated by scientists at ILRAD.

Anaemia in bovine trypanosomiasis

Anaemia is the absolute decrease in the total number of red blood cells per ml of blood, a decrease in the packed cell volume (PCV) due to fewer or smaller red blood cells and a decrease in haemoglobin also due to fewer red blood cells or less haemoglobin per surface area of a cell.

Anaemia develops either as a result of blood loss, accelerated destruction of red blood cells, or depressed red cell production in the bone marrow. The anaemia caused by increased red cell destruction has two forms. In one, red cells are ruptured, whereupon they release free haemoglobin into the plasma of the blood. In the other, phagocytic cells, such as macrophages of the mononuclear phagocyte system, remove red cells by phagocytosis. In the latter kind of anaemia, the haemoglobin of red cells is digested and stored in the macrophages as iron complexes.

As mentioned, anaemia is one of the most important pathological changes in animal trypanosomiasis. Despite the fact that many studies on bovine trypanosomiasis have concentrated on the peripheral blood changes associated with this infection, very few studies have examined the concurrent bone marrow response of infected animals. ILRAD is attempting to bridge this gap in our knowledge with detailed morphological studies of bovine bone marrow responses during acute and chronic trypanosome infections; by adapting *in vitro* cultivation techniques that allow bovine bone marrow cells to be maintained and by investigating the role of the macrophage in causing anaemia. The collective aim of these studies is to determine how the different stages of

anaemia develop and what role an ineffective bone marrow response plays in acute and chronic trypanosome infections.

In addition to the comparative studies of the haematological responses of N'Dama and Boran cattle conducted earlier at ILRAD, two studies have been recently completed in which the peripheral blood and bone marrow responses during the acute phase of infections (46-day studies) of *T. vivax* and *T congolense* were examined. A stock of *T. vivax* that causes a severe haemorrhagic syndrome in dairy calves was chosen as the first study model followed by a second study in which a clone of *T. congolense* was used to infect Boran cattle. Based on these studies, a picture of the anaemia caused by trypanosome infections in livestock is presented here.

The onset of anaemia determined by a drop in the packed cell volume is closely correlated with the onset of fever, and appearance, intensity and duration of parasitaemia. By the second to third week of infection a sharp drop in the red blood cell count and haemoglobin level develop, accompanied by an increase in the circulation of immature red blood cells (a few reticulocytes along with many macrocytes). Other important changes in the blood during the acute phase of the disease involving white blood cells, blood platelets and plasma factors occur simultaneously with the anaemia of trypanosomiasis. The number of white blood cells is reduced to about half the normal number due to a reduction in numbers of neutrophils and lymphocytes. (Monocytes and eosinophils are less severely affected.) The number of circulating blood platelets also decreases early in the infection due to a shortened platelet life span.

The studies of bovine bone marrow obtained from trypanosome-infected cattle revealed that red, lymphoid and monocyte cell lines in the marrow increased during the acute stage of infection. Red cell progenitors in the bone marrow appeared to mature in a normal manner. These findings support the belief that bone marrow of trypanosome-infected cattle is responsive during the early stages of the disease.

Although the number of neutrophils in the blood were observed to decrease in this early phase, the initial neutrophil response in the bone marrow was much weaker than that of the red cell, lymphoid and monocyte lineages. The ILRAD team concluded that production of neutrophils and eosinophils in the bone marrow was being suppressed. Although there was a drop in numbers of circulating blood platelets, the number of progenitor cells in the bone marrow that release blood platelets increased, suggesting that the decrease in circulating blood platelets is due to their excessive removal at coagulation sites or in the circulation by macrophages.

Lymphoid follicles each containing numerous immature lymphocytes developed in the bone marrow of infected calves. Lymphocytes normally comprise about 6% of bovine bone marrow cells. Twenty days after infection with *T. vivax*, lymphocytes made up 24% of the calves' bone marrow cells. The number of monocytes and macrophages in the bone marrow also rapidly increased. Fifteen days after infection, these cells made up 28% of marrow cells; 0-1% is normal. Macrophages in the bone marrow became large and actively phagocytic. Both monocytes and macrophages in the bone marrow of the *T. vivax*-infected calves were observed to remove mature red cells and platelets as well as red cell progenitors, neutrophil progenitors and eosinophils precursors (Figures 1 and 3). Macrophages in the bone marrow began to phagocytose bone marrow progenitor cells before the progenitor cells entered the blood. These results demonstrate that some blood cell progenitors and mature blood cells are destroyed in the bone marrow of trypanosome-infected cattle.

Following the acute phase of trypanosomiasis, characterized by progressive anaemia and a fluctuating parasitaemia of 4 to 12 weeks duration, the packed cell volume of infected cattle may have dropped to 20% or lower. The packed cell volume may

continue to drop resulting in the death of the animal; it may fluctuate at a low level during chronic disease; or gradually improve as the animal recovers.

During the chronic stage of the disease the packed cell volume fluctuates at around 20% and does not improve; there is a low, transient to undetectable parasitaemia. Although there are fewer red cells than normal during the chronic anaemia, the cells are of normal or smaller than normal size and they carry a normal amount of haemoglobin. The bone marrow thus appears to be nonresponsive.

Scientific reports describing chronic trypanosome infections have concentrated on the peripheral blood of chronic anaemia. Changes in bone marrow have rarely been described and simply refer to the bone marrow as being filled with fat and lacking red marrow. The ILRAD group is thus attempting to determine why the bone marrow is unresponsive in cattle chronically infected with trypanosomiasis.

Possible mechanisms for the anaemia in trypanosomiasis

Although the changes in blood and tissues that occur in trypanosomiasis are well characterized, the mechanisms by which these changes occur are poorly understood. Several mechanisms are probably at work, and certain factors may play more dominant roles in infections in one host species, with one trypanosome species or in one stage of the disease compared with others.

The anaemia that occurs during acute trypanosomiasis is due primarily to the removal of large numbers of red cells by macrophages. Several possible mechanisms have been suggested as responsible for this excessive red cell removal by cells of the mononuclear phagocyte system. Such mechanisms include the following.

The membrane of red cells may be physically damaged during infection. Physical alterations in the surface membrane of red cells can lead to their early removal by macrophages. Febrile responses lead to decreased erythrocyte half-life due to increased osmotic fragility, decreased plasticity and increased membrane permeability. In infections that cause extremely high parasitaemias—such as *T. simiae* infections in pigs and some *T. vivax* infections in cattle—disseminated intravascular coagulation may occur, resulting in an accelerated destruction of red cells. This coagulation causes fibrin thrombi to be deposited in small vessels. Red cells are damaged by these partially blocked capillaries, and the aggregation of platelets and fibrin may lead to tissue necrosis and severe reduction in blood platelets. Damaged red cells may then be phagocytosed by macrophages.

Trypanosome antigens or host antibody-trypanosome complexes may bind to red cells. Antibodies and complement have been reported on the surface of red cells from trypanosome-infected cattle. Complement is a system of blood proteins that can be activated by antibody-antigen complexes. Some of the antibodies detected on the red cells were found to specifically recognize trypanosomes. Thus, in addition to helping to clear trypanosomes from the circulation, antibodies produced in trypanosome infection may contribute to the anaemia. It is speculated, for example, that fragments of disrupted trypanosomes may bind to red cells, causing them to be identified as foreign and thus to be ruptured by antibody and complement or (more likely) to be phagocytosed by macrophages.

Aberrant antibodies may bind to the host's own blood cells. ILRAD scientists have detected autoantibodies—antibodies produced against an animal's own proteins—to red cells and blood platelets in *T. vivax*-infected calves. Why autoantibodies appear intermittently in the circulation in some trypanosome infections is still unknown. Trypanosomes may in some way damage red cell membranes, resulting in exposure of normally hidden antibody-binding sites on their surface. Once exposed, such sites

would be recognized by circulating antibodies whose function is to bind to damaged red cells, thus facilitating the removal of old blood cells by macrophages.

Red cells are phagocytosed by activated macrophages. Early in a trypanosome infection, the number of macrophages increases throughout the body. This expanded pool of macrophages actively removes red cells within vessels and tissues in many sites, including the spleen, liver, lungs, lymph nodes and bone marrow, thereby greatly reducing the half-lives of red cells. ILRAD scientists have also observed an increase in numbers of cells of the monocytic lineage in bone marrow, with a resulting destruction of immature blood cells. This observation suggests that trypanosomal infection may cause defective blood cell production. Trypanosomes have been found in the bone marrow (Figure 3), where it is possible that they damage precursor cells by signalling for their early removal by macrophages. But ILRAD scientists believe the more likely explanation for the anaemia is that trypanosome infections in some way cause massive proliferation of macrophages throughout the body and they in turn phagocytose red cells.

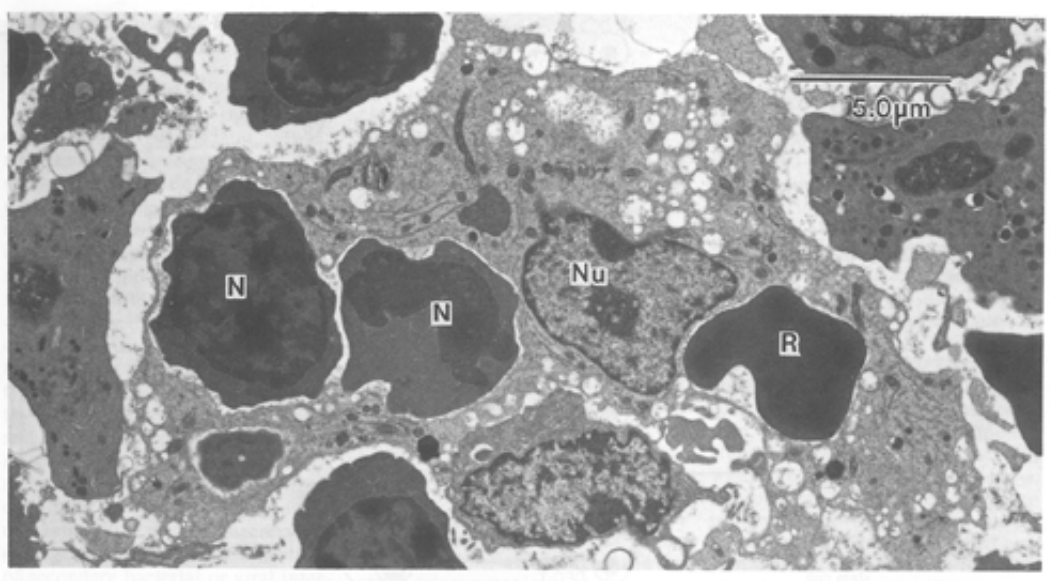

FIGURE 3. *Electron micrograph of a macrophage In the bone marrow of a calf infected with trypanosomes. Note that the macrophage has ingested a mature red blood cell and two immature nucleated red blood cells (normobiasts). Nu: nucleus of macrophage, R: red blood cell, N: normoblast.*

It is not yet known whether there are growth factor(s) produced by host cells or a product of trypanosomes that stimulates macrophages to proliferate rapidly in the bone marrow and other organs during a trypanosome infection. The increases in cells of the mononuclear phagocyte system and phagocytosis of red cells appear to be stronger in cattle that develop anaemia rapidly (when, for example, they are infected with haemorrhagic stocks of *T. vivax)* than they are in cattle that develop anaemia more slowly (such as occurs in *T. congolense* infections in Boran cattle). Whatever the cause of macrophage stimulation, it appears that the bone marrow of infected animals, although responsive during the acute stage of the disease, later simply cannot keep up with the accelerated red cell destruction by macrophages. It is thus possible that trypanosomes, either directly or indirectly, produce factors which induce bone marrow unresponsiveness in chronic infections.

Cytokine mediators of macrophage activation, such as gamma-interferon secreted by T lymphocytes, enhance macrophage phagocytosis and might be partially responsible for the increased removal of both mature and immature blood cells by macrophages in the bone marrow of trypanosome-infected cattle. In other diseases, activated macrophages

have been demonstrated to increase production of cytokines, which in turn act as messengers to other cells in the body. Activated macrophages may also excrete oxygen metabolites that can damage host cells.

ILRAD's approach to studying anaemia

ILRAD has conducted a number of experiments in which the peripheral blood picture has been well characterized in both acute and chronic trypanosome infections in ruminants. Two recent studies, discussed earlier in this issue, have concentrated on the bone marrow changes in cattle during acute trypanosome infections. Both of these studies pinpointed the pivotal role macrophages play in precipitating the anaemia of acute trypanosomiasis. These studies provide evidence that there is destruction of progenitor cells by macrophages in the bone marrow of trypanosome-infected cattle.

Because of their ability to control the harmful effects of trypanosome infections, ILRAD staff chose the N'Dama breed as a model for studies comparing 'trypanotolerant' animals, which tolerate infection with trypanosomes, with trypanosusceptible animals, such as European dairy cattle *(Bos taurus)* and African Zebu *(Bos indicus),* in which the infection causes disease. In 1985, ILRAD staff began a series of experiments comparing the performance of N'Dama cattle with Boran cattle when both breeds were subjected to the same level of tsetse challenge and the same clones of *Trypanosoma congolense.* During the course of six infections, all of the N'Dama cattle controlled trypanosome infections successfully without severe anaemia, while the Boran cattle failed to do this. The reason why N'Dama cattle control trypanosomiasis more effectively is being investigated.

Because of the importance of increased red cell destruction by macrophages as a mechanism of anaemia in trypanosomiasis, ILRAD scientists are conducting *in vitro* comparisons of macrophage function in trypanosome-infected N'Dama and Boran cattle. Attempts are being made in these studies to quantify the rate at which red cells are phagocytosed and destroyed during infection. Phagocytosis of red cells *in vitro* increased during infection. Macrophages from the spleen and bone marrow were more phagocytic than those derived from the blood. Macrophages from infected Boran cattle were more effective at phagocytosing red cells than were macrophages from infected N'Dama cattle. Studies are now being made to identify what factor or factors activate macrophages in trypanosome infections. Differences in macrophage activation may help to explain how N'Dama tolerate infection with trypanosomes whereas exotic dairy breeds and Zebu cattle are more severely anaemic.

Other studies being conducted at ILRAD are designed to investigate the possible role of depressed production of red cells by the bone marrow in trypanosomal anaemia. These studies involve following sequential bone marrow changes in cattle through a trypanosome infection that lasts through the chronic stage of anaemia. These studies require microscopic examination of the blood and bone marrow and culture of the bone marrow cells *in vitro.*

Over the last few years ILRAD scientists have refined and developed techniques to maintain the major bovine bone marrow progenitor cells in short-term culture. As a result of this work, ILRAD staff members have developed a method that enables researchers to maintain red cell and eosinophil progenitor cells of cattle in culture for the first time.

Efforts are now being made to develop monoclonal antibodies that will identify different types of bone marrow progenitor cells. The monoclonal antibodies will be used as markers to study the processes of cell differentiation in bone marrow. The development of short- and long-term bovine bone marrow cultures will be invaluable in exploring the cellular and molecular mechanisms that control blood cell production. Use of these

cultures will allow scientists to study how bone marrow is affected by different cytokines and trypanosome products. Studies comparing how infection changes the bone marrow of trypanoresistant and trypanosusceptible cattle will also be enhanced by use of these culture systems. The aim of *in vitro* bovine bone marrow studies is to identify possible aberrations in the regulation of bone marrow growth that may simulate the pattern of changes seen in trypanosome-infected cattle.

In summary, ILRAD scientists have looked at the responses of bovine bone marrow to infections with *T. vivax* and *T. congolense* during the acute phase of infection. A red cell response does occur, but it is insufficient due to the large numbers of red cells continually being removed by macrophages. An abnormally high number of lymphocytes and macrophages accumulates in the bone marrow. Macrophages within the bone marrow remove many immature and mature blood cells. This causes ineffective haemopoiesis. If scientists can identify the way in which trypanosomes cause massive proliferation of macrophages and can find a way to block this proliferation or the active uptake of red cells by macrophages, they may be able to prevent the development of anaemia.

THE MAIN ARTICLE in this issue is based on reports by ILRAD staff member Linda Logan-Henfrey and former staff member Gerhardt Fritsch. Professor Victor Anosa of the University of Ibadan, Nigeria, collaborated in the study of bone marrow responses of *Trypanosoma vivax*-infected dairy calves. Others whose work is reported here are ILRAD staff members Peter Gardiner, Francis McOdimba, Dean Moloo, Paul Muiya, Jan Naessens, Jim Scow Mike Shaw, Victor Taiwo and Diana Williams and former staff member: Sam Black, Robert Nelson and Robert Paling.

THE EDITOR THANKS Onesmo Ole-MoiYoi and Diana Williams for their comments on a draft of this article.

NOTE TO OUR READERS This issue of *ILRAD Reports* combines Numbers 3 (July) and 4 (October) of Volume 8 (1990). The next issue will be Number 1 (January) of Volume 9 (1991).

Further Reading

The ILRAD scientists whose work is reported on in this issue have published detailed results of their research in scientific journals and proceedings of meetings. Some of their recent publications are listed below. Single copies of reprints may be requested from the ILRAD Library. Please mention the ILRAD publication number given in brackets at the end of the references.

NOTE: If you would like to be put on ILRAD's mailing list to receive regular issues of this newsletter, please write to ILRAD's Information Unit. ASSOKU, R.K.G. and GARDINER, P .R. 1989. Detection of antibodies to platelets and erythrocytes during infection with haemorrhage-causing *Trypanosome vivax* in Ayrshire cattle. *Veterinary Parasitology* 31: 199–216 (577).

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Affected Areas of Africa: Proceedings of a Meeting Held in Nairobi, Kenya, 23–27 November 1987. Nairobi: International Livestock Centre for Africa/International Laboratory for Research on Animal Diseases, pp. 152–160. (594)

MURRAY, M. and DEXTER, J.M. 1988. Anaemia in bovine African trypanosomiasis. *Acta Tropica* 45: 389–432.

PALING, R.W., MOLOO, S.K. and SCOTT, J.R. 1988. The relationship between parasitaemia and anaemia in N'Dama and Zebu cattle following four sequential challenges with *Glossina morsitans centralis* infected with *Trypanosoma congolense.* In: *Proceedings of the International Scientific Council for Trypanosomiasis Research and Control, 19th Meeting, Lomé, Togo,* 1987. Nairobi: Organization of African Unity/International Scientific Council for Trypanosomiasis Research and Control, pp. 256–264 (695).

ILRAD scientists honoured

DR. HIRO HIRUMI, a senior scientist at ILRAD, and MRS. KAZU HIRUMI, a research associate at the Laboratory, were jointly awarded the 16th annual Ohyama Prize for Medical Sciences in a ceremony held on 3 March 1990 at the Nippon Kogyo Club, Tokyo, Japan. The Hirumis, who are Japanese-American, have worked at ILRAD for 14 years in cell biology. They were awarded the Ohyama Prize for their research achievements in developing *in vitro* systems for cultivating African trypanosomes.

The Ohyama Health Foundation was founded in 1973 by Mr. Ohyama, an eminent businessman in Japan, for the purpose of supporting fine medical work, particularly in developing countries. In addition to the Ohyama Prize for Medical Sciences, the foundation annually presents research grants in the field of tropical medicine and hygiene. The foundation's nominating committee consists of prominent Japanese scientists in the medical sciences.

DR. IVAN MORRISON, who left ILRAD in December 1989, was given the first Pfizer Buiatrics Award in London on 11 October. The award is presented for outstanding contributions to the advancement of knowledge in the origin and treatment of bovine diseases. Dr. Morrison spent 15 years at ILRAD, where his research provided new information on the immune system of cattle. He is now head of immunology and pathology at Compton Laboratory, which is part of the Institute for Animal Health, of the Agricultural and Food Research Council, in the United Kingdom.

DR. RONALD KAMINSKY, an ILRAD scientist, and DR. ERICH P. ZWEYGARTH, a scientist at the Kenya Trypanosomiasis Research Institute, in December were jointly awarded a European prize by the Fondation Internationale pour la Substitution de 1'Experimentation Animale, in Luxembourg, for the development and application of *in vitro* techniques to be used for screening and evaluating anti-trypanosomal drug compounds.

The effects of irradiation on the development of *Theileria parva* sporozoites

Irradiation of *Theileria parva* sporozoites is known to modify the infectivity of the parasites. From 1988 to 1990, a study was carried out to investigate the specific mechanisms responsible for this phenomenon. *Theileria parva* sporozoites were irradiated at 13 kRad and analysed to determine the effects of irradiation on the biology of the parasite, specifically on whether irradiation alters the parasite, and if so, what kind of changes it causes. The study also examined whether the irradiated parasites were able to bind to and enter host lymphocytes, the early developmental events that

follow sporozoite entry into lymphocytes, and the ability of laboratory workers to infect and establish cell lines.

No differences were detected *in vitro* in the ability of irradiated and non-irradiated sporozoites to bind to and enter lymphocytes. Between days 1 and 3 after infection, irradiated and non-irradiated sporozoites in lymphocytes developed in a similar way. From day 4 after infection, however, significant differences occurred in their development. Lymphocytes infected with non-irradiated sporozoites had normal schizonts, whereas most cells infected with irradiated sporozoites contained intracytoplasmic particles of varied shapes.

Lymphocytes were infected *in vitro* with irradiated and non-irradiated sporozoites to determine if their early development differed. After 7 days in culture, similar numbers of infected cells were inoculated into autologous cattle. Animals that received cultures derived from irradiated sporozoites survived the infection and resisted challenge with a lethal homologous sporozoite stabilate. Those inoculated with cultures initiated with non-irradiated sporozoites died of East Coast fever.

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