antigens have been successfully introduced and established at 10 of the 11 institutes involved in the validation exercise. The participants reported that in detecting active trypanosome infections, the antigen-ELISAs demonstrated a sensitivity 4–5 times greater than that of the more commonly used buffy coat technique. It was concluded that the antigen-ELISAs clearly have potential use in the development of strategies for monitoring and controlling animal trypanosomiasis.

## Clearance of antibody–VSG complexes by trypanosomes (Ph.D.Thesis)

CATTLE INFECTED with trypanosome parasites are known to produce antibodies against molecules known as VSGs that are located on the surface of trypanosomes. Host anti-bodies bound to parasite VSG mediate the killing of the parasites by other elements of the bovine immune system, such as macrophages and neutrophils. This is the main method by which the immune system clears trypanosomes from the body. It is also known, however, that trypanosomes can remove antibody bound to their VSG. Because this antibody removal has important implications for the control of the parasites, studies were undertaken to determine the trypanosome mechanisms responsible for cleaving VSG-antibody complexes.

The binding of antibodies that specifically recognize VSG molecules exposed on the surface of clones of *Trypanosoma brucei, Trypanosoma congolense* and *Trypanosoma vivax* was investigated using both immunofluorescence (light microscopy and a fluorescence-activated cell sorter) and electron microscopy. In addition, parasites of the *T. brucei S*427 clone 22, which were adapted to cultures containing no feeder cells, were incubated with specific F(ab)2 and Fab antibody fragments or with biotin.

After incubation at 0°C, antibodies, antibody fragments and biotin molecules were observed over the whole parasite surface; fluorescence appeared strongest at the parasite's flagellum. Upon warming to 37°C, surface-bound antibody and antibody fragments were cleared from the parasite surface. Even in the absence of antibody-mediated crosslinking of VSG (i.e., Fab), clearance occurred through the movement of surface-bound Fab-VSG complexes toward the flagellar pocket. Studies of permeabilized trypanosomes using electron microscopy and immunofluorescence showed that after being cleared from the cell surface, small amounts of antibody were located intracellularly between the nucleus and the flagellar pocket. However, when a cocktail of protease inhibitors was added to the culture medium, large amounts of antibody or antibody fragments could be detected within vacuoles situated between the nucleus and the flagellar pocket, suggesting that proteases are required to break down antibody–VSG complexes. Different antibodies were cleared at different rates. Antibodies with both a higher molecular mass and more than one antigen-binding site were generally cleared most rapidly.

Movement of antibody-VSG complexes was inhibited at temperatures below 4°C and by adding 2-deoxy-Dglucose in lieu of D-glucose to the culture medium. Movement was immediately and reversibly inhibited by increasing the NaCl concentration in the medium to 200 mM. Antibody clearance was also inhibited by protein synthesis inhibitors and protease inhibitors. The process was not inhibited by microfilament (cytochalasin B and D) or microtubule (nocodazole) disrupters, nor was it altered by an increase in medium viscosity.

IN SUMMARY, the results of these studies showed that antibody clearance in trypanosomes is a directional, energy-dependent process. It is not dependent on crosslinking of VSG and it is selective: only VSG bound to antibody is cleared.

Antibody clearance may have an important role to play in the process of antigenic switching in trypanosomes in which the parasites periodically remove one coat of VSG and replace it with another of a different antigenic type. An antibody response to a

specific variable antigen type may actually speed up the process of coat substitution. This would be a major advantage to the trypanosome: it would help the parasite evade the host immune response, thus prolonging its survival and increasing its chance of transmission.

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*ILRAD Reports* is published quarterly by the International Laboratory for Research on Animal Diseases (ILRAD), in Nairobi, Kenya. The newsletter is written, designed and electronically laid out to camera-ready stage in house using a personal computer and laser printer. The newsletter is printed in Nairobi by Majestic Printing Works. It has a circulation of 3,500 English and 500 French readers and is available free upon request. Material in the Reports may be reproduced without prior permission, but we ask that credit be given and that two copies of the reprint be sent to ILRAD's Scientific Writer/Editor, Susan MacMillan: telephone: +254 (2) 632-311, telefax: 254 (2) 631-499, telex: 22040 ILRAD, electronic mail: (BT Tymnet) CGI017.

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: trypanosomiasis, transmitted to animals by the bite of a tsetse fly, and East Coast fever, a virulent form of thelleriosis, 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 socioeconomics of animal disease control.

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