quality cattle of defined genotypes, including monozygotic and chimaeric twins produced on the Laboratory's cattle ranch. ILRAD will thus continue to exploit this comparative research advantage to the full.

A preliminary understanding of the role played by gamma/delta T cells in trypanosome and *T. parva* infections is being obtained by research conducted at ILRAD and elsewhere. A thorough understanding of the function of these immune cells is needed because they occur in significantly large numbers in calf spleens, and it is young animals that will be the main target for vaccination. It was speculated that gamma/ delta T cells play a role in enabling young cattle to tolerate or control parasite infection and to develop immunity without developing disease. Low numbers of natural killer cells are also present in cattle blood and are readily activated by cytokines.

A brief discussion was held on the plastic nature of immune responses— that is, that different animals employ different immune cells and mechanisms to protect themselves against *T. parva* and trypanosome infections. In outbred populations, this phenomenon (detected in cell transfer experiments) may be due to the occurrence of parasite epitopes that differ slightly in quantity or quality from one infected animal to another. It was agreed, however, that little merit existed in ILRAD's complicating its investigations of protective immune responses with concerns about plasticity. Rather, ILRAD's approaches to vaccine development will focus on major immune responses in target animal populations and the parasite antigens that elicit them.

Finally, the workshop participants emphasized that a search for protective antigens requires a better under standing of the cell biology of the parasites. This research produces additional information about the parasites and their interactions with the host. Analyses of data obtained from cell biology research help reduce the number of antigens that must be screened for biological characters and may help reveal antigens likely to elicit protective immune responses.

This article is based on workshop reports by ILRAD scientists Tom Dolan, Rob Skilton and Maarten Sileghem. Declan McKeever Is editing the proceedings of the workshop and commented on a draft of this article.

Developing nucleic acid-based methods for typing class II MHC in cattle

The protozoan parasite *Theileria parva* causes theileriosis, an acute and usually fatal lymphoproliferative disease of cattle in Africa, where it is commonly known as East Coast fever. A major goal of ILRAD's research program is development of an improved vaccine that will protect cattle from this parasite.

Significant progress has been made towards achieving this goal (see the July 1992 issue of this newsletter). Administration of a recombinant form of a major surface antigen of the sporozoite stage of the parasite has been shown in experiments to protect cattle against subsequent challenge with normally lethal doses of *T. parva*. lLRAD is developing his antigen for delivery in a first-generation 'subunit' vaccine, which is based on one or more antigenic parasite molecules rather than the whole parasite.

It is well known that the quality of an immune response to infection with an intracellular parasite depends greatly on molecules encoded by genes located in an area of the mammalian genome known as the major histocompatibility complex (MHC). T lymphocytes of the host animal recognize antigens only when these are wedged in the binding clefts of 'self' MHC molecules located on the surface of antigen-presenting cells. The ability of a given parasite antigen to elicit an immune response is likely to be related to the antigen's ability to bind to the MHC molecules expressed by an individual animal.

Two classes of MHC exist and both classes are polymorphic, that is, molecules of each class vary from one individual animal to another. It is possible that MHC polymorphisms within outbred populations of cattle will influence the efficacy of a subunit vaccine in the field. This possibility has led ILRAD scientists to search for reliable techniques with which to type the MHC of cattle.

The typing of bovine class I MHC molecules is in an advanced state. This work has greatly facilitated the characterization of *T. parva*-specific cytotoxic T-cell responses in cattle. In contrast,

available methods for characterizing bovine class II MHC molecules—the MHC class that will influence the efficacy of a p67-based vaccine—have been tedious to use and unsuitable for non-specialized laboratories and field conditions. Recently developed DNA based typing methods have many advantages over the older methods. The availability of these new methods has made development of a simple nucleic-acid-based system for bovine class II MHC typing and characterization a priority for scientists in ILRAD's vaccine development program.

Since 1991, ILRAD has collaborated with the Roslin Institute, in Edinburgh, on a project funded by the Overseas Development Administration (UK) to develop methods for bovine class II MHC typing based on characterization of genes that encode expressed class II products. Scientists at Roslin examined a number of typing techniques based on use of the polymerase chain reaction (PCR) to amplify defined stretches of DNA. Two complementary methods applicable to a range of class II genes were chosen. Types are assigned by analysis of restriction fragment length polymorphism (RFLP) in PCR-amplified segments of the class II gene; the presence or absence of a series of restriction enzyme sites is determined from the sizes of the restriction fragments. The PCR-RFLP assignments are then checked and confirmed by assessment of single-strand conformation polymorphism (SSCP), in which sequence-dependent variation in the migration of the denatured PCR products is used to distinguish different class II genes. Both the PCR-RFLP and SSCP methods for typing have been transferred to ILRAD, where they are being used to evaluate MHC-related effects on the efficacy of ILRAD's experimental vaccine when applied in field trials. Another important aspect of the project has been cloning and characterizing full length complementary DNA for a number of bovine class II genes. More specifically, the ILRAD-Roslin project has established, among many other facts, that class II products from both the DR and DQ loci are expressed by bovine immune cells.

All vaccine development research programs need some way of typing class II MHC molecules. The development of rapid DNA-based methods to define the number and variety of expressed bovine class II MHC genes will provide the basis for rigorous studies of antigen presentation by the products of these genes and an assessment of their consequences in the immune responses of cattle populations to improved vaccines.

The technologies developed in this collaborative research have provided an ideal opportunity to acquire enhanced knowledge of antigen presentation. This, in turn, will improve ILRAD's capacity to evolve improved antigen delivery strategies for the East Coast fever vaccine under development at the Laboratory.

This article is based on a report written by ILRAD scientist Declan McKeever. The scientists at the Roslin institute, in Edinburgh, who developed the improved MHC typing methods described are George Russell and Roger Spooner. This research is funded by the Overseas Development Administration of the UK.