R7365

Identifying antigens of *Theileria parva* that may be used in a vaccine for East Coast Fever*.

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Collaborators
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(*Identification of antigens of *Theileria parva* that are recognised by bovine parasite specific cytotoxic T lymphocytes and evaluation of their vaccine potential in laboratory trials)

Executive Message

- East Coast Fever is (ECF) is a fatal disease of cattle caused by the sporozoan parasite *Theileria parva* and is transmitted by ticks.
- Many of the farmers who suffer stock losses are smallholder dairy producers. The improved higher yielding cross bred cattle they are being encouraged to keep to achieve good returns are very susceptible to ECF.
- This project continues previous DFID research seeking an effective vaccine against ECF.
- Scientists at IAH and ILRI are working to identify antigens from *T.parva* that might become components of a vaccine against ECF. They are using a process called *reverse phase high-performance liquid chromatography* (HPLC) to elute and characterise antigenic peptides that have been isolated from MHC class I molecules.
- So far T cells (white blood cells that are involved in protecting cattle against disease) known to have the ability to kill parasite-infected cells have been isolated. These cells will be exposed to uninfected cells treated with antigen peptides from the parasite. This will allow the identification of key parasite proteins that induce a protective, long lasting immune response. This would mean such proteins could be used in a vaccine to protect the cattle. The screening process for suitable peptides is likely to take several years. Vaccine production is therefore still four or five years away.
- The provision of an affordable vaccine will not only have a positive impact on the livelihoods of smallholder dairy producers in developing countries but could also improve the nutritional status of rural and urban families through a significant increase in the provision of milk.

Smallholder dairy cows are cattle often the victims of ECF

Background

East Coast fever (ECF) is a fatal disease of cattle caused by the sporozoan parasite *Theileria parva.* The parasite, which is transmitted by ticks, has a complex life cycle in which the infective sporozoite and schizont stages have been identified as possible targets as components of future vaccines against ECF.

The principal losses are due to the death of susceptible cattle and substantial production loss. Many of the farmers who suffer losses of cattle are smallholder dairy producers. The improved higher yielding cross bred cattle they are being encouraged to keep, to achieve good returns, are very susceptible to ECF. Any effective vaccine would thus have a dramatic positive effect on the yields and income achieved. Since the only

effective control at present involves extensive use of acaricides, a new vaccine would have positive benefits for the environment.
At the cellular level protection against ECF is associated with MHC class I-restricted cytotoxic T lymphocytes that kill schizont infected cells. The cytotoxic T lymphocytes (CTL) response in immune animals is often restricted by a single MHC molecule, and such responses are usually parasite strain specific. This suggests that, despite the large complex nature of the pathogen, protection may be focused on a small number of antigenic determinants.

Objectives

This project has two aims with the first being the more important, the latter longer term.

- To identify the dominant CTL target antigens from *T.parva*, by isolating and characterising antigenic peptides bound to MHC class I molecules.
- To evaluate the capacity of these antigens to induce a protective, long lasting immune response and thus become potential constituents for a vaccine.

The initial approach is to isolate MHC class I molecules from *T.parva*-infected cell lines, and to elute antigenic peptides from them using reverse phase high-performance liquid chromatography (HPLC). These peptides will be assessed for their capacity to sensitise uninfected cells for recognition by parasite-specific CTL clones. Active peptides identified in this way will be used to target antigenic proteins in the parasite. This will be achieved either by screening sequence databases or cDNA/genomic libraries. Genes identified in this way will be sequenced and characterised. Candidate genes (single, or in combination) will be evaluated as vaccine components in laboratory trials. Information generated in project R7358 (Development of a sub-unit vaccine against East Coast Fever in cattle) may be used at this stage in choosing the most effective immunisation strategy.

Highlights for Year 1

The aim in the first year has been to generate peptide pools from *T.parva* infected cells, initially using cells from A18 homozygous animals that express a single MHC class I gene. Assays are being developed that will enable these pools to be tested for their ability to sensitise uninfected target cells for recognition by CTL clones. Once this is completed the peptide pools will be fractionated and reassessed in order to identify individual/small groups of active peptides. Depending on the outcome, suitable methods will be employed to identify the *T.parva* genes that encode dominant target antigens.

At IAH a great deal of effort has gone into optimising the protocol for lysis of *T.parva* infected cell lines. HPLC has been performed on a number of samples, and the MHC-bound peptide pools generated have been sent to ILRI in Nairobi for analysis. Preliminary experiments will allow optimisation of the methods for drying and reconstituting peptide pools to minimise both loss of material, and toxic effects on cells.
Experiments are being carried out at IAH and ILRI to establish the best method for binding pooled peptides to target cells for assessment of their ability to stimulate antigen-specific CTL. These experiments involve acid-stripping of peptides from infected cells, followed by binding to similarly stripped target cells, using conformation-dependent anti-MHC class I mAbs. All of the initial experiments are being carried out using cells derived from animals homozygous for the A18 MHC class I haplotype. This haplotype is particularly suitable for the planned experiments as it expresses a single class I gene that has been shown to be a dominant allele in its ability to bind and present *T.parva* peptides to T cells. For this reason it is planned to generate additional A18-restricted *T.parva*-specific CTL clones. Animals have been identified that appear to express this haplotype (by mAb recognition). Following confirmation of this using PCR-SSP these animals will be infected and CTL clones will be generated and characterised for future use.

So far so good …….

The aim of this project is to identify components of the *T. parva* parasite that are recognised by the cattle immune system. Many such components are presented at the surface of an infected cell, but few are recognised by the appropriate T cells. Parasite components have been partially purified by a procedure known as HPLC. T cells known to have the ability to kill parasite-infected cells have been isolated. These cells will be exposed to uninfected cells treated with fractions of the extracted parasite components, and in this way it will be possible to define the key parasite proteins that would constitute an effective vaccine.

Impact

It is early days in the life of this project but significant progress has already been made on planned activities towards the development of an

effective vaccine which will ultimately allow the use of improved livestock breeds by smallholders. This will not only have a positive impact on the livelihoods of smallholder dairy producers in developing countries but could also improve the nutritional status of rural and urban families through a significant increase in the provision of milk.

Next …………..

The identification of *T.parva* genes encoding key antigens will be an on-going process, as it is predicted that a number of different active peptides/peptide fractions will be found in the initial screenings. While some genes may be identified through database matches, others will not, and in these cases it will be necessary to make primers and search cDNA/genomic libraries, followed by cloning/sequencing of the genes. This process is predicted to take most of the second year of the project. In addition, further peptide pools may be assessed from cells expressing haplotypes other than A18 e.g. A10, KN104.