Theileria parva: the nature of the immune response and its significance for immunoprophylaxis

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Summary: Theileria parva is a tick-borne haemoprotozoan parasite of cattle and buffalo which is responsible for considerable economic losses to cattle farming in Eastern, Central and Southern Africa. Infection with the parasite results in an acute lymphoproliferative disorder with high mortality, but animals which survive infection are solidly immune to homologous challenge. Such immunity can be reproduced by infecting cattle with the parasite and treating them with tetracyclines or theilericidal drugs, but the widespread use of this technique as a method of control is hindered by its dependence on live parasites which require cryopreservation for maintenance and can give rise to carrier states. In addition, cross-protection between different strains of the parasite is not absolute. These problems have prompted a search for methods of immunisation based on the use of inactivated parasites or their derivatives. Such efforts have met with consistent failure, however, and in recent years scientists have adopted the more rational approach of defining the immune responses of cattle to the parasite with a view to identifying the parasite components which provoke them. These studies have revealed that protection is likely to be mediated by parasite-specific cytotoxic T cells which are restricted by class I major histocompatibility complex (MHC) products. The significance of this observation to the development of an effective subunit vaccine is discussed in the light of current knowledge of the inductive requirements of cytotoxic T cells.


THE PARASITE AND ITS LIFE CYCLE

Theileria parva is a haemoprotozoan parasite which infects cattle and buffalo in large areas of Eastern, Central and Southern Africa. The sporozoite stage of the parasite is transmitted by the three-host tick Rhipicephalus appendiculatus and invades lymphocytes, where its development to the schizont stage is associated with uncontrolled proliferation of the cell. The capacity of the parasite to associate with the mitotic spindle (78) ensures that schizonts are passed on to successive generations of daughter cells, giving rise to a clonal expansion of the initially parasitised
A proportion of parasitised cells undergo merogony, resulting in disruption of the lymphocyte and release of merozoites into the blood. Merozoites then invade erythrocytes and differentiate into piroplasms which constitute the infective stage for the tick.

### PATHOGENESIS

Infection of cattle with *T. parva* gives rise to an acute lymphoproliferative disorder known as East Coast fever (ECF), and the course of this disease has been well characterised in experimentally-infected animals (47). At approximately 8 days after infection, schizont-infected cells are detectable in the lymph node which drains the site of inoculation. Parasitised cells can be detected in other lymphoid tissues throughout the body 2-3 days later. The degree of parasitosis increases with the progress of infection, and after a further 5-7 days over 30% of lymph node cells can be parasitised. Within 14-21 days of inoculation the majority of experimentally-infected animals die in the absence of treatment. On post-mortem examination, large numbers of parasitised cells are found throughout the lymphoid system. Perhaps of more relevance to the cause of death is the infiltration of other tissues, particularly the lungs and gastro-intestinal tract, with parasitised lymphoblasts, and severe pulmonary oedema and associated dyspnoea are commonly observed late in infection (47). A striking feature of the histological appearance of lymphoid tissues at the time of death is a marked depletion of lymphocytes (48, 51), presumably the result of the extensive lymphocytolysis which can be observed in the later stages of the disease and which is not restricted to parasitised cells (7, 47). It is likely that the invasion of haemopoietic tissues by parasitised cells during the course of the disease also contributes to the depletion of cells in lymphoid organs.

The isolation of lymphocyte subsets using the flow cytometer and subpopulation-specific monoclonal antibodies (MAB) has allowed the identification of the cell types which the parasite is capable of infecting *in vitro*. It is clear that all subsets of lymphocytes including those expressing neither B nor T cell surface antigens, are susceptible to infection *in vitro*, while it is not possible to infect monocytes or granulocytes (4). However, phenotypic studies of parasitised cells isolated from infected cattle suggest that very few arise from B cells, and while substantial numbers of infected CD8+ cells are observed, the majority of parasitised cells appear to be derived from the CD4+ subpopulation of T lymphocytes (30). The considerable effect on the immune system which results from the predilection of the parasite for lymphocytes is of obvious significance to the capacity of the infected animal to respond successfully to infection. Parasitised cells in culture have been observed to produce T cell growth factor (TCGF) activity (15) and are known to stimulate the proliferation of naive lymphocytes *in vitro* (38). Such activity *in vivo* might be expected to result in the proliferation of non-infected lymphocytes, perhaps giving rise to a cascade of lymphokine-mediated effects. In addition, when alloreactive T cell clones are infected with *T. parva*, they have been observed to retain the capacity to kill in an antigen-specific manner for 3-4 months (6). It is tempting to speculate that the polyspecific cytotoxic activity observed by Emery *et al.* (29) in the later stages of infection might be the result of the activation of memory cytotoxic T cells by infection with the parasite.
EXISTING IMMUNISATION STRATEGIES

Animals which recover spontaneously from the disease are solidly immune to homologous challenge, and it has been shown that this protection lasts for up to 3.5 years in the absence of challenge (21). Such immunity can also be induced by treatment of the animal with tetracycline or theilericidal drugs at the time of infection, and immunisation by infection and treatment is practised as a method of control in several countries where the disease is endemic. The prophylactic effect of tetracycline was first described by Neitz (60), and is exerted predominantly during the first 4-5 days of infection (64). Originally requiring several doses, the technique has been refined (67) by the use of a single dose of long-acting formulations of oxytetracycline administered at the time of infection. When administered during the early stages of the clinical reaction, the drug parvaquone has been observed (27) to be equally effective in attenuating infection, but the expense of this drug, coupled with the requirement for two mustering in order to complete the immunisation, renders its use somewhat less practical than that of oxytetracycline.

Observations in the early part of this century that inoculation of lymphoid tissues derived from *T. parva*-infected cattle could protect a proportion of animals from challenge (70, 72) suggested that parasitised cells could be used to immunise against the disease. Subsequent studies confirmed this and demonstrated that $10^8$-$10^9$ infected cells were required for protection (63, 80). More recently, following the development of techniques for the culture of *T. parva*-infected lymphocytes in vitro, attempts were made to immunise cattle with parasitised cells derived from culture (12, 13). In spite of an apparent attenuation of the parasite following multiple passage in culture, these efforts did not generate methods with any large-scale applicability because of the large volumes of culture required for individual immunisations. However, they did provide evidence that the schizont stage of the parasite is capable of inducing protective responses and that this process probably involves the transfer of the parasite from the cells of the inoculum to those of the immunised animal (12, 32).

A consistent feature of successful attempts to immunise cattle against ECF has been the requirement for the use of live parasites. This has led to the belief that establishment of infection is a basic requirement for the induction of protective responses. The infection and treatment method remains the most feasible strategy for the establishment of attenuated infections, but several aspects of this technique limit its practical application for field immunisation. The first is that it requires the use of live parasites derived from infected ticks, and in order to maintain viability these must be stored in liquid nitrogen. More importantly, it is clear that solid immunity to one stock of the parasite does not provide protection against all other stocks (24, 65). However, there is evidence (66) that heterogeneity among stocks is somewhat limited, and cocktails of two or three immunising stocks have been used successfully to protect cattle against experimental challenge with a number of isolates from different locations (66). A further cause for concern is the low-grade carrier state which often results from live immunisation (26, 83). This can persist for many months and may contribute to the long-lasting protection against homologous challenge which has been observed for some stocks. In areas where an immunising stock has not been endemic, such carrier animals might represent a source of new parasite strains for resident tick populations.
These limitations highlight an obvious requirement for an effective method of immunisation which does not depend on the administration of live parasite preparations. The development of such a method is likely to require a rational approach involving the identification of the parasite components which give rise to protective immunity. Efforts in this laboratory have focused on defining the elements of the immune response which mediate protection in immune cattle with a view to identifying the parasite antigens which provoke them.

**IMMUNE RESPONSES AGAINST THE PARASITE**

**Serum antibody responses**

Although the serum of immune cattle contains antibody against all stages of the parasite (20), evidence for a role of antibody responses in recovery and protection of cattle from ECF is somewhat limited. Muhammed et al. (57) failed to protect cattle against challenge by the transfer of immune serum. The observation of Emery (28) that protection against concurrent challenge could be achieved by the transfer of thoracic duct lymphocytes from an immune animal to its naive chimeric twin strengthened the belief that immunity was the result of cellular mechanisms. However, the results of the latter study did not exclude a role for transferred B lymphocytes, and the demonstration by Musoke et al. (58) that serum from immune animals which have been repeatedly challenged with infected ticks neutralises the infectivity of sporozoites for cattle suggests that antibody directed at the sporozoite stage of the parasite can be of relevance to protection. Interestingly, in a subsequent study (59), animals repeatedly challenged with a stock of *T. parva* were shown to be immune to challenge with the homologous parasite and to two other stocks against which animals had not been immune after a single immunisation. This observation suggested that protective antibody responses might cross-protect between stocks, although it is difficult to rule out the possibility that other immune mechanisms were involved in the protection of these animals.

Although antibody responses against the schizont stage of the parasite are detectable in recovering animals around the time of the elimination of infection, and indeed provide the most reliable evidence of exposure to the parasite, these mechanisms are not considered to play a role in protection. This is supported by the observation of Emery (28) that animals inoculated with heat-killed schizont-infected cells or semipurified schizont antigens were not protected against challenge in spite of producing titres of anti-schizont antibodies which were similar to those of conventionally immunised animals.

There are several aspects of immunity against *T. parva* which suggest that immune responses directed at the schizont-infected cell play a major role in protection. These include the apparent requirement for the development of this stage of the parasite for the successful induction of immunity, the common occurrence of a low schizont parasitosis before the elimination of infection by immune animals, and the observation that animals immunised by infection and treatment are solidly immune to challenge with up to $5 \times 10^8$ schizont-infected cells (36, 81). This evidence, coupled with the lack of correlation between the presence of anti-schizont antibody and protection, has led to the belief that immunity to *T. parva* in cattle is mediated predominantly by cellular mechanisms directed at the schizont-infected cell.
Cellular immunity

The elucidation of cellular immune responses of cattle infected with *T. parva* has been greatly facilitated by the development of techniques whereby bovine lymphocytes can be infected with sporozoites *in vitro* (14). Pearson *et al.* (61) demonstrated that bovine peripheral blood mononuclear cells (PBM) proliferated *in vitro* to irradiated autologous parasitised lymphocytes. They also observed that a population of the responding cells killed autologous infected cell lines, and to a lesser extent, parasitised allogeneic cells. These results suggested that infection of the lymphocyte with *T. parva* gave rise to an antigenic change on the cell surface which was responsible for the generation of cell-mediated responses, but the possibility that the responses were directed against non-parasite antigens acquired by the cells during long-term culture could not be excluded. This question was resolved when Emery and Morrison (31) reported that schizont-infected cells prepared from various lymphoid organs of lethally infected cattle stimulated the proliferation of autologous PBM's which had been cryopreserved prior to infection. These initial observations prompted several investigations of *in vivo* cytolytic responses to infection or immunisation. Emery *et al.* (29) studied cytolytic activity during the terminal stages of lethal infection and found that PBM contained cells which killed several allogeneic infected cell lines and a mouse tumour cell line, but not autologous parasitised lymphocytes. These responses were in contrast to those in animals undergoing immunisation or challenge, where cytolytic activity restricted to the autologous infected cell lines was detectable in PBM around the period of remission of infection (29, 36). Subsequently, Emery *et al.* (33) provided evidence that this activity resided in the T lymphocyte population of PBM.

The restriction to autologous cell lines of the cytolytic activity detected in immunised animals was similar to that described for cytotoxic responses of mice and humans to virus infections (74, 85), and suggested that its induction might be the result of an association of parasite determinants with MHC molecules on the cell surface. The availability of serological reagents which define polymorphic determinants on bovine class I MHC antigens allows this question to be addressed. Three international comparison tests have grouped available reagents into over thirty class I specificities (18) which are considered to be encoded by one locus referred to as BoLA-A, although there is now good evidence for the existence of a second bovine class I locus (10, 34, 76). Serological typing for class I in conjunction with a knowledge of parentage allows the identification of animals that are MHC haploidentical or which share individual class I antigens.

In a study which involved the assessment of cytolytic activity in the PBM of ten immune heterozygous animals after challenge with homologous sporozoites, it became clear that activity in individual animals was restricted to infected targets derived from animals which shared MHC class I specificities (55). The observation that a MAB specific for a monomorphic determinant on bovine class I molecules was capable of significantly inhibiting this activity, while two class II-specific MAB had no effect, confirmed that class I MHC products were indeed the restricting elements. Another feature of these results was that in most of the animals the restriction of the response was biased towards one or other of the haplotypes. Indeed, of the BoLA specificities represented in the trial, some were consistently seen to dominate over the others as restricting elements (55). This effect was interpreted as being a reflection of a hierarchy in dominance among the BoLA products which restrict the response. A subsequent study of the phenotype of the cells which mediate this class I MHC-restricted cytolytic activity (41) has demonstrated that they are CD8+ T lymphocytes.
Parasite strain specificity

The appearance of this cytolytic activity around the time of remission of infection in immune animals suggests that it may be of importance in the control of infection, and the failure of some stocks of the parasite to protect against challenge with others prompted some analysis of the parasite strain specificity of this response. These studies have made use of the differences in the specificity of immune responses engendered by challenge with the Muguga and Marikebuni stocks of *T. parva*. Cattle immunised with *T. parva* (Marikebuni) are immune to challenge with both stocks, whereas a proportion of cattle immunised with *T. parva* (Muguga) are susceptible to challenge with the Marikebuni stock (46).

The specificity of the response in five animals immunised with *T. parva* (Muguga) and one animal immunised with *T. parva* (Marikebuni) was examined in detail using autologous targets infected with either parasite (52, 53, 55). Cytotoxic T cells prepared from the animal immunised with the Marikebuni stock were capable of killing targets infected with either parasite. However, although three of the animals immunised with *T. parva* (Muguga) had generated cytotoxic activity directed at both stocks, effector cells prepared from the remaining two were capable of killing targets infected with the homologous stock only. These differences were not the result of the two stocks infecting different cell populations, since cloned T cell lines were prepared from each animal for use as targets, and in some cases these targets were again cloned after infection.

The results of these studies suggested that the heterogeneity among individual animals immunised with *T. parva* (Muguga) in their capacity to withstand challenge with the Marikebuni stock might be the result of differences in the specificities of their T cell responses. Recent experiments involving the challenge of Muguga-immunised animals with the Marikebuni stock after determining their cytotoxic T cell specificities (E. Taracha, B.M. Goddeeris and W.I. Morrison, unpublished observations) have provided further evidence that this might be the case.

However, these investigations did not address the possibility that the capacity of the Marikebuni stock to protect against challenge with *T. parva* (Muguga) was due to the presence of additional parasite strains with antigenic similarities to the Muguga parasite. In order to determine whether the cross-immunising potential of the Marikebuni stock was the result of the generation of cross-reactive T cells or merely a reflection of separate populations of cytotoxic T cells with specificities for different components of the stock, it was necessary to develop techniques whereby the cytotoxic T cells could be cloned (39, 42). By repeated stimulation of PBM prepared from immune cattle with autologous infected lymphoblasts, cultures can be generated which are markedly enriched for parasite-specific cytotoxic cells (42). Phenotypic analysis of these cultures reveals that they are composed almost entirely of CD4+ and CD8+ T lymphocytes, and it can be shown by cell sorting experiments that almost all the cytotoxic activity resides within the CD8+ population. It is possible to obtain T cell clones from these cultures after two to four stimulations *in vitro*. In addition to CD8+ cytotoxic T cell clones, such cloning experiments yield a population of CD4+ non-cytotoxic clones (5). In contrast to the cytotoxic clones, the CD4+ clones can in some instances be stimulated by irradiated parasitised cells in the absence of TCGF, and some have been shown to produce TCGF-like activity in the presence of concanavalin-A. In addition, blocking experiments with MAB specific for bovine class II MHC molecules have shown that the CD4+ clones were restricted by these determinants. These observations have led to the conclusion that these clones are helper T cells (5).
Analysis of the parasite strain specificities of cytotoxic T cell clones derived from animals immunised with the Muguga stock of the parasite revealed that the heterogeneity of the response which was observed between individual cattle was reflected at the clonal level. Thus clones prepared from animals whose \textit{in vivo} response was restricted to the Muguga stock were found to be specific for Muguga-infected target cells (42, 54). Similarly, those animals whose PBM were capable of killing either stock yielded at least some clones which were cross-reactive (B.M. Goddeeris, unpublished observations).

Cytotoxic responses induced by immunisation with the Marikebuni stock of the parasite have been analysed at the clonal level in only one animal, and clones were found to kill target cells infected with either \textit{T. parva} (Muguga) or \textit{T. parva} (Marikebuni) (53). This animal expressed the w6.2 and the KN104 specificities, and clones restricted by either of the two specificities were identified. Because clones restricted by the same specificities were available from animals immunised with the Muguga stock, it was possible to compare the parasite specificities of these clones using the same target cells. It was found that w6.2-restricted clones derived from animals immunised with either stock were cross-reactive. In contrast, although a KN104-restricted clone derived from the Marikebuni-immunised animal killed targets infected with either parasite (40), similarly restricted clones from an animal immunised with \textit{T. parva} (Muguga) were specific for this stock. This finding was interpreted as evidence that the specificity of the cytotoxic T cell response was a reflection of the antigenic nature of the immunising parasite, but it also provided evidence that the MHC phenotype of the animal might influence the selection of parasite specificities.

Such interpretations are complicated, however, by the existence of antigenic (43) and genetic (22) heterogeneity within the Marikebuni stock. Recent observations (B.M. Goddeeris, unpublished data) suggest that some Marikebuni-infected cell lines are killed at low levels by clones derived from non-cross-reactive animals immunised with the Muguga stock. In spite of these complications it seems clear that at least two kinds of epitope are recognised by bovine cytotoxic T cells in the Muguga and Marikebuni stocks. One is present in both parasites while the other is restricted largely to \textit{T. parva} (Muguga), with perhaps low-level expression by a component of the Marikebuni stock. The question remains as to why some Muguga-immunised animals remain susceptible to challenge with the Marikebuni stock in spite of the existence of common antigens or epitopes between the two parasites. A likely contributing factor is the MHC phenotype of the animal; studies of the cytotoxic T cell responses of mice to influenza virus have shown that the MHC phenotypes of various strains of mice influence their ability to respond to defined epitopes on antigenic components of the virus (74, 79). This question has been addressed by examining the MHC restriction and parasite strain specificities of a panel of cytotoxic T cell clones derived from four Muguga-immunised animals (43). This was achieved by an assessment of the ability of clones from each animal to kill MHC-matched or half-matched target cell lines derived by \textit{in vitro}-infection of PBM, or in one case a cloned T cell line, with sporozoites of \textit{T. parva} (Marikebuni). Heterogeneity among these Marikebuni-infected targets was confirmed by western blotting analysis using the MAB B5/2.2.5, which recognises a determinant of variable size on the surface of the schizont stage of the parasite (62, 69). A total of 31 clones was tested and the most striking feature of the results was that clones which shared MHC restriction elements also shared parasite specificities, whereas clones which differed in their MHC restriction also
differed in their parasite specificity, even when derived from the same animal. An additional finding was that the set of clones from each animal showed a clear bias towards one MHC specificity as the restricting element. Indeed, only one animal yielded clones restricted by specificities from both haplotypes.

These results provide evidence that by selecting a particular parasite epitope, the restricting MHC molecules can influence the parasite specificity of the cytotoxic T cell response. It is believed that the antigen receptor of cytotoxic T cells restricted by class I MHC products recognises antigenic peptides in association with the restriction element (75). Moreover, there is evidence from the study of influenza virus-specific cytotoxic T cells in mice and humans that this association may be the result of the processing of endogenously synthesised antigens (50, 73). Crystallographic studies of isolated class I MHC molecules have suggested that a cleft formed between alpha-helical structures in the first and second domains of the molecule constitutes the binding site for processed peptides (11). In the light of these observations, the selection of parasite antigenic epitopes by MHC restricting elements might be a reflection of the affinity of different peptides for the binding site on the MHC molecule. Alternatively, this effect may be the result of selection events in the thymus during the generation of the T cell repertoire which result in differences between animals in the frequencies of T cell precursors with specificities for certain peptide-MHC combinations.

The parasite specificity of helper T cell clones derived from *T. parva*-immune animals has been analysed in only two cattle which had been immunised with the Muguga stock of the parasite (5). These animals showed no cytotoxic T cell responses against the *T. parva* (Marikebuni)-infected targets, and this specificity was reflected in the reactivity of the helper clones. In view of the requirement for TCGF which is characteristic of parasite-specific cytotoxic T cell clones, it might be concluded that parasite-specific helper T cell activity would be a prerequisite for the induction of cytotoxic T cell responses. However, studies in mice have demonstrated that virus-specific cytotoxic T cell responses can be generated in the absence of CD4+ T cells (19), and Heeg et al. (44, 45) have demonstrated the existence in mice of a population of CD8+ T cells which produce IL-2 following antigenic stimulation. Nonetheless, in the absence of similar observations in cattle, a role for helper T cells in recovery and protection of cattle from ECF cannot be discounted.

**THE SEARCH FOR PARASITE ANTIGENS**

A great deal of effort has been focused on the identification of parasite antigens which may constitute targets for protective immune responses in cattle infected with *T. parva*. The observations of Musoke and his colleagues (58, 59) that serum from cattle which had been challenged repeatedly with sporozoites was capable of neutralising sporozoite infectivity in vitro and in vivo, and that this activity was cross-reactive between stocks, prompted a search for sporozoite surface antigens which might mediate this activity. By generating MAB with neutralising activity (25, 59), it has been possible to identify a neutralising antigen on the surface of the sporozoite. This molecule, of relative molecular mass (Mr) 67 kiloDaltons (kD), is specific to the sporoblast and sporozoite stages of the parasite, and appears to be invariant between strains.
In contrast, the use of MAB and serum from recovered cattle in attempts to identify parasite-specific target antigens for T cells on the surface of the schizont-infected cell have met with consistent failure. This is not surprising in view of the current perception of the interaction between the T cell receptor and its ligand. As already mentioned, it is believed that T cells recognise processed rather than native antigen, and there is evidence that synthetic peptides of only 13-16 amino acids are sufficient in length to span T cell epitopes of influenza nucleoprotein (8, 75). Indeed, the antigen binding cleft described by Bjorkman et al. (11) between the first and second domains of the class I MHC molecule can accommodate only 20 amino acids. It is unlikely that peptides of this size can be detected by antibody raised against native antigen.

In the light of these observations, it seems likely that in order to define parasite antigens which constitute the targets of T cell responses in *T. parva*-infected cattle it will be necessary to use the effector cells themselves as screening reagents. In the case of helper T cell responses this does not constitute a major problem, since these cells are believed to recognise processed exogenous antigen in association with class II MHC molecules (77). Thus, antigenic fractions of the parasite may be screened simply by their addition to immune T cells in the presence of autologous antigen presenting cells.

In a study which employed helper T cell clones derived from *T. parva*-immunised animals, it was possible to identify two groups of clones on the basis of their reactivity with antigenic fractions prepared from parasitised cell homogenates (17). The first group requires the presence of autologous antigen presenting cells for proliferation, and responds to purified schizonts, schizont membrane fractions and glutaraldehyde-fixed infected cells. The other clones respond to fixed infected cells alone and, in the presence of autologous antigen presenting cells, to the soluble fraction of infected cell homogenates. In a subsequent analysis (16) this soluble fraction was further fractionated by hydroxylapatite chromatography and gel filtration, and was found to contain three peaks of antigenic activity of approximate Mr 43, 12 and 4.2 kD. More recently (D.J. Grab, W.C. Brown and C.L. Baldwin, unpublished observations), this activity has been resolved to a peptide of Mr 10 kD. Further characterisation of this antigenic peptide is in progress.

Another technique which may be used to fractionate parasite antigens for screening by immune T cells is their separation by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transfer to nitrocellulose membranes. Antigen-bearing membranes can be incorporated in T cell proliferative assays either as whole fragments (84) or as floccules (1), and this method has been used successfully to identify antigens of *Mycobacterium tuberculosis* which stimulate T cell responses (37). Identification of parasite antigens by these techniques might be followed by the production of specific antibodies for screening expression libraries of parasite DNA, with the eventual isolation and characterisation of the parasite gene which encodes the antigenic protein. Alternatively, products of expression libraries could be screened directly by inclusion in cultures of immune T cells with autologous antigen presenting cells.

The identification of parasite antigens which elicit cytotoxic T cell responses in cattle challenged with *T. parva* poses a somewhat greater problem. The processing of antigen for recognition by cytotoxic T cells is understood to require the delivery of the molecule to the cytosol of the target cell either by endogenous synthesis (50, 73) or by artificial means (49). Indeed, recent evidence (23) suggests that the association between processed antigenic peptide and class I MHC molecules occurs in the
endoplasmic reticulum. It therefore seems clear that the identification of cytotoxic T cell target antigens will not be accomplished by screening soluble antigenic fractions of the parasite, but will require the expression of parasite genes within mammalian cells bearing relevant bovine MHC molecules. This could be achieved by incorporating parasite DNA into the genome using conventional transfection techniques. Alternatively, appropriate target cells might be infected with recombinant viruses which incorporate parasite genes. However, *T. parva* is a complex organism with an estimated genome size of $10^7$ base pairs (56) and the schizont stage of the parasite is known to express over 200 proteins (71). Dissecting relevant genes for transfection from such a complex mixture might prove a somewhat arduous task. However, it may be possible to circumvent these problems by the construction of libraries of parasite DNA in eukaryotic expression vectors. Relevant constructs might then be selected on the basis of their capacity to render mammalian cells susceptible to lysis by *T. parva*-specific cytotoxic T cells.

**CONCLUDING REMARKS**

The apparent importance of cytotoxic T cell responses in recovery and protection of cattle from challenge with *T. parva* is of considerable relevance to any effort directed at the immunisation of cattle with non-viable parasites or parasite material. It seems likely that the induction of these responses in cattle in the absence of live challenge will necessitate the use of recombinant antigen delivery systems which are capable of expressing relevant antigens within the cells of the vaccinated animal. Recombinant vaccinia viruses have been widely assessed for use as vaccine vectors in several species and have been shown to give rise to protective cytotoxic T cell responses (9). Indeed, a recent report has shown that recombinant vaccinia viruses incorporating the fusion protein or haemagglutinin genes of rinderpest virus induce serum neutralising antibody titres which protect cattle against challenge with the virus (82). Successful use of herpes viruses and adenoviruses as antigen delivery vehicles has also been reported in some species (35). However, there is some public concern over the widespread use of recombinant viruses as vaccine delivery systems, and although several such vaccines have been assessed in clinical and field trials, none has yet been licensed for general use. Nonetheless, in a recent assessment of a limited field trial in the USA which involved a recombinant vaccinia virus incorporating the rabies virus surface glycoprotein gene, the Animal and Plant Health Inspection Service deemed the vaccine to have no impact on the human environment (2). There is perhaps less anxiety about the use of recombinant bacterial vaccines, and the capacity of attenuated *Salmonella* species to invade and colonise mammalian cells in the absence of clinical disease has prompted an interest in their use as vaccine vectors. Experiments in several disease systems have shown that attenuated *Salmonella* recombinants can induce protective antibody (3) and T cell-mediated responses (68) to foreign antigens. It is possible that these vectors might be usefully deployed as antigen delivery vehicles for *T. parva* antigens.

The apparent lack of involvement of sporozoite surface antigens in recovery and protection of cattle from challenge with *T. parva* does not necessarily preclude their inclusion among candidate vaccine antigens. The demonstration of Musoke et al. (58) that repeated exposure of cattle to tick challenge results in high titres of serum antibody
capable of neutralising sporozoite infectivity in vivo suggests that it might be prudent to consider the inclusion of antigens from the surface of the sporozoite in a subunit vaccine against ECF. The generation of high titres of antibody is believed to require the presence of helper T cell epitopes on the immunising antigen, and recent observations in cattle (H. Pereira, unpublished observations) suggest that this is indeed the case with the 67 kD antigen of T. parva sporozoites. Given an appropriate antigen delivery system, it may be possible to generate high titres of serum antibody in cattle to this neutralising antigen. Since it is likely that this response would be boosted by natural challenge in the field, antibody levels sufficient to contribute to protection might be maintained. The characterisation of immune responses in cattle immunised with purified and recombinant 67 kD antigen is in progress.

**THEILERIA PARVA**: NATURE DE LA RÉPONSE IMMUNITAIRE ET CONSÉQUENCES POUR L'IMMUNOPROPHYLAXIE. – D.J. McKeever et W.I. Morrison.

Résumé: Theileria parva est un hématozoaire transmis par les tiques ; les pertes économiques qu'il entraîne pour l'élevage des bovins et des buffles, en Afrique orientale, centrale et méridionale, sont considérables. La maladie due à ce parasite se traduit par de graves troubles lymphoprolifératifs et entraîne une mortalité élevée. Cependant, les animaux survivants présentent une bonne protection à l'égard d'une nouvelle infection par une souche homologue. Cette immunité peut être reproduite chez les bovins par inoculation du parasite, associée à l'administration de tétracyclines ou de produits antitheilériens spécifiques. Cette méthode ne peut pas, cependant, être utilisée pour une prophylaxie généralisée car elle requiert des parasites vivants, conservés par congélation, qui peuvent générer des porteurs. En outre, la protection croisée entre les différentes souches de parasite n'est pas absolue. Ces problèmes ont motivé des recherches sur d'autres méthodes d'immunisation utilisant des parasites inactivés ou leurs dérivés, qui n'ont pas abouti. Aussi, ces dernières années, les chercheurs ont-ils adopté une approche plus rationnelle consistant à analyser la réponse immunitaire des bovins, afin d'identifier les éléments du parasite qui la provoquent. Ces études ont montré que l'immunité résultait probablement des cellules T, qui sont spécifiquement cytotoxiques pour le parasite, et dont l'action est limitée par les produits du complexe majeur d'histocompatibilité (CMH) de classe I. Les conséquences de ces observations sur la mise au point d'un vaccin sous-unitaire sont discutées à la lumière des connaissances actuelles sur les conditions d'action des cellules T cytotoxiques.


**THEILERIA PARVA**: NATURALEZA DE LA RESPUESTA INMUNITARIA Y CONSECUENCIAS PARA LA INMUNOPROFILAXIS. – D.J. McKeever y W.I. Morrison.

Resumen: Theileria parva es un hematozoario transmitido por las garrapatas responsable de pérdidas económicas considerables en la explotación de bovinos
y búfalos en África oriental, central y meridional. La enfermedad que provoca se manifiesta por trastornos agudos linfoproliferativos, con alto índice de mortalidad; pero los animales sobrevivientes cuentan con una buena protección respecto de nuevas infecciones provenientes de cepas homólogas. Tal inmunidad puede reproducirse en los bovinos mediante la inoculación del parásito asociada a la administración de tetraciclínas o de compuestos específicos, pero no puede seguirse este método para una profilaxis generalizada porque requiere parásitos vivos, conservados por congelación, que pueden generar portadores. Por otra parte, la protección cruzada entre diferentes cepas de parásitos no es absoluta. Se trató en consecuencia de encontrar otros métodos de profilaxis, utilizando parásitos inactivados o sus derivados, pero sin resultado. Más recientemente, los investigadores adoptaron la perspectiva más racional de estudiar la respuesta inmunitaria de los bovinos para tratar de identificar qué elementos del parásito la provocan. Estos estudios mostraron que la inmunidad se debía probablemente a células T, específicamente citotóxicas para el parásito y cuya acción limita los productos del complejo mayor de histocompatibilidad (CMH) de clase I. Las consecuencias de estas observaciones en la preparación de una vacuna subunitaria se discuten a la luz de los conocimientos actuales acerca de las células T citotóxicas.


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