Progress towards a vaccine against *Theileria parva* : relevance for heartwater research

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Beaucoup de progrès ont été enregistrés durant les 10 dernières années en ce qui concerne la caractérisation de l'immunité bovine contre *Theileria parva*. Il n'est plus à démontrer que les bovins devenus immunisés après infection peuvent se débarrasser d'infections ultérieures par le déploiement de lymphocytes T cytotoxiques (LTC) spécifiques pour le parasite. De plus, des anticorps neutralisants sont produits à des titres élevés contre la surface du sporozoïte après des infections multiples par le parasite, et peuvent neutraliser l'infection *in vitro*. Bien que cela ne soit vraisemblablement pas significatif dans les circonstances naturelles, on a tiré profit de cette dernière observation pour créer un candidat prometteur pour un vaccin neutralisant basé sur une forme recombinante de l'antigène de surface majeur des sporozoïtes de *T. parva*. On essaie actuellement d'identifier le(s) antigène(s) cible(s) des LTC spécifiques pour *T. parva*, et après réussite un vaccin amélioré visant aussi bien les stades infectieux que pathogènes sera à portée. L'élucidation de la base de l'immunité des ruminants contre *Cowdria ruminantium*, agent causal de la cowdriose, est encore à un stade relativement peu avancé. Néanmoins, on applique déjà à la cowdriose plusieurs des principes et des techniques ayant mené à la compréhension de l'immunologie de *T. parva*, et cela devrait permettre un progrès rapide dans le développement d'un vaccin contre *C. ruminantium*.

Mots clés : Bovin - Cowdriose - *Cowdria ruminantium* - Vaccin - Tique - *Theileria parva* - Lymphocyte - Anticorps - Antigène - Infection expérimentale - Immunité.

INTRODUCTION

Despite the current trend in many Western nations towards the consumption of plant rather than animal proteins, livestock remain a major source of nutritional protein in many Third World countries. The tick-borne diseases of cattle and small ruminants, which include theileriosis, cowdriosis, anaplasmosis and babesiosis, are probably the greatest disease constraint to the improvement of livestock productivity in these countries. The most significant among these diseases on the African continent, in terms of economic losses and restriction of livestock development, are East Coast fever (ECF) and heartwater. Both of these conditions are most severe in animals introduced to endemic locations from areas that are free of the disease.

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ECF is a lymphoproliferative disease of cattle caused by the protozoan parasite *Theileria parva,* which is characterized by high mortality in naive animals. The disease is distributed over large areas of eastern, central and southern Africa, and its principal vector is the brown ear tick *Rhipicephalus appendiculatus.* Heartwater is endemic in a much larger area, occurring throughout most of sub-Saharan Africa, and is also present on some islands to which the major vector *Amblyomma variegatum* has spread (29). Its economic significance is intensified by the fact that sheep and goats are affected as well as cattle. Because young cattle possess an innate resistance to the causal agent *Cowdria ruminantium* (26), heartwater is rarely observed in indigenous livestock in endemic areas, and presents as a problem chiefly in susceptible animals that have been moved to areas where the agent is present.

Control of both diseases is largely dependent on the use of acaricide-based tick control strategies and immunisation by infection and treatment, the administration of live organisms along with chemotherapeutic agents (3, 31). However, the expense of acaricides coupled with the numerous disadvantages associated with infection and treatment have prompted a search for improved vaccines against these diseases.

Immune responses of cattle to *C. ruminantium* are not well understood (34,36). The agent survives intracellularly within vascular endothelial cells, and the pathogenesis of the disease that it causes is believed to be the result of increased capillary permeability (8, 10, 27, 30). After inoculation with *C. ruminantium,* antibody responses are detected in cattle at the height of the febrile response (32), and these are probably generated in response to organisms released following rupture of infected endothelial cells. However, experiments involving the transfer of immune serum or purified g-lobulins have yielded no evidence that antibodies influence the course of infection (1, 9), and there is no apparent correlation between antibody titre as measured by indirect immunofluorescence antibody tests and the immune status of the animal (11). These observations, together with the intracellular location of the agent, have led to the belief that cell-mediated immune mechanisms are required for protection of immune animals from rechallenge.

The life cycle of *T. parva is* more complex than that of *C. ruminantium,* with the parasite progressing through schizont, merozoite and piroplasm stages in the mammalian host. After inoculation by the tick, sporozoites rapidly

invade lymphocytes and differentiate to schizonts (17, 33). This is the pathogenic stage of the parasite, and is associated with uncontrolled proliferation of infected lymphocytes. This feature of the disease is responsible for the majority of its clinical signs (18). Bovine immune responses to *T. parva* are well understood, and much of the available information is derived from studies in animals immunised by infection and treatment. This paper will attempt to summarize the work that has led to the current understanding of bovine immunity to *T. parva,* and to highlight those aspects of it that may be of relevance to heartwater.

IMMUNITY TO *THEILERIA PARVA*

Although serum antibodies against all stages of *T. parva* can be observed in immune cattle, a number of observations indicate that they are not important for the elimination of challenge infections. As observed for heartwater, the transfer of immune serum does not influence the course of infection in naive cattle (22, 35). Several aspects of the immunity seen in cattle after infection and treatment immunisation with *T. parva* suggest that protective mechanisms are directed at the schizont-infected cell. These include an apparent requirement for the development of this stage of the parasite for the generation of effective immunity, the common occurrence of a low schizont parasitosis in immune animals before the clearance of infection, and the observation that cattle immunized by infection and treatment resist challenge with up to $5x10^8$ schizont-infected cells (16, 37). There are no indications that these mechanisms are antibody-dependent. EMERY (13) demonstrated that in spite of generating schizont-specific antibody titres equivalent in magnitude to those produced by conventionally immunized animals, cattle immunised with heat-killed schizont-infected cells or partially purified schizont antigens were not protected against challenge. These observations gave rise to the belief that immunity against *T. parva* was cell-mediated. This was supported by the observation (13) that immunity could be transferred in the cellular fraction of immune thoracic duct lymph, although this fraction did contain B lymphocytes.

Characterization of cellular immune responses against *T. parva* were greatly facilitated by successful infection of bovine lymphocytes with the parasite *in vitro* (5). This allowed PEARSON *et al.* (28) to establish that bovine lymphocytes proliferated in the presence of autologous infected cell lines, and in addition, that lymphocytes derived from immune but not naive cattle developed cytolytic activity in these cultures. A subsequent analysis of the nature of this cytolytic activity confirmed that it was directed at parasite antigens rather than those induced by culture conditions (15). These developments clearly implicated parasite-specific cytotoxic cells in immunity to ECF, and prompted a number of studies of *in vivo* cytolytic responses to infection or immunization. EMERY *et al.* (14) demonstrated that naive cattle generated cytolytic activity only during the terminal stages of lethal infection and that it was not restricted to autologous parasitised lymphocytes, killing in addition allogeneic infected cells and mouse tumour cells. Conversely, in immune cattle undergoing challenge, killing activity was observed around the time of remission of infection, and this was restricted to autologous infected cells. This restriction, coupled with the subsequent localization of the activity to the T cell population of blood lymphocytes, provided a strong indication that parasite-specific killing activity in *T. parva* immune cattle was mediated by class I MHC-restricted cytotoxic T lymphocytes (CTL).

Confirmation of this hypothesis was made possible by the availability of serological reagents for the typing of bovine class I MHC antigens. Three international workshops have grouped these reagents into over thirty specificities, the majority of which are believed to be encoded by one locus, known as BoLA-A (6). The use of these reagents in conjunction with a knowledge of parentage allows the identification of cattle that are MHC haplo-identical or that share individual class I MHC antigens.

In a study of parasite-specific CTL activity in 10 immune cattle that were heterozygous at the BoLA-A locus, it became clear that killing was only observed when target cells shared at least one class I MHC antigen with the donor animal (21). The abrogation of killing by the addition of class I MHC-specific mAbs confirmed that these molecules were indeed the restricting elements. In a later study, it was established that *T. parva*-specific cytolytic activity resides in the $CD8^+$ T cell fraction of bovine lymphocytes. Kinetic analysis of CTL activity in the blood of immune cattle under challenge has shown that peak activity is associated with the disappearance of schizonts from peripheral blood lymphocytes (21), providing further evidence that CTL play a role in protection.

LYMPHATIC CANNULATION

A major advantage held by ruminant immunologists is the capacity to cannulate lymphatics and collect lymph fluid over long periods. It is accepted that immune responses are initiated in the lymph node that drains the site of antigen entry, and the activity within these nodes is reflected in phenotypic and functional changes in efferent lymph cell populations. These systems have been exploited in the study of bovine immune responses to *T. parva*. EMERY (12) examined the kinetics of infection in lymph of naive calves after lethal challenge. He observed a dramatic 7-8 fold increase in cell output that peaked 3-4 days after infection. This was accompanied by an increase in the proportion of blasting cells. Parasitised cells were first detected 8 days after challenge and 60-65 % of lymph cells were parasitised by day 14. Functional parameters

were not examined in these experiments, although it was known that parasite-specific cytolytic activity is not a feature of primary infections of cattle with *T. parva* (14). More recently, we have studied CTL activity in the lymph of immune cattle under challenge with the parasite (McKEEVER, TARACHA, INNES, MacHUGH, AWINO, GODDERIS and MORRISON, submitted). In kinetic studies, we have observed that CTL activity is more marked and peaks one day earlier in lymph than in blood. Furthermore, by limiting dilution analysis of the frequency of CTL precursors, we have established that at the peak of the nodal response to challenge, as many as 1:32 of efferent lymph lymphocytes are parasite-specific CTL. This is up to 25 times the frequency observed in PBM at the same time.

The large numbers of *T. parva*-specific CTL present in responding lymph highlighted the possibility of evaluating their capacity to clear challenge infections *in vivo.* By complement lysis of CD8- lineages in responding lymph, it was possible to prepare up to 7.5 x 10^9 $CD8^+$ T cells from an overnight collection of the fluid. In this way, large numbers of CTL were transferred between immune and naive identical twin calves, such that peak CTL activity in the donor coincided with the emergence of a lethal schizont parasitosis in the recipient. In two such experiments the recipient cleared the challenge after transfer, while challenge control animals developed lethal infections. These observations provide conclusive evidence that *T. parva*-specific CTL, the major cellular effector population in immune cattle, can clear the parasite after challenge.

In the search for an improved vaccine against *T. parva,* considerable effort is now focused on the identification of the parasite components that provoke specific CTL responses. These studies are based on the use of *T. parva*-specific CTL clones and appropriate target cells sensitised either by incubation with peptide fractions of parasitised cells or by the expression of parasite genes from a variety of vector systems.

ALTERNATIVE APPROACHES

In spite of the evidence outlined above for a major role for CTL in recovery and protection of immune cattle from *T. parva,* it should be remembered that immune responses that are protective in the field need not necessarily dictate the nature of an effective vaccine. As mentioned earlier, there is considerable evidence that serological responses against *T. parva* do not play a significant role in protection. However, high titres of antibody against the sporozoite surface are present in sera from cattle in endemic areas or those repeatedly exposed to infected ticks under laboratory conditions (24). These sera neutralise the infectivity of sporozoites *in vitro* and *in vivo.* Their dominant target specificity is a 67 kDa antigen (p67) on the sporozoite surface, and monoclonal antibodies raised against this antigen also effectively neutralise infectivity (25). These observations suggested that immunization with this antigen might give rise to a protective immune response directed at the sporozoite stage of the parasite. The gene that encodes the antigen was cloned and expressed in *Escherichia coli* as a fusion protein with the NS 1 antigen of influenza virus (23), which is a powerful inducer of helper T cell responses (2). The recombinant product has now been used in immunization trials in cattle, and results have been extremely promising; in initial experiments 13 of 21 immunized animals were protected against a measured challenge (23). Further development of this product as a first generation improved vaccine against T. *parva is* underway. These results emphasise the value of exploiting recombinant technology and antigen delivery systems to generate protective responses against antigens that may not be protective under natural circumstances.

RELEVANCE FOR FUTURE STUDIES IN HEARTWATER

Many of the systems and techniques that have led to the current understanding of bovine immunity to *T. parva* are applicable to the development of improved vaccines against heartwater. Major advances have been made in recent years in the molecular and antigenic characterization of *C. ruminantium,* and in the development of *in vitro* techniques for its culture. An immunodominant surface antigen has been described that is serologically conserved among isolates of the agent (19, 20), and this represents a promising candidate for a neutralising vaccine. Protective immunity in the field is likely to be based on cellular mechanisms, and the capacity to infect autologous endothelial cells with the agent (4, 7) has set the stage for a rapid evaluation of the role of cell-mediated responses in immunity of ruminant species to heartwater. If these responses are implicated in protection, the important task will be the identification of the antigens involved in their induction. Because *C. ruminantium is* a less complex organism it is possible that this task will prove less arduous than that of identifying relevent antigens of *T.parva,* and the prospect of an improved vaccine for heartwater may be somewhat less that remote.

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Such progress has been made in the last decade in the characterization of bovine immunity to *Theileria parva*. The evidence is overwhelming that cattle that become immune through infection can clear subsequent challenge infections by deploying parasite-specific cytotoxic T lymphocytes (CTL). Furthermore, high titres of neutralising antibodies are generated against the sporozoite surface after multiple exposure to the parasite, and these can neutralise infection *in vitro*. Although unlikely to be of relevance under natural circumstances, the latter observation has been exploited to generate a promising candidate neutralising vaccine based on a recombinant form of the major surface antigen of the *T. parva* sporozoites. Efforts are under way to identify the target antigen(s) of *T. parva*-specific CTL, and when this has been achieved, an improved vaccine targeted on both infective and pathogenic stages of the parasite will be within reach. The elucidation of the basis of immunity of ruminants to *Cowdria ruminantium*, the causal agent of heartwater, is at a comparatively early stage. However, many of the principles and techniques that have led to the current understanding of the immunology of *T. parva* are already being applied to heartwater, and these should enable rapid progress to be made in the development of a vaccine against *C. ruminantium*.

Key words : Cattle - Heartwater - *Cowdria ruminantium* - Vaccine - Tick - *Theileria parva* - Lymphocyte - Antibody - Antigen - Experimental infection - Protection.

McKEEVER (D.J.). Avance de la vacunación contra *Theileria parva* : importancia para la investigación de la cowdriosis. *Revue Élev. Méd. vét. Pays trop.*, 1993, **46** (1-2) : 231-235

En la última década han habido importantes progresos en la caracterización de la inmunidad bovina contra *Theileria parva*. Parece evidente que los bovinos inmunizados mediante infección, pueden resistir a infecciones futuras, gracias a linfocitos T citotóxicos específicos para el parásito (CTL). Aún más, después de una exposición múltiple al parásito, se generan títulos altos de anticuerpos neutralizantes contra la superficie del esporozoito, lo que permite la neutralización *in vitro* de la infección. Aunque la importancia de lo anterior bajo condiciones naturales es dudosa, parece prometedora para la fabricación de una vacuna neutralizante, basada en una forma recombinante del antígeno mayor de superficie de los esporozoitos de *T. parva*. Actualmente se realizan esfuerzos para determinar el o los antígenos clave CTL-específicos para *T. parva*, lo que una vez adquirido, permitirá la realización de una vacuna específica tanto para el estadio infectivo, como patogénico del parásito. La comprension de las bases de la inmunidad contra *Cowdria ruminantium* (agente causal de la cowdriosis o enfermedad de "heartwater") en rumiantes, se encuentra en un estadio análogo. Sin embargo, muchos de los principios y de las técnicas que han conducido a la comprensión de la inmunología de *T. parva*, se han aplicado a la cowdriosis y deberían permitir un progreso rápido en el desarrollo de una vacuna contra *C. ruminantium*.

Palabras claves : Bovino - Cowdriosis - *Cowdria ruminantium* - Vacuna - Garrapata - *Theileria parva* - Linfocito - Anticuerpo - Antígeno - Infección experimental - Inmunidad.