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Tropical theileriosis: Cytotoxic T lymphocyte response to vaccination

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ABSTRACT

Cattle which survive an infection with *Theileria annulata* become effectively immune to challenge with the same parasite strain, and are thought to be protected against a heterologous strain of the parasite. T-cells play a crucial role in both induction and maintenance of immunity to *T. annulata*. The generation of cytotoxic T lymphocytes (CTL) is closely related to the control of the infection – macroschizont-infected cells are killed in an MHC class I restricted manner. Any strain-specificity induced by immunisation is likely to be manifested by CTL. Besides CTLs, CD4+ T-cells also play an important role in protective immunity to *T. annulata* infection.

1. Introduction

Tick-borne diseases (TBDs) of livestock are responsible for hundreds of millions of dollars loss per year in tropical and temperate areas where they pose a problem. Global loss was estimated at US$ 7 billion a year up to 13.9 and US$ 18.7 billion per annum [1,2]. Theileriosis, caused by *Theileria* parasites, is among the major tick-borne diseases, and economic losses are attributed in particular to those caused by the leukoproliferative *Theileria*, i.e. *T. parva*, *T. annulata* and *T. lestoquardi*. Generally, the life cycle of *Theileria* involves both the transmitting invertebrate tick vector, in which sexual reproduction and sporogony takes place, and the asexual cycle in the vertebrate host, including schizogony and merogony [3–5]. After being inoculated by the tick, *Theileria* sporozoites invade host mononuclear cells (PBMC) [6,7] where they differentiate to the schizont/macroschizont stage which induces transformation and proliferation of the host cell [8]. The subsequent microschizont stage differentiates to merozoites, which are released upon host cell rupture, becoming free to penetrate into erythrocytes where the parasite develops into the piroplasm stage, which is infective to ticks [4].

The protozoan parasite *T. annulata* is the causative agent of the tick-borne disease tropical theileriosis in cattle, causing morbidity and loss of productivity in indigenous cattle and severe and often lethal disease in imported high-grade cattle and cross breeds in a wide geographic distribution ranging from the Mediterranean littoral regions of Europe and Africa to the Near and Middle East to India and China in Asia [9,10]. The disease acts as a major constraint to livestock production and improvement. This is of particular relevance when naive exotic cattle such as Holstein are used to improve the productivity, since these animals are extremely susceptible to this infection.

2. Control of tropical theileriosis

Tropical theileriosis can be contained by targeting the tick vector, by treatment of infected animals and through immunoprophylaxis.

2.1. Vector control

Currently, management of theileriosis and other TBDs is primarily through control of the tick vector using acaricides, which have been applied for tick erradication programmes [11]. The disadvantages of chemical tick control are that acaricides are toxic, raising food safety concerns as they leave residues in meat and milk products and causing environmental pollution [12]. Moreover, increasing acaricide resistance of ticks has been reported [13]. Taken together, acaricides for control of theileriosis and other TBDs is judged as being unsustainable [14].

2.2. Therapy

Several drugs have been used for control of tropical theileriosis. Halofuginone used as a daily drench on day 8, 9 and 10 after infection shows effective results for treatment of *T. annulata* infection [15], but because of a very small margin between therapeutic and toxic dose it is practically limited. Parvaquone and buparvaqune are effective against the schizont stage [16] whereby parvaquone is not able to eliminate the parasites completely. The recovery rate of ani-

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mals treated with a single dose of buparvaquone is approximately 80%, which increases to 100% with a second dose in severe cases under field condition [15,17]. The major disadvantage of treatment regimes is the cost.

2.3. Vaccination

Mainly two methods have been used to immunize against tropical theileriosis. The infection and treatment method is based on inoculating naive animals with tick stabilate and simultaneous treatment with long acting oxytetracycline [18,19]. Under experimental conditions, the treated animals show mild reactions and develop a solid immunity against homologous challenge and are partially protected against heterologous infection.

A further immunization technique is based on the use of attenuated schizont vaccines. It is possible to attenuate *T. annulata* schizonts by prolonged in vitro culture of infected cell lines. Such vaccines are considered as attenuated, when their inoculation into susceptible cattle does not result in sever clinical symptoms. Cell culture vaccines have been successfully used in a number of countries like Israel [20], Iran [21,22], India [23], Russia [24], Morocco [25], Turkey [26], Spain [27], Tunisia [28] and China [29]. The duration of immunity varies between 6 months [30] and lasts as long as 3.5 years [31].

3. Clinical symptoms in immunized animals

In previous studies [32], it has been shown that immunized animals showed mild symptoms of the disease characterized by the swelling of regional lymph nodes and by the appearance of schizonts in lymph nodes (1–2%). In average, the body temperature rose to 40 °C and piroplasms were detected in less than 1% of erythrocytes between days 8 and 11 post immunization. While these immunized animals were protected against challenge with sporozoites of the same parasite stock, naive control animals developed severe symptoms of theileriosis. Body temperature of control animals rose in average to 41.7 °C, more than 60% of the cells of draining lymph nodes were infected with schizonts and a 35% parasitaemia was recorded. To avoid mortality, all control animals were treated with buparvaquone.

Regarding clinical symptoms using allogenic cell lines, the majority of attenuated vaccines used in endemic countries induce short febrile episodes, and vaccination of naive calves results in varying levels of reactions ranging from the absence of fever, schizonts and piroplasms (reviewed in ref. [28]).

4. The immune response to vaccination

Under experimental conditions, it is possible to immunize cattle with 10^2–10^5 autologous cells. In contrast, more heterologous infected cells (10^6–10^7) are required to induce a comparable immunity. In the case of the autologous system, the cells can expand rapidly without being rejected by graft versus host reaction, whereas in the allogenic system the schizonts must first be transferred into the cells of the recipient animal in order to be recognized by T-cells involved in the protection against the infection. This process is an essential prerequiste for the induction of an effective protective immunity.

It is well established that cattle which survive an infection with or are immunized against *T. annulata* develop a solid immunity against the same and to a certain degree against a heterologous parasite strain [33]. Both antibody-dependent and antibody-independent mechanisms are involved in the protection against tropical theileriosis. Antibodies to all stages are detectable at a later phase of the infection and thus at a time when the infection has been controlled. No antibody activity has been detected against the surface of parasitized leukocytes or erythrocytes [34]. However, antibodies are able to neutralize the infectivity of sporozoites and consequently reduce the pressure of infection, allowing the host to develop a T-cell mediated immune response, which plays a crucial role in induction and maintenance of immunity. Based on the cytokine profile and lytic effect of the T-cells, it seems that both CD4+ and CD8+ T-cells are involved in the mediation of immunity [35]. Cytotoxic- and helper-T-cells recognize parasite-antigens, which are presented by the infected cells via MHC I and MHC II, respectively. In vitro, it has been shown that both helper- and cytotoxic-T-cells are responding to the infected cells: helper T-cells proliferate and produce interleukin 2 (IL-2) and interferon γ (IFN-γ) [36]; IL-2 is consumed by the cytotoxic T-cells for their clonal expansion and subsequent killing of their target cells in an MHC class I restricted manner. To date, CD8+ T- (cytotoxic T lymphocytes, CTL) cells are known to be the major anti-Theileria effectors and are activated in both *T. annulata* and *T. parva* infections [37–40].

Taken together, in both cases – immunization using allogenic or autologous infected cell lines – an immune response is mounted against parasite infected cells, however, in the case of immunization with allogenic cell lines, part of this immune response is not protective because the specific CTL response requires the recognition of parasite peptides in the context of MHC class I presentation. Therefore, for investigations on the mechanisms of host immune responses and protection to infection, the autologous experimental system is preferable. In the following, the role of T-cells in the host immune response to immunization with autologous and heterologous *T. annulata*-infected cells is reviewed.

5. The CTL response of immunized cattle to challenge

The CTL response of immunized and control animals in the autologous system was checked by testing the cytotoxic effects of PBMC from these animals. It is interesting to observe that no CTL responses were detected in the PBMC of primary immunized cattle [32]. Similar results were recorded when cattle were immunized by inoculating them with sporozoites of *T. annulata* instead of attenuated culture vaccines [35].

In contrast, in immunized cattle, CTL activities were demonstrated between days 10 and 13 post challenge with maximum lysis of MHC-matched macroschizont infected cells on day 12. Allogeneic target cells infected with the same parasite strain were not lysed [32], indicating that killing is MHC-restricted (Fig. 1). Killing of target cells was not observed when PBMC from naive cattle were used as effector cells.

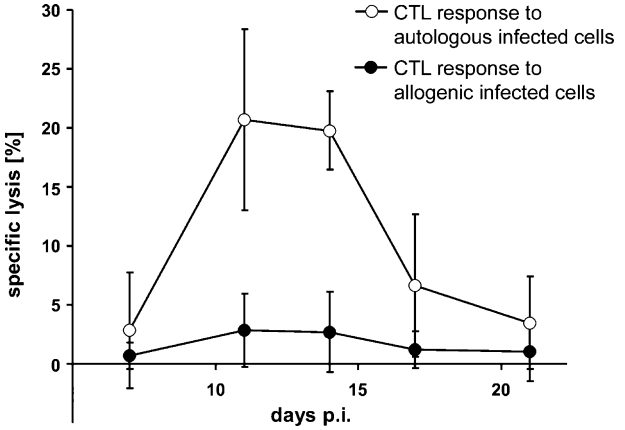

Fig. 1. MHC-restricted lysis of *T. annulata* infected cells. Autologous infected cells are lysed by PBMC isolated from vaccinated and challenged animals (○), whereas allogenic infected cells are not lysed (●).