ORIGINAL PAPER

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Review: the cellular basis of the immunity to and immunopathogenesis of tropical theileriosis

Abstract The intracellular protozoan parasite *Theileria annulata* causes a severe and often fatal disease of pure and crossbred cattle in tropical and subtropical countries. Animals that recover from the infection are immune against challenge with homologous parasite strains. In the present review we refer to the role of immunocompetent cells and their products in containing the infection or in facilitating the progress of the disease. Parasite-infected host cells produce cytokines, which, depending on their concentration and timing of production, may enhance the establishment of the infection. Thus, cell lines producing high levels of proinflammatory cytokines cause severe postvaccinal reactions when inoculated into cattle. This may be supported by an aberrant non-specific activation of naive T-cells, leading to the production of high levels of gamma-interferon (IFN-γ). Under these circumstances development of the specific immune response may be inhibited. At this stage, innate immune reactions are operating to contain the infection. Natural killer cells and macrophages may represent the most important part of this immunity. Antibodies and specific T-lymphocytes, CD4$^+$ T-cells and cytotoxic T-lymphocytes (CTLs), play the most important role in a challenge infection. In this context, CD4$^+$ T-cells produce cytokines required for the clonal expansion of CTLs that kill their target cells in a major histocompatibility complex (MHC) class I-restricted manner. In addition, CD4$^+$ T-cells produce macrophage-activating cytokines such as IFN-γ. Such activated macrophages produce mediators such as NO, which destroy the intracellular schizonts.

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Attempts have been directed toward the identification of parasite antigens involved in the induction of immunity. To date, only a limited number of sporozoite and merozoite antigens have been identified and examined for their immunogenicity, and the protection achieved is partial. An effective vaccine must include schizont proteins, notably, those proteins that are secreted into the host cell cytoplasm because these may have access to the MHC class I and II compartments to be presented to CTLs and CD4$^+$ T-cells, respectively. Several schizont proteins have been identified and these are now under investigation.

Introduction

Theileria annulata, the causative agent of tropical theileriosis or Mediterranean Coast fever, is a tick-borne protozoan parasite. Tropical theileriosis is endemic in a geographic belt extending from North Africa through the Middle East, from the southern countries of the former Soviet Union to India and China, and has been detected in several South European countries. After being inoculated by ticks, the sporozoites of *T. annulata* invade host leukocytes (Fig. 1), where they differentiate to macroschizonts (Schein et al. 1978; Mehlhorn and Schein 1984; Mehlhorn et al. 1988; Mehlhorn et al. 1994). Cattle that recover from a primary infection are strongly immune when challenged with a homologous parasite stock. Immunity against one stock may offer protection against some heterologous stocks (Pipano 1981; Hashemi-Feshkari 1988). The immunological response of the host is directed against all parasite stages: sporozoites, macroschizonts, and merozoites (Figs. 2, 3). Specific antibodies neutralize the sporozoites or enhance their phagocytosis. However, there is increasing evidence for a crucial role of cell-mediated immunity in containment of the infection. Initial studies have shown that remission of the infection is associated with activation of T-cells. In East Coast fever (ECF), which is caused by a closely related parasite (*T. parva*),
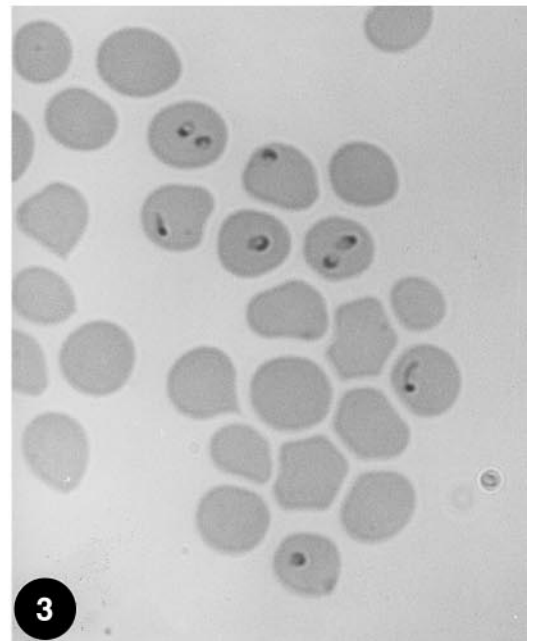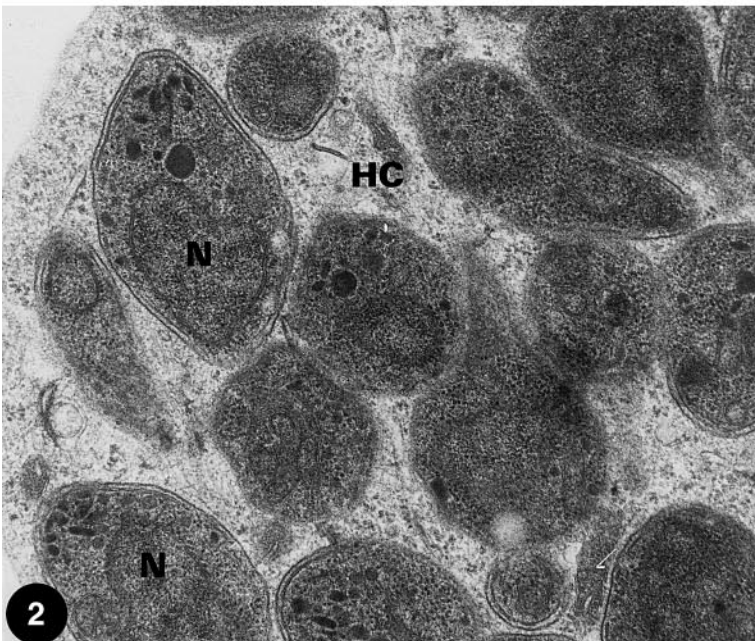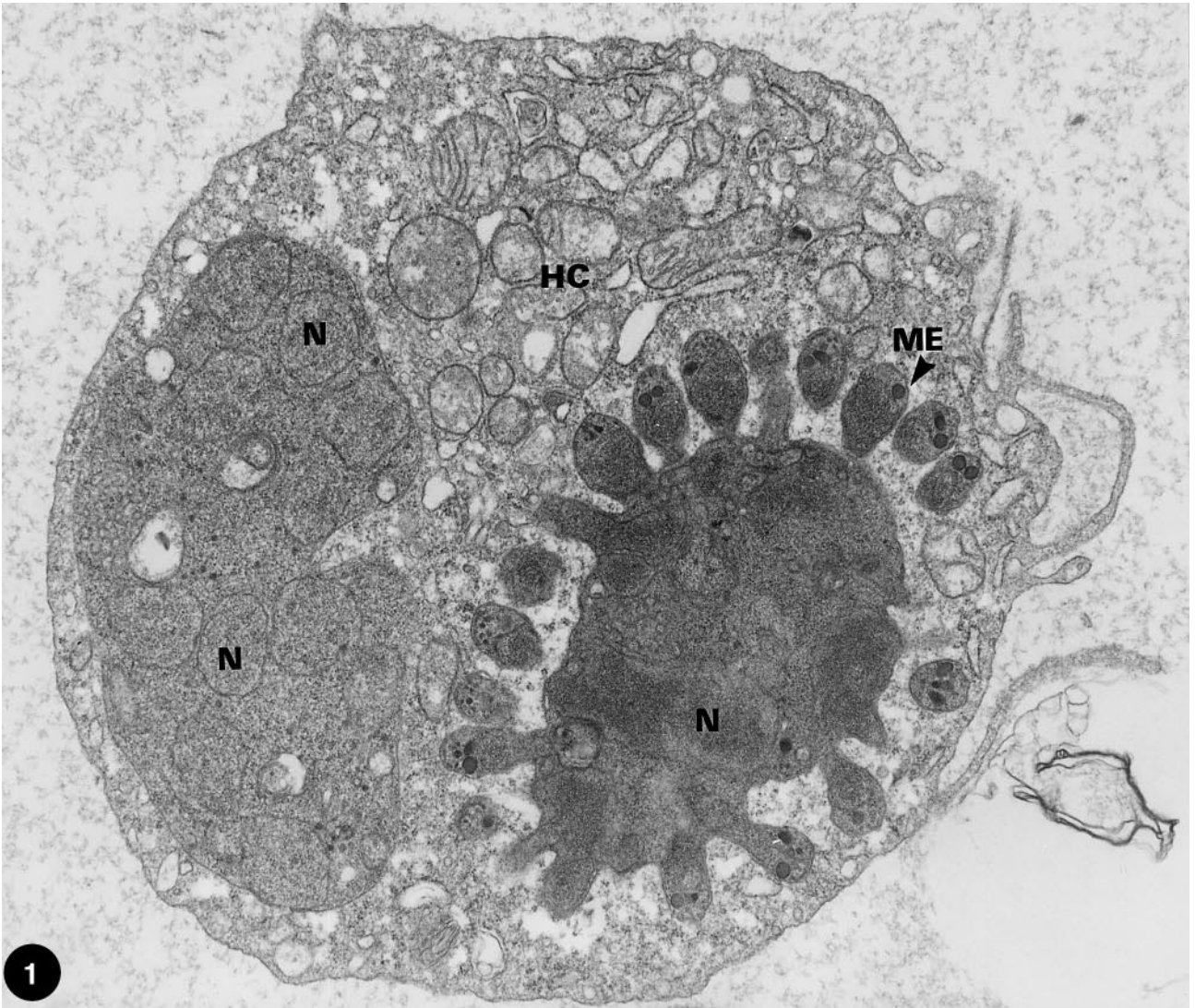
◄
Fig. 1 Transmission electron micrograph (TEM) of a section through a bovine leucocytes containing a macroschizont (*left*) and a microschizont (*right*) of *Theileria annulata*. The latter has begun producing merozoites; both stages are situated immediately in the host cell cytoplasm. (*HC* Host cell, *ME* merozoite, *N* nucleus). ×8,000
Fig. 2 TEM of mature merozoites of *T. annulata* within a bovine leucocyte. Note that the apical pole of the merozoites contains no conoid but displays all other typical organelles such as rhoptries, micronemes, and dense bodies. (*HC* Host cell cytoplasm, *N* nucleus). ×28,000
Fig. 3 Light micrograph of bovine red blood cells containing stages of *T. annulata*. The slender stages represent merozoites; the spherical ones are gamonts or stages prior to division. ×1,400

clearance of the parasite is associated with the activation of cytotoxic T-lymphocytes (CTLs). CTLs have also been demonstrated in *T. annulata*-infected cattle.

Cellular immune responses, however, can augment the pathogenesis of the disease. Therefore, understanding of the protective immune responses and identification of parasite antigens required for their induction are essential for vaccine development. In the present review we refer to the cellular immune reactions that either mediate immunity or favor the disease's progress in tropical theileriosis and try to point out the major differences and similarities between *T. annulata* and *T. parva*. A brief description of the humoral immune response is also included for a better understanding of the overall immune response.

Humoral immune response

During the course of natural infections with *Theileria* the host's immune system is stimulated by the various antigens associated with the respective stages of the parasite's life cycle. Sera from cattle recovering from ECF or tropical theileriosis exhibit a neutralizing effect on sporozoites, thus preventing in vitro infection of lymphocytes by sporozoites (Gray and Brown 1981; Preston and Brown 1985; Ahmed et al. 1988; Musoke et al. 1992). Induction of neutralizing antibodies can be achieved even by immunization of cattle with attenuated schizont cultures (Ahmed et al. 1988), indicating that common antigenic determinants do exist between different stages of the parasite.

Surface structures on *T. parva* or *T. annulata* sporozoites, designated P67 or SPAG-1, respectively, have been cloned and used for immunization trials. Although these antigens induced parasite-specific and sporozoite-neutralizing antibodies, only a portion of the animals become protected (Musoke et al. 1992; Boulter et al. 1994, 1998). An important aspect is that SPAG-1 and P67 contain cross-reactive determinants, since a portion of cattle immunized with P67 could survive a challenge with *T. annulata* sporozoites, and that their sera contain anti-sporozoite antibodies. The most homologous part of these proteins is located particularly in the C- and N-termini, whereas the least homology is in the central domain (Boulter et al. 1998).

T-cells are also activated by sporozoite antigens. Thus, Boulter et al. (1995) expressed a C-terminal fragment of SPAG-1 as a fusion protein in the e-1 loop of hepatitis B core antigen and used this recombinant antigen (HBcAg-SR 1) to immunize cattle. Besides the induction of high levels of neutralizing antibodies, significant T-cell responses to both HBcAg and SR 1 were also observed. The T-cell response requires exogenous interleukin 2 (IL-2). The main conclusion deriving from these experiments is that only partial protection may be achieved after immunization with SPAG-1 or P67.

Within infected cells, macroschizonts differentiate into microschizonts, leading to merozoites that have the capacity to invade erythrocytes (Figs. 2, 3). Very little information is available about the immune response of cattle against this parasite stage. There is no evidence for antibody activity against the surface membrane of schizont-containing leukocytes or infected erythrocytes. Using a chemiluminescence test, Ahmed et al. (1988) could not observe any reaction between the immune serum and infected leukocytes or erythrocytes. Interestingly, the immune serum was capable of reacting with free merozoites isolated from infected erythrocytes, indicating that serum of infected cattle contained antibodies with the capacity to opsonize released merozoites. Recombinant merozoite surface antigens of *T. annulata* (TAMS) have been prepared and tested in immunization trials using different application forms and delivery systems. A degree of protection was obtained using TAMS/ISCOMs when the animals were challenged with blood piroplasms (D'Oliviera et al. 1997). However, this could not be reproduced in other trials (see Boulter et al. 1998). The importance of the humoral immune response probably lies in the prevention of de novo infections of new leukocytes or erythrocytes, thereby keeping the level of parasitemia low, yet it cannot prevent the initiation of theileriosis or substantially reduce the progress of the disease when the infection is established (Muhammed et al. 1975; Ahmed et al. 1988; Boulter et al. 1994; Mehlhorn et al. 1994). Likewise, attempts to induce protective immunity in cattle against *T. annulata* by inoculation of recombinant sporozoite or merozoite antigens were only partially effective. Similarly, immunization trials against *T. parva* using recombinant P67 were also only partially successful. Therefore, any vaccine design must include antigens of all three stages, particularly those of the schizont stage.

Cell-mediated immune response

Early studies provided the first hints for a role of cellular immunity against *Theileria parva* and *T. annulata* infections (Muhammed et al. 1974; Rehbein et al. 1981a, b). The technical breakthrough, which allowed in vitro transformation of lymphocytes from uninfected cattle with *T. parva* sporozoites (Brown et al. 1973), facilitated evaluation of the host's immune responses. Using a mixed

lymphocyte culture (MLC) consisting of infected and uninfected cell populations it was possible to investigate the stimulatory capacity of infected cells, on the one hand, and the response of naive or immune cells, on the other. These studies revealed that schizont-containing cells stimulated autologous as well as allogeneic lymphocytes to proliferate and to produce cytokines. In addition, CTLs (Pearson et al. 1979; Emery et al. 1981; Eugui et al. 1981; Preston et al. 1983; Ahmed et al. 1989a; Innes et al. 1989) and helper T-cells (Baldwin et al. 1987; Ahmed et al. 1989b) were generated in peripheral blood mononuclear cells (PBMCs) of immune animals upon cultivation with macroschizont-infected cells.

Generation of *Theileria*-specific CTLs

Irrespective of the breed, cattle surviving an infection with *T. annulata* develop a long-lasting and solid immunity against challenge with a homologous parasite stock. This protective immunity largely depends on the development of a cell-mediated immunity against the pathogenic schizont stage of the parasites. Thus, the remission of the infection correlates with the activation of T-cells (Rehbein et al. 1981a). With the aid of the MLC technique the kinetics of the CTL response were investigated in peripheral blood of immune cattle. CTLs are detected transiently in the peripheral blood of immune but not naive animals undergoing a lethal infection. CTL activities are first detected on day 10, with peak responses occurring on days 11 and 12 or 13 and disappearing thereafter (Ahmed et al. 1989a; Innes et al. 1989; Conze et al. 1998). With *T. parva*, CTL activity appears on day 7 after challenge, reaching peak levels on days 9 and 11 and disappearing thereafter. CTL responses against *T. parva* are demonstrable 1 day earlier in efferent lymph lymphocytes (ELL) than in PBMCs (McKeever et al. 1994). Taken together, CTLs can be detected in cattle after immunization with sporozoites and subsequent chemotherapy or following inoculation of attenuated macroschizont-containing cells, despite the failure to detect CTLs in a small proportion of *T. annulata*- and *T. parva*-immunized cattle (see below). Thus, the overall results of experiments conducted on *T. annulata* or *T. parva* conclusively establish that CTLs play an important role in conferring protection against tropical theileriosis and ECF. Definitive proof that CD8^{+} T-cells are the major component involved in controlling a *T. parva* infection was presented by McKeever et al. (1994). These authors showed that CD8^{+} T-cells from immune donors exhibiting parasite-specific cytotoxicity offered protection to naive recipient cattle against lethal infections with *T. parva,* whereas the control animals needed to be drug-treated. The CD8-depleted cell population could not offer any protection. These results clearly show that CD8^{+} T-cells are the major component of the protective immunity against *T. parva* infections. This does not mean that CD4^{+} T-cells do not participate in immunity against the parasite (see below).

The CTL responses examined following a *T. annulata* sporozoite challenge were effective only against major histocompatibility complex (MHC) class I-matched, but not mismatched, infected target cells. Moreover, target cells from MHC class II-matched animals were also not killed by the CTLs (Conze et al. 1998). It is noteworthy that a small proportion of cattle that have been immunized against *T. annulata* by inoculation of sporozoites develop a solid immunity to challenge without manifesting a CTL response, despite the increase observed in the number of CD8^{+} T-cells in the blood of one immune animal when cytotoxicity should be expected (unpublished data). In this case, it is possible that CD8^{+} T-cells may have interfered with the growth of the cells by inhibiting the replication of the schizonts without causing lysis of the infected cells. Alternatively, the CTLs were operating in the regional lymph nodes where parasitized cells were present and were therefore not detectable in the peripheral blood at the time of examination.

One of the major differences between *T. annulata*- and *T. parva*-specific CTLs is that memory cytotoxic T-cells (CTL) can be reactivated in vitro by cocultivation with autologous infected cells. This allowed cloning of *T. parva*-specific CTLs, whereas all attempts to clone CTLs with specificity for *T. annulata* failed.

Considering the data published to date, it seems that CD8^{+} T-cells are activated in both *T. annulata* and *T. parva* infections and that they offer immunity by killing their target cells. There is no evidence for another mechanism, for example, as to whether they produce cytokines such as gamma-interferon (IFN-γ), which may activate and enable the target cells to kill the parasites. Neither IFN-γ nor tumor necrosis factor-alpha (TNF-α) appears to play a significant role in the control of ongoing infections, since they have not been capable of inhibiting the growth of established parasitized cell lines in vitro (Ahmed et al. 1992b; Preston et al. 1993). However, in vitro studies have shown that TNF-α prevents the development of trophozoite-infected cells (Preston et al. 1993).

Parasite specificity and MHC restriction of CTLs

In addition to the *T. parva*-specific component of the CTL response, there is clear evidence of a non-specific cytotoxicity directed against allogeneic as well as xenogeneic cells. This nonspecific, MHC-unrestricted cytotoxicity appears to be attributable to natural killer (NK) cells that kill proliferating cells, irrespective of their being infected or not, and their activity is associated with severe symptoms of the disease, for example, lymphocytopenia (Emery et al. 1981; Eugui et al. 1981). Preston et al. (1983) have found that PMBCs from cattle infected with *T. annulata* lyse macroschizont-infected cells in vitro via both BoLa-restricted and unrestricted cytotoxic cells, the latter probably being mediated by NK cells.

The parasite specificity of the activated CTLs is highlighted by several observations. First, autologous uninfected blast cells or parasitized cells treated with buparvaquone are not lysed by CTLs (Ahmed et al., unpublished data). Second, in the majority of animals infected with *T. parva* the CTLs exhibit a parasite strain specificity (Goddeeris et al. 1986, 1990). For example, CTLs generated against the Muguga stock of *T. parva* are specific for this strain and genetically restricted. Only a small proportion of cattle immunized with this strain develop cross-reactive CTL responses (Taracha et al. 1995). When challenged with a heterologous stock, these animals are protected, pointing out a crucial role for CTLs in protection against ECF. The cross-reactivity may be due to the presence of epitopes that might be shared by cross-reactive strains.

In a recent study the strain specificity of MHC class I-restricted CTLs was examined in *T. annulata* infection. CTLs generated through challenge with the Hissar (Indian) strain effectively lysed autologous cells infected with this strain of the parasite. However, CTLs were less effective against cells infected with the Gharb (Moroccan) strain and showed virtually no reactivity against the Ankara (Turkish) strain, providing the first direct evidence for strain specificity in immune responses against *T. annulata* (Conze et al. 1998). Immunization of animals with a virulent strain of the parasite commonly does protect against heterologous strains under laboratory conditions (Brown et al. 1994). As it has been shown that CTLs can exhibit strain specificity, it is thus possible that these cells are not the only mechanism for clearance of the parasites during such challenge infections. The results of Conze et al. (1998) may offer an explanation for the lack of protection against heterologous challenge in attenuated-cell-line-immunized cattle recently described by Dargouth et al. (1996) and indicate that other types of immune response may augment the CTL response against *T. annulata*. Indeed, there is evidence for a non-MHC-restricted killing of parasitized cells (Preston et al. 1983), although not at the time points examined in the experiments of Conze et al. (1998), and for inhibition of the growth of infected cells by PBMCs of immunized animals (Ahmed et al. 1989a) and by macrophages (Preston and Brown 1988). Nonetheless, for the first time the results of Conze et al. (1998) demonstrate strain specificity in a recognized arm of the immune response against infection with *T. annulata* and have implications for vaccine design, especially with regard to efforts directed toward identification of peptides recognized by CTLs. Any peptide-based vaccine designed to induce CTL-mediated immunity must include a cocktail of different parasite strains.

T-helper cells

Another subset of T-cells has been detected in cattle immunized either by infection and treatment or by inoculation of macroschizont-infected cells. When incubated with autologous parasitized cells these T-cells proliferate and produce IL-2 and IFN-γ (Ahmed et al. 1989b). These cells have been characterized as CD3$^+$ CD4$^+$ cells expressing high levels of IL-2R, MHC class II antigen, and CD45RB (Conze 1995; Bußler et al. 1997). Macroschizont-containing cells can also induce naive T-cells to proliferate and to secrete Th1 cytokines (Campbell et al. 1995). The proliferation of naive T-cells can be blocked by anti-MHC class II or anti-CD4, but not anti-MHC class I or anti CD8, monoclonal antibodies (Campbell et al. 1997b). These authors suggest that the activation of naive T-cells may be mediated by superantigens in a manner similar to that observed in many other bacterial or viral infections. Superantigens activate T-cells bearing specific Vβ gene products in their TCR (Campbell et al. 1997b).

PBMCs of *T. annulata*-immune cattle, in which both CD4$^+$ and CD8$^+$ T-cells were present, killed MHC class I- but not class II-matched parasitized target cells (Conze et al. 1998). This may be an indirect evidence for the inability of CD4$^+$ T-cells to exert killing of target cells since such cells recognize peptides in association with MHC class II antigens. There is no direct evidence for the involvement of CD4$^+$ T-cells in the clearance of the parasites, although they are certainly activated and produce cytokines such as IFN-γ and IL-2. However, none of these cytokines has ever been shown to destroy the schizonts or to inhibit the growth of parasitized cells. In contrast, TNF-α, IL-2, and, in some cases, IFN-γ enhance the growth of infected cells, even though a population of PBMCs was found to exhibit a cytostatic effect on the growth of both autologous and allogeneic *T. annulata*-infected cells. These cells were detectable in the blood of the animals at days 11–24 after a primary infection. Interestingly, this cytostatic activity disappeared as the animals contained the infection (Ahmed et al. 1989a). A possible role for CD4$^+$-positive T-cells in immunity to *T. annulata* may involve their production of macrophage-activating cytokines. Indeed, cytostatic macrophages have been demonstrated in *T. annulata*-immune cattle (Preston 1981; Preston and Brown 1985; Preston et al. 1992b). On the basis of the capacity of such macrophages to produce TNF-α (Preston et al. 1993) and NO (Visser et al. 1995), it has been suggested that CD4$^+$ cells produce IFN-γ, which participates in the activation of such macrophages to produce NO and to kill the schizonts (Preston et al. 1997; Richardson et al. 1998). This is a very attractive hypothesis, but it needs to be confirmed, since recently published results indicate that IFN-γ may support the pathogenesis of the disease. For example, Campbell et al. (1998) have found that far from having antiparasitic effects, IFN-γ may actively stimulate parasitized-cell growth. Supporting these observations, we have found that neither TNF-α nor IFN-γ can inhibit the growth of parasitized cells (Ahmed et al. 1992b). In addition, we have observed that supernatants of immune PBMCs stimulated with autologous *T. annulata*-infected cells do not interfere with the growth of infected cells, despite the presence of IFN-

γ in these supernatants (manuscript in preparation), even though immune cells harvested from immune cattle can inhibit the growth of the infected cells, indicating that cell to cell contact is necessary to inhibit the growth of the target cells.

CD4$^+$ helper T-cells are also activated in *T. parva*-immune animals (Baldwin et al. 1987; Baldwin et al. 1992). They do not appear to be directly involved in the clearance of the parasites, since transfer of immune cells depleted from CD8$^+$ T-cells to naive cattle did not offer protection against a lethal infection with *T. parva* (McKeever et al. 1994). A proportion of CD4$^+$ T-cells exhibit a killing activity that is, however, not parasite-strain-restricted (Baldwin et al. 1987). Accordingly, they act rather as helper cells, producing IL-2, which is required for the clonal expansion of CD8$^+$ T-cells. That CD4$^+$ T-cells are important in mediating immunity to both *T. annulata* and *T. parva* is substantiated by the observation that IgG antibodies are produced, since production of these antibodies requires the participation of CD4$^+$ T-cells.

MHC class II-associated peptides have not yet been identified for *Theileria*-infected cells. However, in an attempt to identify *T. parva* proteins relevant for the induction of cell-mediated immunity, a soluble cytosolic parasite antigen was fractionated by several biochemistry techniques and used to induce proliferation of helper T-cell clones. Fractions ranging between 10 and 24 kDa were identified as stimulators of the T-cell clones. An antiserum that was raised against the 24-kDa fraction detected a 30-kDa native schizont protein (Grab et al. 1992; Brown et al. 1995). Further improvements are required for protein purification and for the establishment of effective antigen presentation systems.

Pathogenesis of theileriosis

The pathogenesis of theileriosis is primarily due to infection of host's leukocytes by macroschizonts. The macroschizonts of *Theileria parva*, *T. annulata*, and *T. lestoquardi* (syn. *T. hirci*) live within the cytoplasm of the host cells, in which the parasites proliferate synchronously with their host's cells. Although the macroschizonts are the most pathogenic stage of these species, merozoites also seem to be involved in the pathogenesis of the disease via tissue injury and anemia. During the course of tropical theileriosis and ECF a dramatic decrease occurs in the cellularity of lymphoid tissues (Preston et al. 1992a). For example, the count of blood leukocytes falls to less than 10^3 cells/mm^3 (Rehbein 1981). At the same time an increase in the percentage of parasitized cells is noted in the regional lymph nodes and in the spleen, thymus, bone marrow, and lung (Irvin and Morrison 1987).

T. annulata and *T. parva* may infect and transform a variety of cell types. However, there are some differences between the two species. *T. parva* preferentially infects T-cells, whereas *T. annulata* mainly transforms MHC class II-positive cells (Spooner et al. 1988). Recently it has been confirmed that macrophages (Campbell et al. 1994; Sager et al. 1997) and B-lymphocytes are targets of *T. annulata* (Sager et al. 1998). Moreover, it has been shown that inoculation of cattle with T-Parva-infected B-cells causes a mild and self-limiting infection (Morrison et al. 1996), indicating that the pathogenesis of the disease is associated with infection of T-cells. These data confirm earlier observations on the pathogenesis of ECF (Irvin and Morrison 1987).
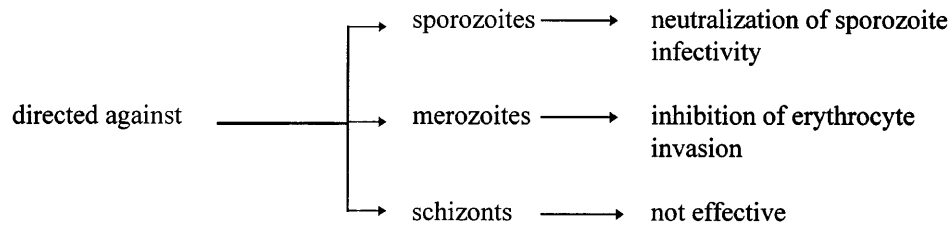
The massive, uncontrolled cell proliferation observed during infection with *Theileria* schizonts is the most important pathogenic aspect. The mechanisms underlying the proliferation of the infected cells is not quite clear. However, it has been shown that growth factors such as IL-2 can enhance the proliferation of parasitized cells. This growth factor seems to exert its enhancing effect via IL-2 receptors (IL-2R) that are expressed on the parasitized cells (Dobbelaere et al. 1988; Hermann et al. 1989; Ahmed et al. 1992a). Although a great number of *T. parva*-infected cells express IL-2, none of them has definitely been proven to secrete IL-2. In a single study it has been shown that anti-IL-2 antibodies may inhibit the growth of one cell line. The same antibody, however, did not inhibit the growth of a great number of *T. parva*-infected cell lines, regardless of their high levels of IL-2 expression. Moreover, none of the *T. annulata*-infected cell lines expressed or secreted IL-2. A growth factor with the biological activities of IL-2 has been demonstrated in supernatants of some, but not all, *Theileria*-infected cells (Brown and Logan 1986; Ahmed et al. 1987, 1992a; Dobbelaere et al. 1988). On the other hand, it is well documented that exogenous IL-2 can enhance the proliferation of infected cells. Thus, it is possible that uninfected cells being stimulated, for example, by the parasitized cells produce IL-2, which then enhances the growth of parasitized cells in a paracrine manner, meaning that such a growth factor plays a role in the pathogenesis of the disease (Dobbelaere et al. 1988; Ahmed et al. 1992a).

T. annulata- and *T. parva*-infected cells were examined for their capacity to produce IFN. All *Theileria*-infected cell lines of T-cell origin produced IFN-γ as demonstrated in bioassays and at the molecular level. None of the *T. parva*-infected B-cell lines or *T. annulata*-infected cells expressed IFN-γ mRNA. In contrast, the majority of these cell lines produced type I IFN (Ahmed et al. 1993). The significance of IFN for *Theileria*-mediated cell transformation is not yet clear. Recombinant bovine IFN-γ did not inhibit the growth of *Theileria*-infected cells in vitro. In some cases it rather enhanced their proliferation (Ahmed et al. 1992b). There is no clear correlation between the production of IFN-γ and the control of a *T. annulata* infection. Despite a significant degree of expression of IFN-γ, naive cattle are not protected against a severe primary infection with *T. annulata*. The greatly elevated amounts of IFN-γ seem to enhance the growth of the parasitized cells (Campbell et al. 1998).

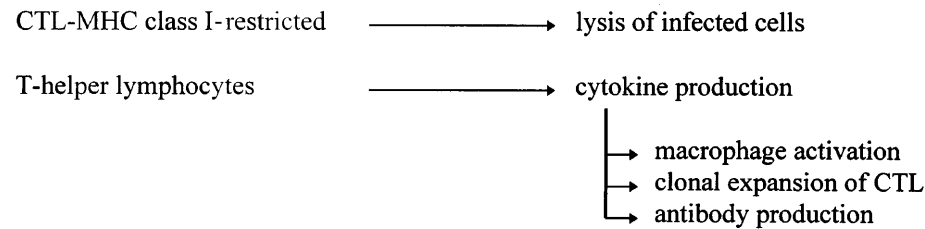Different effects of exogenous TNF-α have been observed. This cytokine may prevent the development of

Possible immunization mechanisms in *Theileria annulata* infection
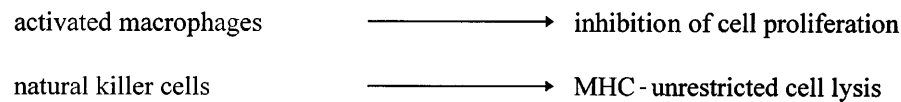
Antibody-mediated immune response

directed against

→ sporozoites ──→ neutralization of sporozoite infectivity

→ merozoites ──→ inhibition of erythrocyte invasion

→ schizonts ──→ not effective

Cell-mediated immune responses

a) specific mechanisms:

CTL-MHC class I-restricted ──────→ lysis of infected cells

T-helper lymphocytes ──────→ cytokine production

→ macrophage activation
→ clonal expansion of CTL
→ antibody production

b) Nonspecific mechanisms:

activated macrophages ──────→ inhibition of cell proliferation

natural killer cells ──────→ MHC-unrestricted cell lysis

Fig. 4 T- and B-lymphocytes are activated during the infection with *T. annulata*. 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. However, antibodies can neutralize the infectivity of the sporozoites but do not prevent initiation of an infection. T-cells participate in immunity to *T. annulata* by acting as cytotoxic T-lymphocytes (CTLs) and as T-helper lymphocytes. Thus, T-helper cells produce IL-2, which is required for the clonal expansion of CTLs, and IFN-γ, which activates macrophages to produce NO. The latter destroys the schizonts within the infected cells. CTLs kill their infected target cells in an MHC class I-restricted manner. Cytokines are also required for the induction of parasite-specific antibodies. The role of NK cells is not clear. They may act as effector cells in controlling the infection by nonspecific lysing of parasite-containing cells or they may activate macrophages via production of IFN-γ. Cytostatically acting macrophages have been described to inhibit the in vitro growth of *T. annulata*-infected cells

trophozoites, but it does not interfere with the proliferation of previously established cell lines infected with *T. annulata* or *T. parva* (Ahmed et al. 1992b; Preston et al. 1993). In some experiments, exogeneous TNF-α even enhanced the growth of infected cells in vitro.

However, TNF-α has not been detected in supernatants of *T. annulata*-infected cell lines (Ahmed et al. 1992b), despite its expression at the mRNA level (Campbell et al. 1994). Considering that this cytokine is a potent inducer of fever and has been shown to play a role in anemia, muscle wasting, and necrosis (Sileghem et al. 1994) – symptoms that are observed in tropical theileriosis as well – it is reasonable to suggest that TNF-α is involved in the pathogenesis of the disease.

A significant aspect of *T. parva*-induced transformation is the up-regulation of IL-10 in all cell lines tested. IL-10 mRNA was also detected in one *T. annulata*-infected cell line and in three clones derived from this cell line. Due to a lack of solid in vivo data, it is difficult to understand the significance of these in vitro observations. Nevertheless, these data raise the possibility that IL-10 may influence the immune response of naive cattle to challenge (McKeever et al. 1997). This is in line with the finding that human IL-10 inhibits bovine CD4^{+} T-cell responses as well as CD8^{+} T-cell proliferation (Brown et al. 1994). Accordingly, McKeever et al. (1997) have suggested that IL-10 produced by *T. parva*-infected cells may prevent the generation of parasite-

specific cytotoxic cells in naive cattle and may therefore be involved in the pathogenesis of ECF.

There is a direct association between increase in the number of infected cells and lymphocytolysis observed during the course of the disease. Although the mechanism responsible for the cytolysis is only poorly understood, there is some evidence for the involvement of the immune response. Thus, besides the destruction of the cells due to their infection with the parasites, natural killer (NK) cells have been to shown to lyse their target cells indiscriminantly, whether they are infected or not (Emery et al. 1981; Eugui et al. 1981).

There is no evidence for the involvement of antibodies in the lysis of *T. annulata*- or *T. parva*-infected cells, since neither anti-lymphocyte antibodies nor antibody-mediated cytolysis of infected cells has been demonstrated (Duffus et al. 1978; Ahmed et al. 1988). In a mixed lymphocyte culture, infected cells stimulate their uninfected counterparts to proliferate (Ahmed et al. 1981) and to produce IL-2 and IFN-γ (Ahmed et al. 1989a). Recently, the responder T-cells were characterized as CD3-positive and it was found that these cells express high levels of IL-2R, MHC class II, and CD45RB (Conze 1995; Bußler et al. 1997).

Stimulation of T-lymphocytes in a mixed lymphocyte reaction with schizont-containing cells is nonspecific because lymphocytes of *T. parva*-infected and uninfected animals show a similar magnitude of proliferation upon incubation with *T. parva*-infected cells (Pinder et al. 1981; Goddeeris and Morrison 1987). However, the proliferative response of *T. annulata*-immune peripheral blood lymphocytes to *T. annulata*-infected cells is substantially stronger than that of lymphocytes from naive cattle (Ahmed et al. 1989a). Rintelen et al. (1990) found that elimination of *T. annulata* schizonts by buparvaquone prevented the generation of a mixed lymphocyte reaction in vitro. The same drug, however, had no influence on the capacity of uninfected bovine lymphocytes, which had previously been activated by the T-cell mitogen concanavalin A, to stimulate autologous or allogeneic cells. Accordingly, the authors postulated that antigens presented by infected cells, which are supposed to be responsible for the stimulation of uninfected lymphocytes, are induced by the permanent presence of schizonts within the cytoplasm of the host cells. Removal of parasites leads to inhibition of the expression of the neoantigenic determinant on the surface membrane of the cells. Anti-MHC class II and anti-CD4 monoclonal antibodies blocked the activation of naive T-cells. In contrast, anti-MHC class I monoclonal antibody did not inhibit the proliferation of naive T-cells (Campbell et al. 1997b). These authors suggested that the activation of naive T-cells may be mediated by superantigens in a manner similar to that observed in many other bacterial or viral infections. Superantigens activate T-cells bearing specific Vß gene products in their TCR. In vivo, T-cells are also activated by parasitized cells. This is one of the major components of the pathogenesis of *T. annulata* and *T. parva* infections

(Campbell et al. 1997a). Gamma/delta TCR+ lymphocytes may also be stimulated to proliferate by autologous *T. annulata*-infected cells. This response requires the presence of IL-2 (Collins et al. 1996).

Taken together, the nature of the cells, the cytokines they produce, and their capacity to induce a nonspecific activation of naive T-cells to proliferate account for the pathogenesis of tropical theileriosis. The role of NK cells remains to be determined and has not clearly been associated with the pathogenesis of the disease as has been suggested for *T. parva*.

Conclusions

It is possible to immunize cattle against tropical theileriosis using cultures of schizont-containing cells or by a combination of experimental infection and subsequent treatment. Although such vaccines may be effective, the carrier status of the immunized animals is one of the major problems of postvaccinal reactions. Irrespective of the breed of the animals, immunization leads to the activation of T-lymphocytes, which act either as effector CTLs or as helper T-cells. With some exceptions, control of the challenge infection generally coincides with the generation of cytotoxic cells that are capable of killing of parasitized cells in an MHC class I-restricted manner. Moreover, these CTLs exhibit a certain degree of strain specificity. In addition, there is evidence that besides CTLs, other cells might be involved, such as NK cells, CD4+ T-lymphocytes, and macrophages. All these components and the humoral immune response may cooperate to contain the infection (Fig. 4).

Parasite antigens recognized by T-cells, CTLs and CD4+ T-cells are candidates for vaccine design. Because of the strain specificity of the CTLs, various antigens must be considered. To achieve this goal, techniques of protein identification, purification and presentation systems, and delivery systems must be developed. Therefore, a successful immunization strategy must include antigens expressed in the schizont stage, but sporozoite and merozoite proteins should be taken into consideration as well. Partial protection has been achieved by immunization with SPAG-1 (Williamson et al. 1989). Screening of high-performance liquid chromatography fractions of *Theilaria annulata*-infected-cells by CTLs has led to the identification of two fractions that seem to contain MHC-restricted parasite peptides (Conze 1995). Another important finding is the identification of several parasite genes. One of them is expressed in the schizont stages of both *T. annulata* and *T. parva* and has now been completely sequenced (Ahmed et al. 1997; Shayan et al. 1998; Schnittger et al., in preparation).

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