Vaccination against Tropical Theileriosis

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ABSTRACT: *Theileria annulata*, the cause of tropical theileriosis is propagated in cattle with stage-to-stage transmission by *Hyalomma* ticks. Three stages in the life cycle of the parasite—tick-derived sporozoites, intramononuclear schizonts, and erythrocytic merozoites—infect cattle. When cattle are inoculated with schizont-infected cells, the parasite is transferred from the donor cell to the recipient. The main pathological damage in cattle is induced by the schizont stage. Each development stage of *T. annulata* elicits a specific immune response. Schizont-infected lymphoid cells can be grown indefinitely in culture and prolonged cultivation results in loss of virulence. Blood-derived schizonts induce stronger immunity than culture-derived schizonts, which suggests that restrictions on the parasite population or antigenic variation occur during prolonged cultivation. The duration of immunity following sporozoite or schizont infections has not yet been determined, but does not appear to be lifelong. The attenuated, culture-derived anti-theileria vaccine proved to be safe and effective in prevention of field theileriosis in large enzootic areas.

INTRODUCTION

Tropical theileriosis is a cattle disease caused by the protozoan *Theileria annulata*. The disease occurs around the Mediterranean basin and in vast areas of Asia. It is transmitted by several tick species of the genus *Hyalomma*. Endemic regions of *T. annulata* and *T. parva* do not overlap except, perhaps, in a restricted area of East Africa. On the other hand, *T. annulata* and *T. sergenti* occur concurrently in large regions in the Far East. Tropical theileriosis is maintained in nature by a cattle–tick–cattle cycle, both taurine (*Bos taurus*) and zebu (*Bos indicus*) cattle being susceptible to the disease. The yak (*Bos grumii*) is also highly susceptible and suffers severe clinical symptoms and mortality following infection with *T. annulata*. The water buffalo (*Bubalus bubalis*) does not usually exhibit clinical manifestation on infection, but may act as a reservoir for the infection of ticks.

Tropical theileriosis is among the most serious of constraints on the livestock industry in the regions where it occurs. As with other tick-borne diseases, tropical theileriosis can be prevented by control or eradication of the vector ticks or by prophylactic immunization. The efficacy of acaricides against several *Hyalomma* spp. has been studied recently, but adequate treatment regimes to control or eradicate the vector ticks have not yet been critically evaluated.

Vaccination against tropical theileriosis was initiated by a group of French investigators headed by Edmond Sergent, working in North Africa. They isolated field...
strains of naturally low virulence, which they maintained in the laboratory by needle passages in calves. After several tens of passages, only schizonts, and not erythrocytic merozoites, could be detected in the infected calves, and blood drawn during the acute period of the infection was used for vaccination of susceptible cattle.

The method just described was adopted in Israel, but the North African strain conferred only partial protection against the local Israeli isolates. Therefore, a virulent Israeli isolate (Tova) was administered 1–2 months after the primary inoculation with the North African strain in order to reinforce the immunity. The North African strain was maintained by passages in calves (up to passage 420) until the beginning of the 1960s when no schizonts were found in lymph nodes and liver smears of subinoculated animals. This event triggered research to develop an alternative method for vaccination, and *T. annulata* was successfully cultivated *in vitro* by Tsut-Tchernomoretz, who used explants of infected tissues. This was the starting point for development of a culture-derived vaccine against tropical theileriosis.

**LIFE CYCLE**

*T. annulata* has a complex life cycle in the bovine and tick hosts. The various development stages differ in their pathogenicity to cattle and in their immunogenic attributes (both in their role in causing disease and in their capacity to induce immune response and confer protection).

*T. annulata* is transmitted by two or three host *Hyalomma* sp. ticks by stage-to-stage transmission. A feeding period of 48 to 72 hours is required, to enable maturation and produce infective sporozoites in the salivary glands of the tick. However, maturation of sporozoites can also occur when ticks are held at elevated temperatures or in proximity to cattle. It seems, therefore, that blood feeding of the tick is not a prerequisite for development of infective sporozoites. This finding has an important epidemiological implication: ticks may bear *T. annulata* infective stages before they attach to cattle, and infection may be transmitted as soon as the tick begins to feed. Under such circumstances there is little chance to prevent transmission of *Theileria* by acaricide treatment of the bovine host.

After their inoculation into cattle, sporozoites invade mononuclear cells. *In vitro* studies have shown that sporozoites penetrate cells within a few minutes and preferentially parasitize monocytes, macrophages, and B cells, rather than T cells. Inside the host cell, the sporozoite becomes enlarged and loses the two inner membranes of its pellicle. Simultaneously, nuclear division starts, resulting in the formation of schizonts (meronts). During the early stages, the schizonts possess relatively large and sometimes irregularly shaped nuclei (the macroschizont). This stage is followed by schizonts with relatively smaller nuclei (the microschizont). The microschizonts produce merozoites by a budding-like process of the nuclei. Beginning from the eighth day after the sporozoite infection, the merozoites are released from the mononuclear cells and invade the red blood cells to become erythrocytic merozoites (piroplasms). In addition to the tick-derived sporozoites, the *Theileria* stages developing in cattle (schizonts and erythrocytic merozoites) can also produce infection in bovines when inoculated intravenously, intramuscularly, or subcutaneously.
DETECTION OF *T. ANNULATA* INFECTION IN CATTLE

To obtain laboratory confirmation of infection, thin blood films and smears of lymph node or liver tissues obtained by needle biopsy are prepared. The presence of schizonts in lymph nodes and the liver is sufficient for the diagnosis of acute theileriosis. The infection rate of the red blood cells declines after recovery from acute infection, and erythrocytic merozoites may not be found several weeks later. However, they may be detected intermittently in subsequent examinations. It appears that cattle remain carriers for life, and relapse can be induced by splenectomy. Detection of parasitemias at a level as low as 0.000048% has been successfully achieved by means of a polymerase chain reaction (PCR), using primers derived from a gene encoding for a 30-kDa merozoite antigen.

The most widely used serological test for detecting antibodies against *T. annulata* is the indirect fluorescent antibody (IFA) test, in which both schizonts and erythrocytic merozoites have been used as antigens. The IFA test can be considered specific only in areas where *T. annulata* alone occurs, since antibodies to other *Theileria* species crossreact with *T. annulata* antigen. The IFA test is useful for identifying herds that contain carriers of *T. annulata*, but is not always sufficiently sensitive that it will detect all infected individuals. Both schizont and merozoite IFA antigens have failed to detect antibody in some animals carrying patent infection with erythrocytic merozoites.

The enzyme-linked immunosorbent assay (ELISA) has been successfully adapted for detection of anti-*T. annulata* antibody. In contrast to the IFA test, schizont antigen used in ELISA was less sensitive and specific than the antigen prepared from erythrocytic merozoites, since the latter detected antibodies for a longer period.

**VIRULENCE, PATHOGENICITY, AND ANTIGENIC VARIATIONS**

Sporozoites are believed to be more pathogenic to cattle than schizonts. Sergent *et al.* found that mortality of cattle under experimental conditions was 28.4% when they were infected by ticks versus 15% when infected by inoculation of blood containing schizonts. However, no quantification of the parasites used for the infections was possible.

Field isolates showed various degrees of virulence when inoculated into cattle: mortality ranging from 3.2 to 49% was observed among 939 calves inoculated with blood infected with five different North African isolates. An obvious variation in the virulence of four Israeli isolates was observed, following inoculation of schizont-infected blood into cattle. Similar variations in virulence have been reported from other geographical areas. Attempts to alter the virulence of *T. annulata* by serial passages in calves were unsuccessful. An Israeli isolate, Tova, that had been passaged 240 times in calves, induced mortality of 39.5% in cattle inoculated with schizont-infected blood of passages 203 to 240. However, it should be mentioned that field isolates with low initial virulence did not become more virulent when passaged through susceptible cattle.

Sporozoites and schizonts of *T. annulata* were exposed to various doses of gamma irradiation in an attempt to alter their virulence. The irradiated parasites generally caused milder clinical manifestations than the non-irradiated organisms, and
in most instances, engendered resistance to reinfection with non-irradiated sporozoites. Successful attenuation of *T. annulata* schizonts has been obtained by long-term passage in cell culture,\(^{37}\) a topic that will be further discussed in the section dealing with development of culture-derived vaccine.

**ANTIGENIC VARIATION**

Antigenic differences among isolates of *T. annulata* are based mostly on cross-immunization trials in calves. Most field isolates conferred a high degree of reciprocal immunity,\(^{8,38–40}\) and it has been suggested that a constant mixing and crossing of genetically different parasite populations occurs in nature.\(^{41}\) Most isolates are, therefore, likely to consist of a mixture of “strains” and will, consequently, confer a relatively wide range of protection. On the other hand, several experiments detected *T. annulata* isolates with a distinctly low capacity for reciprocal immunity. A noticeable instance is the difference in the immunogenic relationship between Algerian and Israeli isolates studied by Sergent and coworkers\(^{8}\) and by Adler and Ellenbogen.\(^{42,43}\) In another experiment, an Israeli field isolate with naturally low virulence induced partial immunity (six out of 10 inoculated animals exhibited clinical theileriosis, but none died) when challenged with a more virulent laboratory stock.\(^ {31}\)

Culture techniques have been used in attempts to distinguish among different *T. annulata* parasite populations: isoenzyme electrophoresis revealed differences among schizonts from Turkish, Iranian, and Indian isolates as well as among six isolates from Sudan.\(^ {44,45}\) A series of monoclonal antibodies, reacting with intracellular macroschizonts, was used to examine the level of antigenic diversity between and within stocks of *T. annulata*. The binding of the antibodies varied when tested against different stocks. Some monoclonal antibodies failed to react against a number of stocks and others recognized the macroschizonts of all stocks but revealed difference among stocks in their degree of antibody reactivity. In addition to the antigenic variability between stocks, variations were also observed within the stocks. This variability segregated when the stocks were cloned, indicating that the original stock consisted of several types of parasites.\(^ {46}\) Fifty-three Tunisian isolates have been characterized by antiparasitic monoclonal antibody reactivity, isoenzyme electrophoresis, and Southern blotting: no identical isolates were detected by these methods and the majority of isolates contained more than one parasite population.\(^ {47}\) Based on up-to-date experience from anti-*T. annulata* vaccination campaigns, it appears that the diversity in field parasite populations detected by laboratory techniques is not necessarily related to immunogenic differences and, therefore, has had a limited impact on immunization against tropical theileriosis.

**IMMUNITY INDUCED BY DIFFERENT DEVELOPMENTAL STAGES OF *T. ANNULATA***

Each of the three developmental stages of *T. annulata* that are infective for cattle (sporozoites, schizonts, and erythrocytic merozoites) induces a specific immune response that may result in partial or no protection against infection with
the heterologous stages. Sergent et al. reported that cattle that recovered from tick-induced T. annulata infection were protected more effectively against challenge by T. annulata-infected ticks than cattle that had recovered from blood- (schizont) induced infection. The infection of cattle with sporozoites results in the development of macroschizonts, microschizonts, and erythrocytic merozoites. It appears that cattle recovered from sporozoite infection are immune to all development stages of T. annulata.

Theilerial infection induced by inoculation of virulent schizonts results in multiplication of schizonts in the host mononuclear cells, followed by the appearance of erythrocytic merozoites in red blood cells. The recovery from schizont-induced infection engenders a stronger immunity to reinfection with schizonts than to that with sporozoites. However, the immunity induced by schizonts is usually strong enough to prevent severe clinical manifestation, following an infection with sporozoites. This phenomenon provides the basis for vaccination against tropical theileriosis with a schizont vaccine.

The immunogenic relationship between schizonts and sporozoites was studied by using lyophilized and disrupted schizont-infected cells plus Freund’s adjuvant (killed schizont vaccine) for immunization of calves. A partial to complete immunity to schizont infection was induced by the killed schizonts. The animals in which live schizonts became established following the challenge with virulent schizonts were highly resistant to subsequent challenge with sporozoites, whereas the animals that were totally protected against the virulent schizonts succumbed to the sporozoite infection. It seems, therefore, that dead schizont vaccine engenders protection only against the homologous developmental stage. However, the results of recently published investigations indicate that immunization with the killed vaccine may have generated an immune response not only against the schizont antigen but also against the infected cells. Therefore, cattle were protected against the schizont challenge not only by the acquired antiparasitic immunity, but also by the blocking of the transfer of the virulent schizonts by the allogeneic response to the inoculated cells.

The persistence of the erythrocytic merozoites for years—probably for life—in cattle recovered from tropical theileriosis indicates that the immunity against the schizonts has little or no effect on the erythrocytic merozoites. Reciprocally, the erythrocytic stage does not confer any protection against homologous schizonts, since splenectomized calves carrying this stage for 41 to 165 days succumbed to schizont infection. Western blot analysis showed that three antigens of between 71 and 73 kDa are common to the three infective stages of T. annulata (sporozoites, schizonts, and merozoites). An antigen of 32 kDa was specific for merozoites only. Antibodies generated by schizonts or sporozoites reacted with all stages of T. annulata in the ELISA test, regardless of the method of immunization. An important feature of the antibodies was that animals inoculated with schizonts exhibited antibodies to the sporozoite antigen, although they had not been exposed to this stage of T. annulata.
ISOLATION AND CULTIVATION OF T. ANNULATA

Isolation of T. annulata parasites from the field can be achieved from cattle suffering from tropical theileriosis or from T. annulata-infected ticks. Field studies have shown that a considerable percentage of Hyalomma spp. ticks harvested in barns or in the field are infected with T. annulata. Therefore, a high probability for isolating this parasite can be expected.

The techniques for cultivation of T. annulata schizonts have been reviewed by Brown, as well as by other investigators. Cultures of T. annulata can, in principle, be initiated from any tissue containing schizont-infected cells, but in practice material for starting cultures has been obtained mainly from three sources: (1) peripheral blood leukocytes (PBL), (2) internal organs such as lymph nodes, liver, and spleen of theileriosis-sick cattle, and (3) by infection in vitro of bovine PBL with sporozoites harvested from infected ticks.

Several methods have been used to initiate cultures from PBL of infected cattle. The first monolayer culture of T. annulata-infected cells was derived from kidneys, liver, and spleen of a calf dying from tropical theileriosis (unpublished data). A notable improvement for harvesting material from internal organs that does not require the killing of infected calves was the use of liver tissue obtained by needle biopsy for initiation of cultures. The probability of obtaining schizont-infected cultures by the above methods is very high, as shown by a comparative study of techniques using PBL and liver tissues from infected calves.

The technique for using tick-derived sporozoites to establish schizont-infected cultures was first developed by Brown et al. for T. parva and subsequently also used for T. annulata. The presence of the schizonts in the mononuclear bovine cells stimulates the growth and division of these cells, and they are theoretically capable of unlimited multiplication cycles. Division of the schizont-infected cells has been studied by means of Giemsa-stained preparations of dispersed cells that were collected and fixed onto slides, or by means of cells fixed in situ and stained with acridine orange.

For most of the infected cells there is a balance between the division of the cell and the size of the schizont. The number of nuclei in individual schizonts is relatively constant, varying between 4 and 16, with an average of 12.2 nuclei per schizont. In a few cells very large schizonts are present; it may be assumed that such cells are no longer capable of dividing and are destroyed by the schizont. Destruction of cells also occurs in newly established cultures and this may result in selection of schizont populations.

GROWTH REQUIREMENT FOR T. ANNULATA SCHIZONTS

T. annulata-infected cell lines have been grown successfully in a wide variety of synthetic media. In a comparative study with nine culture media, the Leibovitz L-15 medium gave a maximum yield after seven days of incubation at 37°C. According to other investigators RPMI-1640 was also highly efficacious in supporting the growth of schizont-infected cell lines. Although fetal bovine serum exhibits the
best support for multiplication of schizont-infected cells, normal bovine serum also
gives very satisfactory results. Media supplemented with horse, sheep, and goat
sera supported long-term cultivation of infected cells lines, but the use of sheep
serum was associated with a lower population of schizont nuclei per cell.67

ATTENUATION OF THE VIRULENCE OF T. ANNULATA SCHIZONTS

Previous investigations demonstrated that the virulence of T. annulata schizonts
became attenuated following growth and passage of the schizont-infected cells in
culture. The degree of attenuation was assessed by periodic inoculation of
2–5×10⁶ schizont-infected cells into susceptible calves. Three main stages in the
attenuation process were described. During the initial period of cultivation the sch-
izonts produce clinical theileriosis and eventually death in the inoculated calves.
After further subcultivation milder clinical manifestations occurred occasionally.
Lymph node or liver biopsy showed various rates of schizont infection, and erythro-
cytic stages of T. annulata were detected in blood films. After a period of several
weeks or months, only a portion of the inoculated animals exhibited fever, and sch-
izonts were not detected in all of them. Erythrocytic stages appear in the cattle,
sometimes after a prolonged prepatent period. Attenuation is complete when the
inoculated calves show no clinical manifestation (except sometimes a transient rise
in temperature), and neither schizonts nor erythrocytic stages are detected in
smears. After this stage, reversion to virulence of the schizonts was not observed
during further subcultivation. According to some authors, no indications were found
to suggest that the time required for attenuation depends on the initial virulence of
the schizonts, but other authors reported that a longer period of subcultivation was
required for highly virulent isolates. Since these results were not obtained under
identical conditions, other factors may have influenced the attenuation process.

Although the nature of the attenuation of virulence has not been fully elucidated,
several recent investigations indicate mechanisms that may be involved in this phe-
nomenon. Schizont-infected cells produced a series of proteases that were not
detected in uninfected bovine mononuclear cells, and the enzyme activities of the
infected cells varied between cell lines. Long-term culture of infected cell lines
resulted in a gradual and substantial reduction in the activity of the proteases. It
was suggested that since proteases may be involved in the pathogenic effect of the
schizonts, the reduction of their activity may contribute to the decrease in virulence
and pathogenicity. Attenuation has also been associated with reduced ability of
the schizonts to differentiate into microschizonts and to produce merozoites. This reduced ability might be adequate with culture passages, but on the other hand,
in vivo passaged schizonts may lose their ability to produce merozoites while con-
serving various degrees of virulence.

The results of a recent investigation demonstrate that schizont-infected cells
lines derived from theileriosis-sick cattle are initially more virulent than correspond-
ing cell lines obtained by in vitro infection of peripheral blood leukocytes with
sporozoites. Selection of parasite populations and reduced transcription of particular
genesis of parasite origin appear to occur during in vitro cultivation of schizonts. Two distinct novel parasite antigens on the surface of the parasitized mononuclear
cells were detected by monoclonal antibodies; one was present in all passages of the
cell lines examined, the other recognized macroschizonts and part of the cell surface
of lines that cause severe theileriosis. It appears that attenuation was accompanied
by altered schizont gene expression, resulting in reduction of the antigenic compo-
nents. This loss of antigens may explain the reduced immunogenicity of attenuated
schizonts compared with the parent virulent organisms.77

PREPARATION AND TESTING OF THE SCHIZONT VACCINE

The description of the techniques used for large-scale production of the schizont
vaccine for tropical theileriosis is beyond the scope of this paper. However, the basic
principles of preparation and testing of the vaccine are described here. Most of the
particulars reflect the Israeli experience in production and application of the schizont
vaccine.78,79

Before starting vaccine production, “seed material” with known characteristics is
required. Three types of seed material are distinguished: the master seed, the work-
ing seed and the production seed. The master seed represents schizont-infected cells
from a specific passage that have been selected and permanently stored, and from
which all other seed passages are derived. The working seed represents schizont-
infected cells at passage levels between the master seed and the production seed. The
production seed represents schizont-infected cells from a specific passage level that
are used for the preparation of a fraction or batch of vaccine. For this purpose the
cells of the last passage of the production seed are propagated in stationary (mono-
layer) cultures or in suspension. The schizont-infected cells from all vessels are har-
vested, pooled, and the total number is computed. Alternatively, about 20% of the
cells may be seeded again to prepare another fraction of vaccine. Several fractions
can be produced using a portion of the production seed as working seed. Since pro-
longed subcultivation may generate alterations in the features of the schizonts, such
as immunogenic capacity, after several fractions, additional batches of vaccine are
produced again from the master seed.

The vaccine is stored in liquid nitrogen in 1.8- or 3.6-ml vials containing 10 or 20
doses, respectively. The proper number of infected cells per dose is a subject of con-
troversy.80–83 It appears that virulent schizonts are more infective than attenuated
ones, and infectivity might be inversely proportional to the degree of attenuation.

Testing of T. annulata schizont vaccine has been discussed elsewhere, but
54,78 only recently have internationally accepted standards been published.54 The frozen
vaccine is usually produced in batches of several thousands of doses, which makes
the full testing of each batch impracticable for economic reasons. It is, therefore, rec-
ommended that the master seed be tested for safety and efficacy, and that each fraction
be tested for sterility and potency. This recommendation is based on the fact that once
the cultured schizonts became attenuated, reversion to virulence was not observed
during further cultivation. As far as efficacy is concerned, no obvious alterations in
the immunogenic properties are expected during a limited number of passages.
Safety

Safety (freedom from properties causing undue local or systemic reactions) is tested by inoculation of susceptible cattle with a tenfold greater dose than that recommended for immunization. This dose should not produce clinical manifestation beyond a transient rise in temperature. With vaccine produced from completely attenuated master seed no schizonts or erythrocytic merozoites are seen in the immunized cattle.

Efficacy

Efficacy (the capacity to protect against naturally transmitted theileriosis) is tested by challenging cattle that have been immunized with a standard dose, by infecting them with sporozoites. The mode of sporozoite infection (tick bite or inoculation of macerated infected ticks) is subject to controversy, since macerated ticks paradoxically generate a more severe disease than live ticks.

Potency

Potency (viability of schizont-infected cells) is tested by assessing the plating efficiency of the schizont-infected cells.85

Sterility

Sterility (freedom from viable contaminating organisms) is usually ensured by specialized microbiological laboratories.

IMMUNE RESPONSE AND PROTECTION CONFERRED BY THE SCHIZONT VACCINE

According to the concept of premunition (coinfection immunity) formulated by Sergent et al.86 the immunity against *T. annulata* has been regarded as coincident with and dependent upon the continued presence of living parasites in animals that have recovered from infection.8 Culture-derived schizonts that have retained a certain degree of virulence multiply when inoculated into cattle and can easily be detected by conventional techniques. On the other hand, parasites are usually not detected after inoculation of completely attenuated cultured schizonts. It can be assumed, however, that such schizonts undergo only a limited number of multiplication cycles in cattle and, therefore, do not reach the size of population necessary for detection by microscopic examination.89

Recently, PCR was used for detection of the carrier state of theilerial infection that results in immunity. However, it appears that PCR performed with blood samples is dependent upon the presence of erythrocytic merozoites and not necessarily of schizonts. The detection of latent schizonts in recovered or immunized cattle remains a problem, since culture techniques usually detect schizonts only few weeks after recovery or immunization.87

Inoculation with culture-derived schizonts elicits production of antibodies that have been detected by the IFAT88 and ELISA techniques.29 Anti-theilerial antibodies
exhibited a neutralizing effect on sporozoites in vitro, preventing their penetration into bovine peripheral blood leucocytes. The IFAT based on schizonts derived from culture or infected cattle detected considerable levels of antibodies in cattle immunized with attenuated schizonts, which provides a means for assessing the results of the vaccination. Although the antibodies detected were not necessarily an indication of immunity to infection, the very fact of the presence of this antibody indicates that a multiplication of schizonts occurred in the vaccinated animals.

However, cell-mediated immunity appears to play the major role in the protective immune response against virulent T. annulata parasites. Recovery of cattle from tropical Theileriosis has been attributed to the acquisition of cellular immune response rather than anti-T. annulata antibody. Lethal infections are characterized by a severe leucopenia associated with absence of pan-T, mature T, CD4+, and CD8+ cells as well as large mononuclear cells (monocytes and macrophages).

The phenotypic profile of cell lines derived from T. annulata-infected cattle proved to be similar to the profile of normal bovine cells infected in vitro by sporozoites. The phenotypic analysis of cells from tissues of infected cattle demonstrated that schizont-parasitized cells express the myeloid cell marker CD11b and the lymphoid antigen CD2. The cytokine profiles produced by T. annulata-infected mononuclear cells were characteristic of macrophages, but not of T- or B-lymphocytes. These profiles include interleukins (IL-1α, IL β, IL-6, and IL-10) tumor necrotic factor α (TNFα) and interferon (INF) types α and β. Macrophages from infected cattle spontaneously produce TNFα and nitric oxide (NO), which are known to be lethal to intracellular protozoa. Both factors inhibit the invasion of sporozoites into bovine cells in vitro.

Substantial evidence suggests that both innate and adaptive immune responses appear to be involved in engendering protective immunity against T. annulata theileriosis. The innate immune mechanism involves stimulation of macrophages to produce TNFα and NO, production of INFγ by macroschizont-infected cells, and cytotoxic natural killer cells. The adaptive immune response, in cooperation with innate mechanisms, mounts a directional T-helper 1 response, and production of TNFα, INFγ, and cytokines, which activate macrophages to induce NO, which, in turn, inhibit the development of trophozoites to schizonts and cytotoxic T-cell responses.

Culture-derived, attenuated schizonts engendered an almost total immunity against homologous virulent schizonts and a lesser degree of immunity against virulent heterologous schizonts. However, the immunity engendered to tick-transmitted infection is of greater practical interest. Various degrees of immunity have been observed in laboratory trials in which immunized calves were challenged by a bite from infected ticks or by a cryopreserved stabilitate of such ticks. The trials were performed with different Hyalomma spp. under variable conditions and with no exact calibration of the infective doses used. A proportion of the vaccinated cattle remained asymptomatic following the challenge infection, but in most instances transient fever and mild parasitemia occurred. A lesser degree of protection was witnessed when cattle immunized with attenuated schizonts were challenged with sporozoites originating from a relatively remote geographical area or were subjected to heavy tick challenge.
Field observations have shown that local, as well as exotic, cattle from theileriosis-free geographical areas, were protected by the schizont vaccine when introduced into theileriosis-infected pastures. According to the few data available, the duration of immunity induced by the schizont vaccine varies between one and three years. Field observations in Israel have shown that immunity after a single inoculation of schizont vaccine is not lifelong, and that revaccination might be required for cattle in herds with a low tick infection rate. Outbreaks of theileriosis have also been observed among cattle vaccinated with blood-derived vaccine. In practice the response to primary immunization with the schizont-infected cells is not influenced by the histocompatibility of the schizont-infected cells and the recipient animals. However, together with the immune response to the schizonts, an allogeneic response occurs to the cells carrying the schizonts. On revaccination the allogeneic responses can block the transfer of the parasite to the recipient animal and prevent the enhancement of immunity if the second vaccination is performed with the same culture-derived vaccine as the first. On the other hand, a heterologous schizont-infected cell line from low culture passage considerably boosted the immunity against sporozoite infection.

A technique that involves infecting cattle with sporozoites and then mitigating the response by chemotherapy was elaborated for immunization against *T. parva* infection. This technique, reviewed by Pipano, has also been evaluated for *T. annulata*, but without much attention to practical aspects.

**FUTURE PROSPECTS**

Tropical theileriosis is one of the first protozoan diseases against which a commercial vaccine, manufactured *in vitro*, has become available. However, more research should be invested in the development of an improved product. Additional efforts should be focused on the elucidation of the mechanism of the attenuation of the schizonts, especially the antigenic and immunogenic alterations that occur during the attenuation process. Specific questions concern the relationship between virulence and immunogenicity, and whether avirulent schizonts may, under specific conditions, induce a similar immunity to that induced by virulent blood-derived schizonts. Alternatively, techniques for reinforcement of immunity engendered by avirulent schizonts should be sought, as discussed elsewhere. The determination of the duration of the immunity induced by the schizont vaccine, and techniques for detecting a decrease in the immunity of vaccinated animals are of vital importance for protection of cattle against tropical theileriosis.

Consistent data have been accumulated to indicate that the allogeneic response generated in cattle after a primary immunization with a schizont-infected cell line can block parasite transfer and enhancement of immunity when a second immunization with the same cell line is administered. This can probably be prevented by a heterologous vaccine. However, an immunization regime that will allow effective revaccination of cattle has still to be elaborated.

The results summarized in this paper show that the live culture-derived schizont vaccine can adequately protect cattle against tropical theileriosis until more advanced techniques become available for vaccine development and production.
REFERENCES


