Theileria annulata: control measures, diagnosis and the potential use of subunit vaccines

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Summary: Theileria annulata is an important pathogen of domestic Bovidae across a broad region of the world with some 250 million cattle at risk. Our basic knowledge of the incidence and mortality is reviewed together with an outline of current control measures, including the use of acaricides, chemotherapy and attenuated cell line vaccines. The limitations and potential problems of these control measures are discussed and considered in relation to the use of a subunit vaccine.

Research over the last ten years on the protective immune response to the disease in both laboratory and field studies suggests that a humoral immune response to the sporozoite could be protective although the primary protective response appears to be mediated by cytotoxic T-cells recognising the macroschizont-infected lymphocyte stage. Recent work on the study of protective antigens using recombinant DNA and hybridoma technologies is discussed together with the potential advantages and problems of such approaches. Possible future developments in our understanding of the immune response and the identification of the relevant antigens are considered in addition to the use of new techniques for diagnosis and the analysis of parasite diversity.

KEYWORDS: Control methods - Diagnosis - Immunity - Recombinant DNA - Surface antigens - Theileria annulata - Vaccines.

INTRODUCTION

The protozoan parasite Theileria annulata infects wild and domestic animals across an area stretching from Southern Europe and Northern Africa, through the Middle East and Southern Russia to India and probably Southern China (37). It is transmitted by ticks of the genus Hyalomma (26, 38) and more than 250 million domestic cattle are at risk from the disease. The economic losses caused are difficult to calculate as no recent, extensive studies on the incidence, loss of production, or mortality are available. Such estimates are further complicated by the differences in susceptibility of Bos indicus cattle, imported Bos taurus cattle and crosses between these two breeds. Perhaps the most accurate data on mortality come from control animals in vaccination trials where figures of 27-40% mortality have been reported (30) although higher figures have been reported for the susceptible Bos taurus and lower figures (<5%).

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mainly in calves, for *Bos indicus*. The incidence of infection is difficult to assess; however, figures available from Turkey (40) showed that 18% of a random sample of 996 cows were infected while some 53% of cows brought to clinics at the Ankara Veterinary Faculty were infected. Similar high figures (64%) have been reported for animals brought to the Animal Diseases Diagnostic Laboratory, Anand, over a four-year period (46), although these cattle were all cross-bred or exotic. Such figures, although not extensive, suggest that the incidence of tropical theileriosis is high in endemic areas and that mortality can cause serious losses in exotic breeds or crossbred animals. This is without considering loss of productivity in those animals not suffering from a fatal infection.

These considerations highlight two main points: firstly, the need for more extensive data on the incidence, mortality and loss of production from tropical theileriosis and, secondly, the probable seriousness of the disease and therefore the need to consider the most effective means of control. In this review, we will briefly examine the available methods of control and diagnosis and then consider recent advances in our knowledge of immunity to the disease and the potential for new control measures and diagnostic techniques based on the application of recombinant DNA and hybridoma technology. Our aim is to highlight possibilities for the future so that those with practical experience, much greater knowledge of the field situation and detailed knowledge of current control (and diagnostic) measures can assess the possible value of these future developments.

**LIFE CYCLE**

The complex life cycle of *Theileria* is illustrated schematically in Fig. 1. The two main stages in the tick — gametogony and sporogony — have been described in detail elsewhere (7, 24) and we shall be primarily concerned with the stages in the bovine host. The bovine host is infected by the inoculation of sporozoites by infected ticks during feeding and the sporozoites invade leukocytes, probably within 5-60 minutes (22). Recent research (14, 51) has shown that *T. annulata* preferentially invades MHC-class II positive cells and not T-cells; however, cytotoxic T-cell lines can be infected at high densities of sporozoites (20). Thus, the parasite infects both monocytes and B-cells with high efficiency. These experiments were undertaken *in vitro* and further showed that the infected cells ceased to express a number of surface markers (49) including those for monocytes and B-cells (50), thus making it difficult to determine the cell type infected *in vivo*. After entry into the host cell, the sporozoite develops into the trophozoite which after nuclear division develops into the macroschizont, inducing the host cells to become large lymphoblastoid cells which divide in synchrony with the macroschizont. As a result of the parasite-induced lymphoproliferation, a large population of parasitised cells develops in the infected animal. These further develop into microschizonts and ultimately merozoites which are released from the lymphocyte. The merozoites invade erythrocytes and develop into piroplasms, this stage completing the life cycle within the bovine host. Although the lymphoproliferation has pathogenic consequences for the host, the anaemia induced by the destruction of the high levels of infected erythrocytes is probably the major pathogenic change in the infected animal (26).

A priori, a protective immune response could arise to either the extracellular stages (sporozoite or merozoite) or to antigens exposed on the surface of infected host cells.
FIG. 1
Life-cycle stages of *Theileria annulata*
(macroschizont or piroplasm stages). Protective immunity clearly occurs as cattle, after recovery from primary infection, are immune to homologous challenge and often to challenge with heterologous stocks of the parasite (reviewed in 16). Such immunity can be induced naturally as a result of sporozoite-induced infections (42), by artificial inoculation using ground-up infected tick stabilates (12) or by infection with attenuated culture-derived macroschizont-infected leukocytes (18, 30, 47, 52). The role of the immune response to sporozoites in protection has not been specifically evaluated; the ability to protect using macroschizont-infected cell lines implies that immune responses to the sporozoite in natural immunity may be limited, although Pipano (30) reported improved immunity if sporozoite immunisation was undertaken in addition to schizont-infected cell line immunisation.

**CURRENT CONTROL MEASURES**

The main control measures available for tropical theileriosis are acaricides (applied by dipping), chemotherapy with or without low levels of sporozoite-induced infection and vaccination by attenuated cell line vaccines. The latter control measure is by far the most widely used against *T. annulata*; successful cell line vaccines have been developed in Israel (30), Iran (18), India (47) and the USSR (52).

Although acaricides have been applied successfully against *T. parva* they have not been used extensively for the control of tropical theileriosis. There are a number of logistical and theoretical problems associated with their use:

(i) a highly organised dipping programme needs to be set up to ensure that all cattle are treated

(ii) long-term application can lead to acaricide resistance in ticks

(iii) if tick density is significantly reduced and the cattle become free of *T. annulata*, then over time these animals will become highly susceptible to the disease as a result of waning immunity due to the reduction in challenge. Thus, if infected ticks migrate into a treated area after cessation of acaricide treatment, many animals will become infected with a high level of mortality.

The major advantage of acaricide-based control methods is that simultaneously all tick-borne diseases are controlled, thus reducing the need for different measures for each disease.

Chemotherapy too has been used only sparingly for the treatment of *T. annulata* infections although the efficacy of drugs such as parvaquone and halofuginone (9, 41) is established and these drugs have been used successfully in the treatment of *T. parva* infections. However, Hashemi-Fesharki (18) reports that in Iran, treated cases can still die from acute disease and this has been reported by others (53). Despite reports (41, 46) of the effectiveness of chemotherapy, for many developing countries the cost of chemotherapy is considered to be too high in relation to animal health budgets and the system of livestock rearing.

The most widespread control measure used against *T. annulata* infection has been the use of attenuated macroschizont cell lines as vaccines. It has been clearly established that the vaccination of animals with such cell lines leads to the establishment of
infection and the transfer of the parasite from the donor cells to those of the recipient (2, 4). No reports are available to show that such vaccines 'break down' against either heterologous challenge (12) or from a decreased immunity with time. The vaccine lines can be cryopreserved (29, 47) for storage and transport, although once thawed the vaccine has a limited shelf-life unless further culture is initiated. The ability to cryopreserve the vaccine circumvents many of the problems of transport, although this is a potential limitation in some parts of the world. The limitations and potential problems of such cell line vaccines are primarily theoretical. However, they should be borne in mind as the use of the vaccine becomes widespread. The testing and long culture period needed to produce a vaccine is a limitation, as well as the possibility that indefinite culture may lead to the loss of the ability of the macroschizonts to transfer to the recipient cells, thus reducing the ability to immunise. Markers for attenuation and the definition of attenuation at a molecular level could speed production of cell line vaccines. A further potential problem is that vaccination on a large scale may lead to the infection of cattle which therefore would allow transmission to continue. This is probably not a problem where fully attenuated cell lines are used as these do not produce the infected erythrocyte stage (30) and so transmission cannot occur. However, if avirulent strains of the parasite are used for immunisation (27), the infected erythrocyte stage occurs after immunisation and vector transmission can occur. Furthermore, although cattle are protected by the attenuated cell line vaccine, it does not prevent the appearance of the erythrocytic stages when animals are challenged. If the parasite is maintained in this way, the potential for reversion to virulence or for antigenic variation to occur is present and, if either or both occurred, the consequences would be severe. In addition, one must also consider the possibility of transferring other diseases by the use of such vaccines, although this could be eliminated by appropriate screening (if available). Clearly the issues raised here need to be investigated in order to obtain further information and the improvement of the cell line vaccines. However, none of these considerations should detract from their undoubted value.

**DIAGNOSIS**

The simplest diagnostic test available is the use of either clinical signs or Giemsa-stained blood or tissue smears in order to detect either macroschizonts or piroplasms within an infected animal. As sick animals usually have relatively high parasitaemias, such tests are effective and have the added advantage of detecting active infections. In addition, indirect immunofluorescence antibody tests have been used, based on those developed for *T. parva* (5) using either piroplasms or cultured schizonts as antigen. In this context, antigens derived from schizont cell cultures (3) are suitable, as schizonts are relatively abundant in infections and thus infected animals produce high titre antibodies to this stage. The use of a serological test, however, suffers from the disadvantage that it is not possible to distinguish between animals with an active infection and those which are recovering or immune.

With the availability of monoclonal antibodies to both the schizont (44, 45) and the piroplasm stage (13), it would be possible to develop ELISA assays aimed at detecting circulating antigens or immune complexes and thus to detect active infections. Similarly, with the availability of cloned parasite genes (17, 23, 56) it would be possible
to develop sensitive methods based on DNA hybridisation to detect parasites within samples of blood or tissues. At the present time, the need to develop such sensitive tests for active infection has not been apparent. However, if low parasitaemias needed to be detected, such methods could have the sensitivity that would make them superior to the methods currently used. In this context the extra-chromosomal element described by Hall et al. (17) might be very useful as it is present in all life-cycle stages, all strains of T. annulata examined and in multiple copies per genome. This latter property would increase the sensitivity of any test using DNA hybridisation with a cloned fragment of the element.

**IMMUNITY AND IMMUNE MECHANISMS**

It is clear that animals can become immune to tropical theileriosis either naturally, by vaccination using macroschizont-infected cell lines or by immunisation with sporozoites (39, 48), although the latter method is almost certainly not based purely on an immune response to the sporozoite. These observations raise a number of questions: for example, what is the identity of the antigens involved, is the immunity cell-mediated or humoral and is the protective immune response directed at specific stages? The answers to such questions would provide a basic understanding of the immune response to tropical theileriosis which could be exploited to develop more effective methods of protection. To review our current state of knowledge concerning these questions, it is convenient to consider first what is known of the humoral response and, secondly, what is known of the cell-mediated response.

**Humoral response**

Much of the work on the role of antibody in protective immunity has been undertaken with T. parva (5) although there is no reason to believe that the conclusions reached do not also apply to T. annulata. Antibodies reacting with the macroschizont and piroplasm stages of the parasite are consistently observed but two sets of observations suggest that these do not play a role in the protective immune response. Firstly, such immune sera do not recognise the surface of infected mononuclear cells in T. parva (8) or T. annulata (43) nor the surface of T. annulata piroplasm-infected red blood cells (Glascodine, quoted in 16). On the assumption that reaction with the surface of infected host cells would be a prerequisite for antibody-mediated lysis of infected cells, it has been argued that this shows that the humoral response to these stages is not protective. Secondly, experiments using lysates or inactivated schizont material to immunise animals have not induced a protective response in either T. annulata (31) or T. parva (55). Similarly, attempts to immunise using extracts of piroplasm (6, 54) have failed. Thus, it has been generally concluded that the humoral response to these two major pathogenic stages is not protective. While this is a reasonable conclusion, it should be pointed out that none of the experiments conclusively exclude a role for the humoral response as the lack of protection using extracts of infected cells may be due to dose, presentation or a number of other reasons; however, the inability to demonstrate antibodies to the surface of infected cells is more compelling.

The possibility of a protective role for the humoral response to the sporozoite is a real one. *In vitro* studies using immune serum have shown that the invasion of
mononuclear cells by sporozoites can be prevented (15) and that this neutralising activity increases after sequential sporozoite challenge (33). The latter work demonstrated that two inhibitory mechanisms were operating, the first reducing sporozoite invasion and the second reducing transformation of trophozoite-infected cells to proliferating macroschizont-infected cells. On the assumption that the inhibition of sporozoite invasion is antibody-based, these results show that antibodies are generated to the sporozoite which could be protective. A similar inhibition of invasion has also been demonstrated using antibodies raised to an expressed, recombinant sporozoite surface antigen gene fragment (56), providing additional support and direct evidence for the potential protective role of antibody to the surface of the sporozoite. It is unknown at the present time what the relative importance of the humoral response to the sporozoite is in animals which have become immune under field conditions or whether a strong humoral response to the surface of the sporozoite would lead to protective immunity.

One further stage is exposed to the immune system, namely, the merozoite. Until very recently, it has not been possible to prepare merozoites in sufficient quantities and thus examine the role of anti-merozoite antibodies in protection. However, the ability to produce this stage of the parasite routinely in culture (11, 13) allows such studies to be undertaken, particularly if an in vitro red blood cell invasion assay can be established. No published studies are available on the humoral response to the infected red blood cell surface, although unpublished studies (Glascodine, personal communication) have shown that immune sera do not recognise viable infected red blood cells.

Cell-mediated response

Interest in the role of the cell-mediated response in protective immunity arose as a result of the apparent lack of evidence for a humoral response and from the demonstration, in T. parva, of the generation of both specific and non-specific cytotoxic cells in immune animals (28) and the demonstration that immunity could be transferred by inoculating susceptible cattle with leukocytes from immune animals (10). A large body of work with many interesting findings has been undertaken with T. parva (reviewed in 25) but as the research on this parasite species is described in another paper in this volume, the discussion here will be limited to work undertaken with T. annulata.

It has been shown that calves, recovering from infection initiated with sporozoites, generate cytotoxic peripheral blood lymphocytes (35, 39, 48) which lyse T. annulata-infected lymphoblastoid cells in vitro. Preston et al. (35) showed that the level of cytotoxic cells increased after challenge and that two peaks of cytotoxic cells are generated, the first being BoLA (the equivalent of bovine MHC Class I antigens) restricted while the second showed restriction in some animals but not in others. This latter non-restricted population has been considered to be due to the generation of natural killer cells by analogy to other systems. In the same study, two peaks of cytotoxic cells with similar properties were also observed in animals during infection and recovery prior to challenge. Such studies have led to the conclusion that the main protective response to the infected lymphocyte is mediated by cytotoxic T-cells and natural killer cells. Using Theileria-infected cell lines to immunise cattle followed by subsequent sporozoite challenge, it has also been shown that cytotoxic cells are generated which are stimulated on challenge (20, 34). In the absence of a detectable humoral response to the surface of the infected lymphocyte, it can be concluded that
the cytotoxic (presumed T-cell response) is one of the main protective immune responses to the infected lymphocyte; it should be noted, however, that cell transfer experiments have not been undertaken to prove this.

The work of Innes et al. (21) also elucidated the effect of match or mismatch of BoLA antigens between the donor cell lines used for vaccination and the recipient animals. Although cytotoxic cells were generated in both situations, the mismatched combination showed less severe disease and a high level of anti-BoLA cytotoxic T-cells was initially observed. Challenge of these animals with sporozoites primarily stimulated the cytotoxic anti-parasitised cell response. It is not clear, at the present time, why this 'graft rejection' type response should be associated with the very mild clinical symptoms observed (compared to the BoLA matched group), particularly as the parasite-infected cell-specific response was similar between the two groups. These results clearly warrant further investigation, as in another paper (19) the opposite effect is reported, i.e. the mismatched group showed more severe clinical symptoms. It is interesting to consider that a similar mismatch of BoLA types is likely to be encountered in the use of attenuated cell line vaccines as the probability of a match between the cell line used for vaccination and thousands of recipient animals is small. While it is clear that parasite-specific cytotoxic cell responses are generated in immunised animals, their appearance tends to be transient and therefore other effectors of the cellular immune system may be involved in protection. In this context, Preston (32) has provided data showing that adherent cells have a cytostatic effect on schizont-infected cell lines. It has been clearly demonstrated (34) that adherent cells (presumed to be macrophages) isolated from the peripheral blood of calves immunised using either schizont-infected cell lines or sporozoites, exhibit strong cytostatic effects on schizont-infected cell lines. The cytostatic cells were active against both autologous and allogeneic cell lines, were induced on challenge and shown to mediate the cytostatic effect via a soluble factor. The relative importance of the cytotoxic cells and the cytostatic soluble factor in protective immunity is unclear.

The importance of these two immune mechanisms in protective immunity needs to be established, as does the identity of the cytostatic factor and the mechanisms by which it is generated. In addition, on the assumption that these responses are induced by infection-specific neo-antigens on the schizont-infected cell surface, it is necessary to identify these antigens if the immune response is to be understood and potentially manipulated. In this context, studies with *T. annulata* have identified a number of polypeptide differences at the surface of the infected cells (43, 45) using a combination of infection-specific monoclonal antibodies, surface labelling and lectin affinity chromatography. Two glycoproteins of molecular mass 100-125 kDa and 80 kDa have been identified on the surface of the infected lymphocyte which are not present on uninfected cells; the 100-125 kDa molecule is recognised by the monoclonal antibody 4H5 (45) and has been shown to vary in size between different cell lines. Although it has been shown (36) that this monoclonal induces complement-mediated lysis of infected cells, the role of the infection-specific antigen in generating an immune response is unclear. In addition, it is unknown at the present time whether these infection-specific molecules are gene products of the parasite or host genome. As the primary cytotoxic response to infection is BoLA-restricted and probably mediated via cytotoxic T-cells, the antigens priming this response are likely to be small peptides presented in the context of the bovine MHC, rather than large polypeptides such as those identified to date. However, the role of these molecules in immune recognition and the response to the infected cell cannot be excluded until more data are available on their functional significance and structure.
One approach to both understanding and defining the immune response to *T. annulata* infection is the isolation, characterisation and production of specific antigens by recombinant DNA techniques. Furthermore, such antigens, if shown to be protective, could provide the basis for a subunit vaccine. The value of such an approach is primarily in defining and characterising the antigens recognised by the host as well as examining the immune response to antigens not normally 'seen' by the host immune system. Rather than discuss, in any detail, the arguments for and against the use of recombinant antigens as vaccines, we will confine ourselves to reporting the current state of knowledge as regards the characterisation of antigen genes and their products. A priori, four stages of the parasite life cycle in the bovine host are exposed to the immune system: the sporozoite, the macro schizont-infected lymphocyte, the merozoite and the infected red blood cell. From the studies described in the previous sections there is evidence for the role of antibody in blocking sporozoite invasion of leukocytes and for cytotoxic cells in lysing infected leukocytes but little evidence for protective immune responses to either the infected red blood cell or the merozoite. However, on a simplistic view that the surface of any stage of the parasite or parasitised host cell is a potential target for a protective immune response, studies have been initiated to define these surface molecules, isolate the genes coding for them and ultimately examine whether the immune response to them can be protective.

Monoclonal antibodies have been raised to the sporozoite (56) and screened for activity in an *in vitro* leukocyte invasion blocking assay (33); two monoclonal antibodies were identified as having activity in this assay and were used to screen a lambda gt11 genomic expression library in order to isolate the genes coding for the epitopes recognised. One of these monoclonals (1A7) recognised two recombinant clones which were isolated and subjected to further characterisation. These studies have led to the isolation of the complete gene for this sporozoite antigen which has now been completely sequenced (Hall, unpublished observations). Results from these studies show that antibodies raised (in rabbits) to the polypeptide encoded by a 300bp fragment of the gene (expressed as a recombinant antigen) block invasion of leukocytes by sporozoites and recognise a complex of polypeptides derived by proteolytic processing of a single gene product (56; Hall, unpublished). The gene is present as a single copy in the genome but shows restriction fragment length polymorphisms between the genotypes within a single stock and between isolates from different geographical regions. The gene is expressed to a significant degree only in the sporozoite and not in the schizont or piroplasm stages. Current research is aimed at expressing the complete gene and assessing both the response of the bovine host and the role of this response in protection.

Attempts to isolate the genes coding for the infection-specific polypeptides on the surface of the infected lymphocyte (see previous section) have been unsuccessful and, until this is achieved, it will be difficult to define their role, if any, in the immune response. Monoclonal antibodies have been generated to piroplasms and shown to react with *in vitro*-derived merozoites but not the macro schizont (13); one of these monoclonals has been shown to react with the merozoite surface and detects an abundant antigen of molecular mass 30 kDa. These studies, by analogy with those undertaken with the sporozoite, raise the possibility of isolating the genes determining the surface polypeptides of the merozoite and then examining their role in protective immunity.
Thus, at a molecular level, studies are currently aimed at the isolation of the genes for surface antigens of the sporozoite, merozoite and those specific to the infected lymphocyte. Once these have been isolated and expressed, further studies can be initiated on the protective role of the immune response to these antigens. It will also be at this point that the possibilities of a subunit vaccine can be seriously examined and factors such as host immune responsiveness, antigenic diversity of the parasite and use of adjuvants considered.

MECHANISMS OF ATTENUATION

At the present time little is known of the mechanism by which continuous culture of schizont-infected cell lines leads to attenuation. However, as the attenuated cell line vaccine is being applied extensively in a number of countries, it is worth considering and investigating the mechanisms of attenuation in order both to improve the production of such cell lines and to examine whether attenuation is a stable character of the inoculated schizonts or whether such parasites could revert to virulence. Attenuation is defined on the basis of the reduced clinical symptoms of the disease when naive animals are infected with cell culture lines. It is unclear whether this is as a result of reduced virulence or increased immunogenicity, but it is worth briefly reviewing the experimental observations that have been made during attenuation of cell lines before going on to consider a general model of the potential factors altered in attenuated lines, proposed as a basis for future investigation.

Early studies (1, 42) had shown that there were naturally occurring strains of the parasite which had low virulence and these were used as part of the vaccination strategy in Israel (30). It was noted in these studies that serial passage through calves resulted in the loss of the ability to produce merozoites and, furthermore, that prolonged passage could lead to the total loss of infectivity of the schizont to inoculated animals. More recently results of experiments using infection with schizont-infected cell lines established in vitro from sporozoites have shown the existence of avirulent lines (27) without prolonged passage, although some lines derived from the same stock of parasites were virulent. Overall these studies show that virulence varies between different stocks of the parasite and that if stocks of low virulence can be isolated they can be used to provide protection without needing to be attenuated.

The attenuated cell lines used in Israel, Iran and India have been developed by continuous passage in culture and tested by infection of calves after different passage levels. The general observation is that mortality and the severity of clinical symptoms decreases with increasing passage number until, after several months to two years, virulence was completely lost as was the ability to produce the erythrocytic stages of the parasite on inoculation of susceptible calves (30). Similarly the two strains used in Iran (18) have lost the ability to produce the erythrocytic stages. During intermediate stages of passage, these cell lines still produce the intra-erythrocytic piroplasm stage of the parasite. Although not specifically reported (27), the naturally less virulent non-attenuated lines most likely produce the erythrocytic stages in infected animals but at lower levels than virulent parasites. Such lines, therefore, differ significantly from fully attenuated cell lines. These clinical observations lead to a number of conclusions:
(i) fully attenuated cell lines do not produce the erythrocytic stages of the parasite
(ii) attenuated and avirulent cell lines infect the cells of the recipient animal
(iii) virulence is a phenotype which shows variation between different strains or isolates of the parasite but can be distinct from full attenuation
(iv) prolonged syringe passage using calves can lead to the complete loss of infectivity of the macroschizonts to the recipient animal.

These observations suggest that a number of processes are involved in prolonged passage and that the mechanisms of attenuation may be complex. The potential factors involved in attenuation are illustrated diagramatically in Fig. 2. When a macroschizont-infected cell line is used to infect or immunise an animal, the initial events involve the transfer of the schizont by an unknown mechanism to the recipient monocytes (a) which will then express infection-associated antigens (b) and proliferate (c) as a result of parasite infection. At a later stage of the infection the schizont differentiates within the lymphocyte and yields merozoites (d) which are liberated and subsequently invade (e) red blood cells. In addition, a cellular immune response is generated both to the injected cells (f) and to the infected recipient cells (g). Alteration in the level or rate of any one of these processes (a-g) would potentially affect virulence and could be altered during passage to yield an attenuated cell line. The level of the immune response could potentially be modulated by either the number of infected recipient cells generated (determined by the rates of (a) and (c)) or by the level and nature of the infection-associated antigens (b). In the case of the fully attenuated cell lines prolonged culture has resulted in the cessation of either (d) or (e) although whether this is the sole reason for the reduced virulence remains to be examined. In the non-attenuated avirulent cell lines, reduction of the rate or level of any one of these processes could potentially lead to reduced virulence.

Although this model does not define what is altered in attenuated lines, it sets out a conceptual framework within which, by experimental measurement, it should be possible to define the alterations determining attenuation. While this may seem, at first sight, to be an academic exercise, understanding the mechanism of attenuation could have practical consequences in terms of producing attenuated cell lines more rapidly and cost-effectively.

CONCLUSIONS AND PROSPECTS

Our knowledge of the immune effector mechanisms involved in protective immunity and the mechanisms of attenuation are clearly rather limited and a considerable further research effort is required to understand and define these mechanisms more fully. Given that the results obtained with the cell culture vaccine suggest that this is a very effective means of disease control, one may question whether such research effort is warranted. However, there are a number of drawbacks to these vaccines not least of which is that they do not prevent the transmission of the disease, a factor which raises the possibility of the parasite circumventing the immunity. In this context, further understanding of what is involved in attenuation is required so that improvements can be made in the generation and production of attenuated cell lines. Recent research using recombinant DNA techniques has led to the isolation
FIG. 2
Factors potentially involved in attenuation
of a sporozoite surface antigen gene and future work is directed towards the isolation
of merozoite surface antigen genes. The ability to produce recombinant antigens in
quantity to these two invasive stages will allow the examination of the immune response
to them and the assessment of its protective value. Potentially, a vaccine based on
such antigens would be of considerable value as it would be cheap to produce and
likely to have a long shelf-life.

The application of both recombinant and hybridoma technologies has produced
a series of reagents (DNA probes and monoclonal antibodies) with which both parasite
diversity and numbers could be determined at a high level of sensitivity. Further
technical development of such reagents would be required before they could be used
in the field. Before this is undertaken, it would also be necessary to assess whether
current diagnostic methods needed improvements. The high levels of parasitaemia
encountered in infected exotic breeds and cross-breeds, argues that current diagnostic
methods are satisfactory and, as the current focus is on disease control rather than
eradication, the need to identify all infected animals is limited. However, if a vaccine
was developed which produced sterile immunity, then the prospect of eradication of
the disease would become a possibility and would generate the need to identify all
infected animals and hence more sensitive diagnostic methods.

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THEILERIA ANNULATA: MESURES DE PROPHYLAXIE, DIAGNOSTIC ET
PERSPECTIVES POUR L'EMPLOI DE VACCINS SOUS-UNITAIRES. – A. Tait et
F.R. Hall.

Résumé: Theileria annulata est un important agent pathogène des bovidés
domestiques, présent dans une vaste partie du monde où il représente un risque
pour 250 millions d'animaux. Les informations de base sur l'incidence et la
mortalité sont passées en revue ainsi que les mesures de prophylaxie actuelles,
y compris le recours aux acaricides, à la chimiothérapie et aux vaccins préparés
à partir de souches atténuées. Les limites et les problèmes potentiels de ces
mesures prophylactiques sont examinés et comparés à ceux résultant de l'emploi
d'un vaccin sous-unitaire.

Les recherches menées ces dix dernières années sur les réactions de défense
immunitaire, tant en laboratoire que sur le terrain, laissent à penser que la
réponse humorale vis-à-vis du sporozoite pourrait jouer un rôle protecteur bien
que la principale réponse immunitaire semble être assurée par l'intermédiaire
des cellules cytotoxiques T qui réagissent dès que les lymphocytes sont infectés
par les macroschizontes. Des études récentes sur les antigènes qui ont un pouvoir
protecteur, menées à l'aide de technologies faisant appel à l'ADN recombinant
et aux hybridomes, font l'objet d'une discussion ainsi que les avantages et les
inconvénients inhérents à ces approches. Les perspectives de progrès en matière
de connaissance de la réponse immunitaire et d'identification des antigènes en
cause sont évoquées, ainsi que l'emploi de nouvelles méthodes de diagnostic
e d'analyse de la diversité de ce parasite.

MOTS-CLÉS : ADN recombinant - Antigènes de surface - Diagnostic -
Immunité - Méthodes de prophylaxie - Theileria annulata - Vaccins.

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THEILERIA ANNULATA: MEDIDAS DE PROFILAXIS, DIAGNÓSTICO Y PERSPECTIVAS DE LA UTILIZACIÓN DE VACUNAS SUBUNITARIAS. – A. Tait y F.R. Hall.

Resumen: Theileria annulata es un importante agente patógeno de los bóvidos domésticos presente en gran parte del mundo que significa un riesgo para 250 millones de animales. Los autores dan cuenta de las informaciones básicas de que se dispone sobre incidencia y mortalidad y pasan revista a las medidas de profilaxis actuales, como el recurso a acaricidas, la quimioterapia y las vacunas preparadas a partir de cepas atenuadas. Estudian los límites y problemas potenciales de esas medidas y los comparan con los que presenta la utilización de una vacuna subunitaria.

Las investigaciones de campo y en laboratorio sobre reacciones de defensa inmunitaria que se llevaron a cabo durante los diez últimos años permiten pensar que la respuesta humoral al esporozoito podría desempeñar un papel protector, aunque la principal respuesta inmunitaria parece obtenerse por medio de las células T citotóxicas que reaccionan en cuanto los linfocitos son infectados por los macroesquizontes. Los autores discuten las ventajas e inconvenientes de estudios sobre antígenos con poder protector realizados recientemente a partir de técnicas que recurren al ADN recombinante y los hibridomas y se refieren a las perspectivas de progreso en el conocimiento de la respuesta inmunitaria y en la identificación de los antígenos, así como al uso de nuevos métodos de diagnóstico y de análisis de la diversidad del parásito en cuestión.


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REFERENCES


