IMMUNOLOGY AND IMMUNOLOGICAL CONTROL OF TROPICAL BOVINE THEILERIOSIS

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Tropical bovine theileriosis, a tick-borne disease caused by the protozoan parasite, *Theileria annulata,* is probably the most important of all the tick-borne disease of domesticated cattle. This disease is of major economic importance in Asia and has always been a formidable barrier to the survival of exotic and crossbred cattle in India. Losses of US $800 million per annum due to tropical theileriosis are reported in India (Devendra, 1995). In view of the importance of the disease, the immune response of cattle to theileriosis has to be reviewed first, in an attempt to understand the acquisition of immunity to theileriosis.

Immune response to *Theileria annulata*

Animals undergoing primary infections with *T. annulata* develop serum antibodies capable of neutralizing the infectivity of sporozoites in vitro (Gray and Brown, 1981). Although antibodies to schizont infected cell are present in serum of animals that have recovered from infection with T. annulata, these antibodies seem to be directed at the parasite itself, rather than the infected cell and so there is no correlation between parasite specific antibody titres and the degree of protection (Pipano, 1981). Similarly, piroplasm and merozoite specific antibodies are detectable in recovered animals, but they do not contribute to the protection that is observed in immune animals. This led to the belief that immunity is probably cell mediated and studies have shown that cell mediated immune responses directed against the antigens on the surface of the macroschizont infected cell are important in immunity.

There is strong evidence that cell mediated immune responses, which involve T lymphocytes, are essential for the recovery and protection of cattle from *T.annulata* infection. The effector cell that mediates these responses is likely to be a subpopulation of T lymphocytes known as Cytotoxic T Lymphocytes (CTL). T lymphocytes cannot recognize free antigens but require that antigen first be processed to peptide fragments by an antigen-presenting cell and expressed on the cell surface in association with major histocompatibility molecules. The latter are

glycoproteins encoded by a family of genes known as the Major Histocompatibility Complex (MHC). Cytotoxic T lymphocytes recognize foreign antigens that have been synthesized within the target cell only when these are found in association with Class I MHC molecules. In contrast, helper T cells, the other major subpopulation of T lymphocytes, recognize external soluble antigens that have been taken up and processed by antigen presenting cells before being expressed on their surface in association with class II MHC molecules. The antigen receptor on T cells recognizes a combination of antigenic peptide and self MHC molecules, and CTL from an immune animal will identify and destroy only infected cells carrying MHC molecules identical to its own. This phenomenon is known as MHC restriction.

Infections with *T. annulata* stimulate innate and adaptive immune responses. Protective immunity to *T. annulata* depends upon cooperation between innate (T cell independent) and adaptive (T cell dependant) immune responses. The innate immune responses stimulated by infections with *T. annulata* sporozoites or schizont infected cells include activated cytostatic macrophages which suppress the proliferation of schizont infected cells and produce tumour necrosis factor, nitric oxide and natural killer cells, that lyses schizont infected cells. Adaptive immune responses include T cells cytotoxic for schizont-infected cells, antigen sensitized lymphocytes and CD4+ T cells. Adaptive immune responses promote macrophage anti-*Theileria annulata* activity (Preston, 1998).

The mechanisms that regulate immunity to *T. annulata* appear to be further complicated by the nature and behaviour of parasitized mononuclear cells. First infected cells can stimulate uninfected macrophages and cytokines to produce cytokines. Secondly, infected cells might induce infected natural killer cells (NK) to become lytic and to produce interferon (IFN-γ), indirectly causing macrophages to synthesize nitric oxide and induce T helper into CD4+ or CD8+ cells. Although many types of cells are invaded by sporozoites not all transform into continuous growing cell lines. Before we go into factors that control this transformation the type of cells that *T. annulata* transform should be known. *T. annulata* infects and transforms macrophages, monocytes, B cells but never T cells. In contrast, *Theileria parva* infects mainly T cells and B cells and not macrophages. *Theileria* infected cells are not only transformed but are also metastatic. This host cell transformation is reversible and the host cell can revert back to its resting state following the death of the parasite by treatment with antiparasitic agents. The alterations to the host cell apparently do not involve such permanent

changes to the host cell genome such as mutations or chromosomal translocations.

The ability of *Theileria* transformed cells to become metastatic is due in part to the parasite inducing the host cell to secrete the matrix metalloproteinase MMP9. At this juncture, it is worthy to note that *Theileria* is the only eukaryote known to induce uncontrolled host cell proliferation or in other words immortalize the host cells. The host cell and schizont divide in synchrony resulting in the clonal expansion of infected cells. Schizont infected cell lines behave like immortalized cells, exhibiting several phenotypes characteristic of cancerous cells. Association of the schizont with the host cell nuclear spindle ensures that daughter host cells remain infected during cytokinesis. Although schizont and host cell divide synchronously, schizont DNA synthesis occurs as the host cell enters mitosis and is immediately followed by division when the host cell is in metaphase. The host could counter this uncontrolled host cell replication by initiating its own cell death termed as programmed cell death or apoptosis. The parasite evades this by activating NFaB pathway which plays a crucial role in the survival of *T. annulata* transformed cells by conveying protection against an apoptotic signal that accompanies parasite mediated transformation.

Immunodiagnosis

The most commonly used serum antibody assay for *Theileria* has been the schizont antigen indirect fluorescent antibody test (IFAT). Although widely applied, the test is cumbersome and relies on the subjective observation of degrees of fluorescence. Secondly, it lacks specificity in that it crossreacts with other *Theileria* species. ELISA allows the processing of large numbers of samples than the IFA, and being objective suffers less from inter operator variation. Improved diagnosis of theileriosis can be achieved using monoclonal antibodies, DNA probes and Polymerase chain reaction. MAbs to *T. annulata* have been produced against stage specific antigens viz., sporozoite, schizont and piroplasm. MAbs are widely used which permits the selection of highly specific antigens by recognition of unique epitopes or with the appropriate antibody. Such antibodies can directly be used in antigen trapping or sandwich ELISA to detect persistent circulating antigens in an infected animal. ELISA using specific MAbs that specifically react with parasite antigens having a molecular mass of 32 kDa is employed for detection of T. annulata. A highly sensitive test such as the Polymerase chain reaction

(PCR) is useful when low numbers of parsite are present as in a carrier animal.

Immunization

In the early 1920s, blood from cattle infected with milder stocks of the parasite was used to immunize cattle. Subsequently, an improved method of immunization was made possible by the establishment of *Theileria* schizont infected lymphoblastoid cell lines. This in vitro system consisted of immortalized schizont infected mononuclear cells, lymphoblastoid in nature in which the schizont and host cell divisions were synchronous. *Theileria* infected cells grown in vitro were used by Pipano (1981) to infect and immunize cattle. After a period of culture, the parasite lost its ability to produce merozoites, when inoculated into cattle. After prolonged culture, there is a loss in the virulence and this allowed the development of a cell culture vaccine, which was used first in Israel and subsequently in other countries, including India (Singh, 1990). An interesting feature of *T. annulata* culture induced infections of cattle is that unlike infections induced by sporozoites, they are not quantum dependant. In practice, 1 ml containing 10^6 cells is used in order to ensure that infectivity, which is reduced by attenuation is maintained. The cultures are cryopreserved and used for immunization on resuscitation. A similar cell culture vaccine could not be developed for *T. parva* owing to MHC class I restriction.

As T. annulata is an intracellular parasite, conventional approaches to identification of parasite antigens have not been very successful. Only a handful of genes have been sequenced, and to date only one sporozoite-specific surface protein (p67) that can be targeted by antibody has been identified. Likewise, parasite antigens expressed in schizont-infected cells have been difficult to identify. Analysis of the complete genome sequence, along with micro array based studies of gene expression throughout the life cycle, will be used to identify antigens for vaccine development.

A recombinant vaccine will have distinct advantages of stability (obviating the need for cold chains), being nonliving (preventive reversion to virulence or contamination) and or having a defined composition with simple quality control. The three potential targets for such a vaccine are the sporozoite, (to reduce the infective dose), the schizont (to control proliferation of infeted mononuclear cells and pathology) and the merozoite/piroplasm (to reduce infection of erythrocytes and anaemia,

and to limit transmission. Recombinant vaccines against *T. annulata* should comprise of a number of antigens viz., Sporozoite antigens that will initiate CD^4 T cell responses that will generate parasiticidal cytokines, such as tumour necrosis factor α and interferon (INF-γ) or activate macrophages to produce nitric oxide. Schizont antigens that will stimulate CD4+ T cells to produce interleukin 2 and IFN-γ. The IL 2 will enhance T cell proliferation and the IFN-γ will activate macrophages to produce factor such as tumour necrosis factor α and nitric oxide and IL-12 to activate natural killer cells to produce interferon (INF-γ). Antigens stimulating the production of cytotoxic T cell that will eliminate schizont infected cells and generation parasiticidal products like INF-γ, TNF-α and NO. Immunization with sporozoites or cell lines confers resistance to stocks from distant geographical regions, presumably because both cytostatic macrophages and cytotoxic T cells act on heterologous parasites, encouraging the hope that a single recombinant vaccine could be effective throughout endemic areas. The partial protection elicited by the defined stage specific antigens encourages the view that a recombinant vaccine against *T. annulata* is a distinct possibility. Trials with two recombinant antigens, SPAG-1, (from the sporozoite surface) and TAMS (from the merozoite surface) induced partial protection against sporozoite or merozoite challenge, rerspectively, but efficacy depended upon the delivery system. Although vaccinia recombinants have been shown to be efficicacious, none has yet to be licensed. Experiments have shown that delivery system based on Salmonella typhimurium can induce protective antibody and and T cell mediated responses to antigens. These subunits vaccine were given with adjuvants such as saponin or ISCOMs. In trials with SPAG significant levels of anti-SPAG antibodies, sporozoite neutralizing antibodies and T cell proliferative responses were obtained but did not correlate with the level of protection induced. Similarly, in trials with TAMS, protection and antibody production were not correlated. Analysis of the complete genome sequence, along with micro array based studies of gene expression throughout the life cycle, will be used to identify antigens for vaccine development.

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