



# Cattle Specific Immune Mechanisms used against the Protozoan *Theileria annulata*

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**Abstract**— *Theileria annulata*, the causative agent of tropical theileriosis, is an intracellular protozoan parasite transmitted by ticks of the genus *Hyalomma*. This tick-borne disease (TBD) exerts a high impact on livestock production in many developing tropical and subtropical countries. With an intricate life cycle and wide distribution around the world, many advances were made to restrict the impact and to control this TBD through the use of acaricides, chemotherapy and attenuated vaccines. However, an overreliance on these chemicals has meant new approaches for developing more effective vaccines are needed. Decades of studies support the idea that the humoral immune response elicited against the sporozoite stage of the tick life cycle may protect the host from infection. Further protective responses provided by cytotoxic T-cells, macrophages, and Natural Killer cells have also been identified as critically important during *T. annulata* infection. Here our focus will be the bovine immune response upon *T. annulata* infection, particularly the differential humoral and cellular immune responses. Our aim is to highlight the importance of the mechanisms potentially involved in protective immunity as well as significant findings, which may be incorporated into novel strategies for tropical theileriosis control.

**Keywords** — *Theileria annulata*, tropical theileriosis, immune response, humoral response, cellular response, cattle.

## I. INTRODUCTION

THE phylum Apicomplexa is comprised of eukaryotic obligate parasites of vertebrate and invertebrate hosts.

Morphologically, they are characterised by the presence of an apical complex with secretory organelles. This feature may be involved in the invasion and/or establishment of the parasite in the host species [1]. Within this phylum, the group Piroplasmorida, known as piroplasms because of their pear-shaped intraerythrocytic stage, includes the *Theileria* genera transmitted by ixodid ticks [1]. Although several species of *Theileria* are able to infect cattle, *T. parva* and *T. annulata* are the most pathogenic and economically important of domestic livestock in Old World tropical and subtropical regions [2].

*Theileria annulata* are tick-borne protozoan that infects wild and domestic Bovidae, transmitted by ticks of the genus

*Hyalomma* (mainly *Hyalomma anatolicum anatolicum*) and are responsible for the lymphoproliferative disease called tropical theileriosis [3]. This pathogen, thought to have originated from the Asian water buffalo (*Bubalus bubalis*), can be found in several regions of the world [4]. Cattle are more susceptible to *T. annulata* infection, but the disease can also occur in yaks, water buffalo, camels, sheep and goats, though is usually subclinical. In susceptible cattle introduced to endemic areas or crossbred animals, losses in production and mortality rates, varying from 40 to 90% depending on the country, are major concerns [3, 5, 6]. In the susceptible *Bos taurus* species, infection with *T. annulata* induces a severe inflammatory response, leading to high levels of fatality; whereas in the *Bos indicus* species, the Sahiwal, that lives in endemic areas, the pro-inflammatory cytokine dependent acute phase response is controlled and survival rates are higher [4]. In the mammalian host, *T. annulata* infection occurs through the sporozoite stage present in the saliva of a feeding tick and undergoes sequential development within the mononuclear cells where the macroschizont stage develops, with posterior invasion of the erythrocytes [7]. Tropical theileriosis might manifest as a subclinical to an acute disease, occasionally fatal, and accompanied by anaemia caused by the destruction of high levels of infected erythrocytes. Other clinical changes that can occur in these animals include: leukopenia, inappetance, cachexia, mucous membrane discharge and haemorrhagic diarrhoea, amongst other signs [5, 8]. Animals that recover from the disease are supposedly protected against homologous *Theileria* strains [9].

In this review, we will explore the current state of knowledge regarding the bovine immune response to *T. annulata* during the intricate interaction between this protozoan and its host, describing the differential humoral and cellular immune responses. To date, methods of controlling ticks and tick-borne diseases rely heavily on chemical acaricides; however, their continued use is unsustainable due to widespread cross-species resistance and growing environmental concerns [10, 11]. *In vitro* culture derived-vaccines, known as attenuated vaccines, are efficient at preventing theilerioses but they are not used on a large scale [15]. Alternative cost-effective and environmental-friendly control measures such as more effective and reliable vaccines are urgently needed. Thus, our objective is to highlight the importance of the mechanisms that might be involved in protective immunity and discuss key findings aiding the development of novel vaccine-based strategies to control tropical theileriosis.

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## II. CATTLE SPECIFIC IMMUNE MECHANISMS USED AGAINST *THEILERIA ANNULATA*

### A. *Theileria annulata* distribution and life cycle

Tropical theileriosis affects *Bovidae* and is present in most of the areas where the tick vector of the genus *Hyalomma* exists. Widely distributed in tropical and subtropical regions of the world, this disease has been reported through the years in areas of India, Middle East, North Africa, Russia, Southern Asia, and Southern Europe [3, 12].

The *Theileria* spp. life cycle is very complex and includes three typical phases: schizogony, gametogony and sporogony. Like all intracellular parasites, *T. annulata* transmission and survival is dependent on the ability of the invasive stages - sporozoites and merozoite in the mammalian host, and the zygote and kinete in the tick vector - to recognize and invade specific host cells [2]. Here, our main focus is to describe these stages in the bovine host. Approximately 3 to 5 days after tick attachment, infection of the vertebrate host occurs when a *Theileria*-infected nymph or an adult tick inoculates infective sporozoites in the saliva during a blood meal [2]. The sporozoites then invade a diverse range of cells, including peripheral blood monocytes, bone marrow-derived macrophages, and lymphocytes; inducing them to proliferate in an unregulated way [13]. This results in a rapid clonal expansion of parasitized cells in the lymphoid tissues [2, 5]. In the cytoplasm of host cells the sporozoite develops into trophozoite, which after nuclear division, develops into the intracellular macroschizont stage [2]. The macroschizonts stimulate host cells to undergo a rapid synchronised cell division and become large lymphoblastoid cells. Here the protozoa ultimately develops into the merozoite stage [3]. When the infected cells rupture, the merozoites become free and are released to actively penetrate erythrocytes, forming piroplasms approximately 8 days after infection [2, 3]. The parasite completes its life cycle within the vertebrate host where most relevant pathogenic effects occur during the phase of intralymphocytic schizogony, also called the erythro-destructive stage, leading to anaemia and often to death [14].

### B. Humoral immunity

Host immunity towards *T. annulata* is a necessarily elaborate response due to the combination of a complex parasite life cycle and diverse antigenic heterogeneity. Each of the parasite life stages might display a distinctive group of antigens that require a specific immune response from the host. Likewise, the immune response triggered against one developmental stage may not be effective or provide protection against other stages [15]. Protective immunity seems to be determined by a variety of immune responses against the sporozoite or merozoite extracellular stages, or against the antigens exposed on the surface of macroschizont or piroplasm-infected cells [3].

The humoral immune response is triggered after *T. annulata* infection with the synthesis of antibodies that recognise surface epitopes of the sporozoite. In a preliminary study on the effect of immune cattle sera against *T. annulata* sporozoites, a clear

neutralization of sporozoite infectivity was observed when cultured lymphocytes were incubated at 37 °C with bovine serum from uninfected animals. It was also observed that the transformation of lymphocytes into macroschizont-*T. annulata*-infected transformed lymphoblastoid cells was inhibited [16]. Similarly, in a different study it was found that the monoclonal antibody 1A7 was able to inhibit approximately 66% of sporozoite invasion, whereas other parasite stages, such as macroschizont were not recognised by the antibody [7]. Boulter et al. (1999) evaluated the potential of the major sporozoite surface antigen, SPAG-1, to be included in a subunit vaccine [17]. After four vaccination trials conducted in cattle under different delivery systems and adjuvants, using the recombinant SPAG-1 of *T. annulata*; it was observed that none of the tested conditions provided complete protection to sporozoite challenge. The reduction of early piroplasm parasitaemia and the presence of neutralising antibody titres after a single inoculation suggested, however, that partial protection was achieved.

Even though an anti-sporozoite antibody alone is not sufficient to prevent infection because some sporozoites might escape neutralization, these results underline the important role of the humoral response in *T. annulata* in reducing the infection during the early stages of invasion. The role of antibodies reacting against the macroschizont and piroplasm stages of the parasite has been difficult to define. Shields et al. (1989) characterised surface polypeptides of the different life stages of *T. annulata* and during this study found that antibodies present in immune sera failed to recognise the surface of infected mononuclear cells, resulting in a lack of antibody-mediated lysis of infected cells [18]. Furthermore, the inoculation of calves with killed schizonts failed to induce a protective response when these animals were challenged with ticks infected with *T. annulata* [19]. Although several factors may affect these results, including the dose of inoculated schizonts, all of the available data on this, suggests it may be possible that the humoral response to either macroschizont or piroplasm stages is not able to protect the host from disease.

### C. Cell-mediated immunity

Innate and adaptive immune responses are thought to simultaneously act to protect cattle against *T. annulata*. Studies on the immune response to *T. parva* identified the major determinant of protection as the cytotoxic T-lymphocyte-directed response to the schizont stage [9]. It is likely that similar T-cell-mediated immunity plays a comparably dominant role in protecting the host from *T. annulata*. Different strains of *Theileria* exhibit varying tropism towards host cells. After infection, *T. annulata* schizonts inhabit host macrophages and B-cells, whilst *T. parva* invasion mainly targets T cells [13, 20, 21]. Following primary exposure to sporozoites, infected macrophages (MΦ) direct anti-microbial activity towards invading schizonts and trophozoites through production of nitric oxide (NO), destroying the parasites and leading to apoptosis of infected cells. Cytokines produced by these MΦ stimulate further innate responses and concurrently mobilise the adaptive arm of the immune system. Trophozoite-infected MΦ produce interleukin (IL)-12 and tumor necrosis factor (TNF)-α whilst interferon



(IFN)- $\alpha$  is secreted by those harbouring schizonts [20]. These cytokines stimulate Natural Killer (NK) cells to directly lyse both types of infected M $\Phi$  as well as produce IFN- $\gamma$ , which further stimulates trophozoite-infected M $\Phi$  to produce more NO [20]. The exact role NK cells have in the immune response towards *T. annulata* was, in fact, originally inferred from evidence of an NK-like activity [22]. Though a subsequent study failed to produce any data confirming NKs are directly involved in clearance of this parasite [23]. A recent study finally provided some solid evidence of a role for NKs in eliminating *T. annulata* (as well as *T. parva*). In these experiments, both conventional (NKp46<sup>+</sup> CD3<sup>-</sup>) cattle NK cells and a novel non-conventional (NKp46<sup>+</sup> CD3<sup>+</sup>) T-cell subset exhibited cytotoxicity towards *T. annulata*-infected cell lines, which were originally derived from autologous cattle bovine peripheral blood mononuclear cells (PBMCs) [24]. Furthermore, blocking the NKp46 receptor led to a partial reduction in cytotoxicity towards these targets, confirming it plays a direct role in the recognition and lysis of *Theileria*-infected cells [24]. None of these responses were major histocompatibility complex (MHC) class-I restricted. Uninfected M $\Phi$  also play a key role in clearing a primary sporozoite infection. They produce the same cytokines as infected M $\Phi$  (IL-12, TNF- $\alpha$  and IFN- $\alpha$ ), so also contribute to activating NKs. In addition, they produce two further pro-inflammatory cytokines (IL-1 $\beta$  and IL-6) and NO. The former induce NO production in adjacent infected macrophages whilst the latter provide extraneous cytotoxicity to these same cells and free merozoites [20]. NO also inhibits the invasion of PBMCs by sporozoites. Uninfected M $\Phi$  stimulated with IFN- $\gamma$  phagocytose and clear intraerythrocytic piroplasms [20]. As mentioned above, the T-cell response is particularly critical in providing protection. This occurs during both the initial phase of infection and when resisting subsequent challenges. Direct lysis of schizont-infected M $\Phi$  is mediated by cytotoxic CD8<sup>+</sup> T-cells. This response is MHC class I-restricted and leads to clearance of the parasite [25]. Using animals immunized with either sporozoites or an infected cell line, both from a population of cloned *T. annulata* C9, parasite-specific CD8<sup>+</sup> T-cell lines exhibit greater cytotoxicity towards parasitized cells [26]. In agreement with previous work, these T-cell responses are MHC-restricted [25, 27]; however, crucially, they are also antigen-specific as they were restricted by parasite strain [26]. This would be an important consideration when developing anti-parasite therapies such as a vaccine. In fact, *T. annulata* vaccine trials have shown a more effective induced immune response towards homologous parasite strains than those derived from a heterologous strain [28]. Subsequent to confirming the strain specificity of T-cell responses, MacHugh et al. (2011) identified three *T. annulata* antigens by screening a parasite cDNA library with CD8<sup>+</sup> T cell lines. Using T-cells restricted by the A10 MHC class-I haplotype, a single dominant epitope from only one of the three antigens was revealed as the target for these T-cells [29]. Furthermore, this antigen displayed considerable polymorphism with clear evidence of positive selection driving this diversity. These findings would be especially important for designing effective vaccines, though cross-protection with different isolates has been observed [15]. CD4<sup>+</sup> T-cells also play a vital role in clearing

*Theileria*. Antigen presented by M $\Phi$  and other APCs as well as production of IL-1 $\beta$  and IL-12 mobilises CD4<sup>+</sup> T-cells to produce IL-2 and IFN- $\gamma$ . Secretion of IL-2 induces CD8<sup>+</sup> T-cell proliferation leading to the aforementioned effects. Infected and uninfected M $\Phi$  are further activated by IFN- $\gamma$ , enhancing NO production to promote destruction of intracellular trophozoites (directly) and schizonts (indirectly via healthy M $\Phi$ ) [20]. Preston et al. (1983) found that when *T. annulata*-infected calves recovered from the disease, the macroschizonts in the lymph nodes had disappeared which also coincided with an increase of cytotoxic cells in the blood and lymph nodes, and the subsequent development of immunological memory. By contrast, the animals that presented acute and fatal disease had increased parasitaemia with no detectable cytotoxic cells in the blood or lymph nodes. After primary infection, challenged calves rapidly produce bovine leucocyte antigen (BoLA)-restricted cytotoxic CD8<sup>+</sup> T-cells and non-restricted NK cells [22]. This rapid response is mediated by parasite-specific CD4<sup>+</sup> memory T cells, which produce IFN- $\gamma$ , enhancing (uninfected) M $\Phi$  activity. CD8<sup>+</sup> T-cells and NKs also produce this interferon, which along with NO directly eliminates trophozoite-infected cells that have evaded the humoral response [20, 22]. Solid protection is afforded to cattle that recover from the primary infection, however, as alluded to earlier in this section, this only extends to homologous strains with exposure to heterologous challenge rendering some animals susceptible [30]. An overproduction of TNF- $\alpha$  and IFN- $\alpha$  by parasitized mononuclear cells, in particular, macrophages, accounts for most of the clinical disease and tissue pathology observed in *T. annulata* infection [31]. Interestingly, different species of cattle exhibit differential disease resistance phenotypes for tropical theileriosis. The Sahiwal (*Bos indicus*) species are inherently more resistant to *T. annulata* than *Bos taurus* animals [4]. A functional genomics approach to understanding the key genotypic differences regulating tolerance identified members of the signal regulatory protein (SIRP) family and MHC Class II (BoLA-DQ) [4] as variable between species. The transcriptome of M $\Phi$  from *Bos taurus* cattle revealed much higher production of pro-inflammatory mediators such as cytokines (amongst others) than is found in the same cells in Sahiwal animals [4]. Levels of the transforming growth factor (TGF)- $\beta$ 2 are also significantly different between infected individuals of the two species and exist as direct correlates of disease susceptibility [32]. Collectively, these data indicate the more stringent regulation of inflammation by *Bos indicus* underpins their superior resistance to tropical theileriosis.

### III. CONCLUSIONS

Infectious diseases caused by protozoan parasites including tropical theileriosis caused by *T. annulata* continue to have a major impact on global animal health. In underdeveloped countries the disease has a tremendous economic impact on the subsistence of communities that live from livestock production. To date, vigorous immunogenicity has been difficult to achieve in order to provide complete immune protection against *T. annulata* infection. Several factors such as antigenic heterogeneity among strains and different life stages have



contributed to this. Past research reinforce the idea that antibodies produced against the surface epitopes of the sporozoite may aid in the reduction of the infection during the early stages of invasion, highlighting the important role of the humoral response in *T. annulata*. Furthermore, protective mechanisms provided by T-cells, NK cells and their products, also contribute towards adaptive immune protection. A combination of surveillance after vaccination with molecular characterisation of the field circulating strains, alongside a deep understanding about the host immune mechanisms, are the key measures in controlling bovine theileriosis. Nevertheless, the precise effector mechanisms underlying protective immunity have yet to be fully elucidated, warranting a requirement for further research.

The authors declare no competing personal or financial interests.

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