Int.J.Curr.Microbiol.App.Sci (2018) *7*(11): 2814-2821

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 11 (2018)**
Journal homepage: http://www.ijcmas.com

Review Article https://doi.org/10.20546/ijcmas.2018.711.324

Theileria-Induced Leukocyte Transformation: A Parasite Model for the Study of Lympho Proliferation

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Keywords

Theileria parva and *Theileria annulata*, Exotic (taurine), P 53, immortalization, NF-kB

Article Info

Accepted:
22 October 2018
Available Online:
10 November 2018

A B S T R A C T

The genus *Theileria* includes tick-transmitted Apicomplexan parasites of ruminants with substantial economic impact in endemic countries. Amongst the Apicomplexa phylum, *Theileria* is the only genus which transforms its mammalian host cells. *Theileria parva* and *Theileria annulata* infect leukocytes where they induce phenotypes that are shared with some cancers such as immortalization, dissemination and hyper proliferation. Transformation of lymphocytes is induced during the schizont stage. The mechanism of host cell transformation induces constitutive activation of bovine transcription factors (NF-kB) and associated signal transduction pathways. P53 plays an important role in proliferation and apoptosis and thus represents a key molecule in tumor formation. Moreover *T. annulata* schizonts induce a Warburg effect in host cells by a shift in ATP generation from predominantly oxidative phosphorylation to glycolysis and *Theileria* parasites avoids autophagic clearance by directly blocking autophagy. *Theileria* parasites secrete a protein, prolyl isomerase for maintaining the transformation of host cells. The host cell transformation by *Theileria* parasites and the comparison between cancer biology and host-*Theileria* interactions can help in revealing chemotherapeutic targets. This review is about host cell manipulation induced by *Theileria* parasites and the phenotypes, *Theileria* infected cells share with many cancers.

Introduction

Tropical theileriosis and East Coast Fever are tick-borne diseases of cattle caused by the protozoan parasites *Theileria annulata* and *Theileria parva*, respectively. They cause lymphoproliferative disorders of cattle (Irvin and Morrison, 1987). Exotic (taurine) breeds of cattle and their crosses are more susceptible to the disease and its clinical course may

prove fatal. However, Indian breeds of cattle are relatively resistant and act as reservoir hosts. Pathology of the disease is associated with the presence of intra-macrophage stage of the parasite (schizont) but the exact mechanisms remain unclear (Preston *et al.,* 1999; Glass, 2001; Preston, 2001). *Theileria* parasites enter the bovine hosts during tick feeding as sporozoites, which rapidly invade mononuclear leukocytes in the lymph glands nearest to tick bite. Unlike many apicomplexans, *Theileria* resides in the host cytosol instead of inside a parasitophorous vacuole and during host cell mitosis, the schizonts bind to the host mitotic spindle, ensuring segregation into both daughter cells with great efficiency to maintain the infection rate. Macroschizonts develop further into microschizonts and ultimately into merozoites, which are released from the leukocyte. The merozoites invade erythrocytes and develop into piroplasms (Tait and Hall, 1990). *T. annulata* infects monocytes/macrophages and B cells of bovine, ovine, and caprine origin (Dobbelaere and Heussler, 1999; Schnittger, 2000), but only bovine leukocytes are transformed. The macroschizont stage is largely responsible for the pathology of *Theileria* infections (Hooshmand-Rad, 1976). The intracellular multinucleated parasite induces the proliferation of its host cell, leading to the clonal expansion of the parasitized cell population (Hulliger *et al.,* 1964; Nichani *et al.,* 1999). The synchrony between host cell and parasite cell cycles, together with the close association of the macroschizont with the host cell spindle microtubules, ensures that each daughter cell inherits a parasite at the completion of mitosis (Hulliger *et al.,* 1964). The conversion of *Theileria* schizont-infected cells into immortal cell lines depends on acquired characteristics that are remarkably similar to those exhibited by some cancerous cells, such as immune evasion and resistance to apoptosis, and possibly provides a better understanding of the molecular interactions underlying these phenotypes (Hulliger *et al.,* 1966; Schmuckli-Maurer *et al.,* 2008; Shiels *et al.,* 1992). Like Plasmodia, in their mammalian hosts, *Theileria* parasites are haploid. Diploid parasites and genetic recombination only take place in the insect vector that are ticks—*Hyalomma* for *T. annulata* and *Rhipicephalus* for *T. parva*. It is the distribution of the two tick species that determines the distribution of the two diseases.

Host cell transformation

Theileria-induced transformation leads to activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB)-dependent proliferative signaling. Transformation of *T. annulata* and *T. parva*-infected leucocytes induce constitutive activation of bovine transcription factors and associated signal transduction pathways (Shiels *et al.,* 2006). *Theileria* schizonts constitutively activate the IKK complex on their cell surface by trans-autophosphorylation and IkB kinase (IKK) complex activates NF-kB (Hayashida *et al.,* 2013). NF-kB leads into the nucleus and transcriptional activity starts and during host cell mitosis, the schizonts bind to the host mitotic spindle ensuring segregation into both daughter cells with great efficiency to maintain the infection rate (Von Schubert *et al.,* 2010). NFkB (nuclear factor, p50 and p65) is an important transcription factor (a protein that interacts with the promoter of genes and stimulates gene expression). NFkB is bound by IkB (its inhibitor) which retains it in the cytoplasm and keeps it inactive and Phosphorylation followed by ubiquitinylation and degradation of IkB leads to import into the nucleus and transcriptional activity. *Theileria* interferes with this pathway by causing the destruction of IkB and it is a major player in the stimulation and clonal expansion of lymphocytes (Von Schubert *et al.,* 2010).

While bovine leukocytes transformed by *T. parva*, but not by *T. annulata* can produce interferon g (IFN-g) and interleukin 2 (IL-2), both produce and respond to tumor necrosis factor a (TNF-a). However, it has been suggested that some *Theileria*-transformed cell lines may grow independently of growth factors (Reddel, 2000; Ahmed *et al.,* 1987).

Immune evasion in *Theileria*-infected cells

Immune evasion may involve modulation of the host immune response. Even though possible molecular mechanisms are unclear, some patterns are emerging that inflammatory cytokine production (e.g., IFN-g) is somehow delayed by the parasite in vivo until after schizont development, when signaling is not as effective (Campbel *et al.,* 1999). This may be one reason why *T. annulata* is not cleared by a Th1 response, even though this is typically the case for other macrophage-resident protozoan infections. *T. annulata*-infected bovine macrophages down regulate some macrophage markers and lose functions such as Fc- mediated phagocytosis and the production of antimicrobial molecules including nitric oxide and TNF-a (Jensen, K. *et al.,* (2009). The inflammatory cytokine production (e.g., IFN-g) is delayed by the parasite until after schizont development when signaling is not as effective and *Theileria* infected activated T cells migrate from the lymph nodes to the efferent lymph downregulate CD2 a crucial adhesion molecule for cytolytic activity. Parasite-transformed cells cause marked proliferative response of uninfected lymphocytes this phenomenon is called the autologous Theileria mixed-lymphocyte reaction (Nichani *et al.,* 1999; Campbel *et al.,* 1999). *T. parva*-infected lymphocytes can also upregulate several immunoregulatory molecules, including IFN-g and IL- 2, both of which improve the transformation efficiency of host lymphocytes (Shayan *et al.,* 1999). Because MHC I is essential for *T. parva* invasion of bovine lymphocytes and IFN-g is known to upregulate MHC I (Shaw *et al.,* 1995; Zhou *et al.,* 2009), the expression of IFN-Y is an apparent mechanism by which the parasite can increase the susceptibility of circulating lymphocytes to infection, and proliferative cytokines likely aid the division of parasitized cells. However, cytokine profiles often vary among *T. parva*-infected bovine T cell clones.

Pathogens and tumors have also been known to use other immune evasion strategies, such as sequestration, dormancy, antigenic variation and failure of antigen display. Investigations into these potential mechanisms of immune evasion in cattle and buffalo could lead to insights that are crucial for the improvement of vaccine regimens.

Role of p53 gene in survival of Transformed lymphocyte cell

P53 is important protein involved in proliferation and apoptosis and thus represents a key molecule in tumor formation. It arrests cell division and to initiate apoptosis in the case of cell stress and DNA damage, protecting the integrity of the genome. The central function of p53 is preventing the onset of cancer (Levine, 1997). *Theileria* schizonts induce anti-apoptotic proteins such as cellular FLICE-like inhibitory protein (cFLIP) and cellular inhibitor of apoptosis proteins (cIAPs) by activating host IKK complexes and upregulating or maintaining high c-MYC expression. *T. annulata* schizont leads to cytoplasmic sequestration of the majority of host cell p53 resulting in the inhibition of p53-mediated apoptosis and promotion of host cell survival. Elimination of the parasite leads to nuclear translocation of p53 resulting in the upregulation of the proapoptotic proteins and the downregulation of an anti-apoptotic protein (D Haller *et al.,* 2010) (Fig. 1 and 2).

Fig.1 (NF-kB)-dependent proliferative signaling

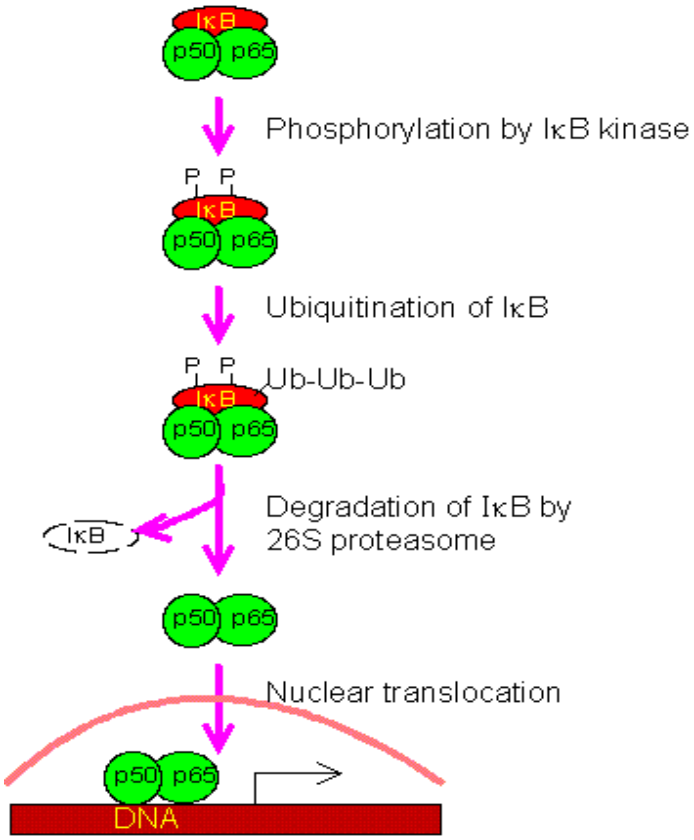
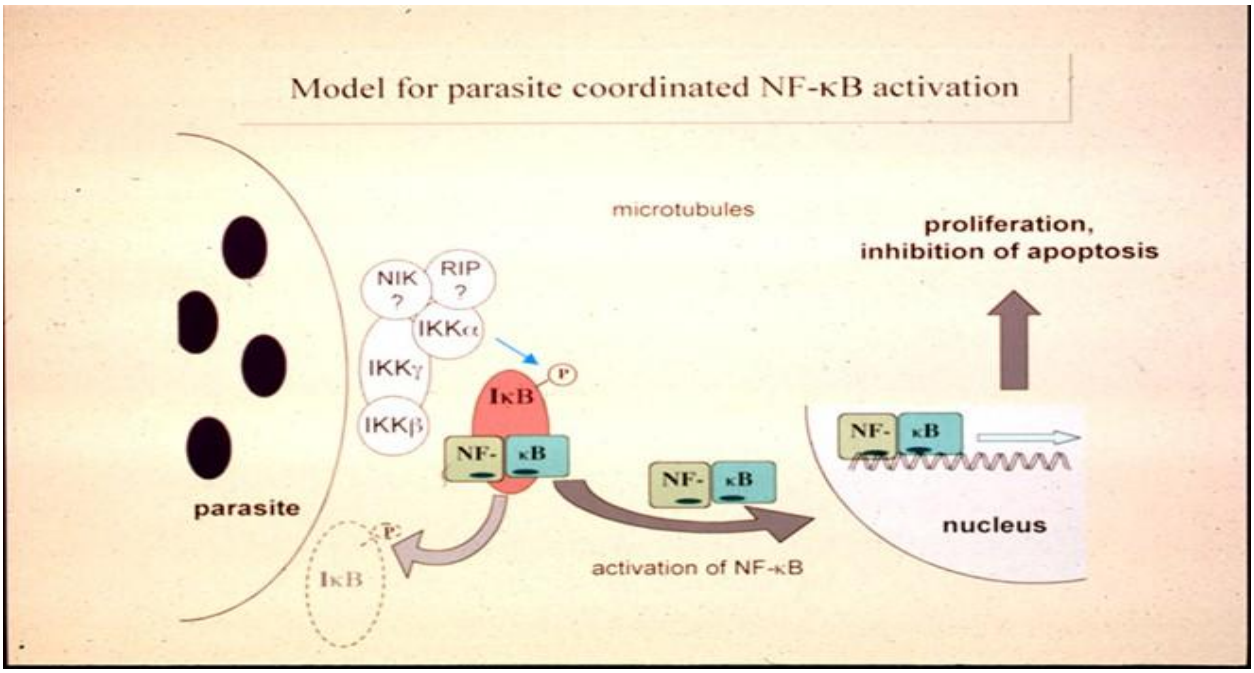


Fig.2 Parasite coordinated NF-KB activation



Deregulation of cellular energetics

Theileria parasites take nutrients from cytosol of cell and the acquisition of metabolites from the cytosol depletes nutrients from the host. Moreover, *T. annulata* schizonts induce a Warburg effect in host cells defined by a shift in ATP generation from predominantly oxidative phosphorylation to glycolysis. This metabolic switch is associated with a deregulation in the concentration of reactive oxygen species (ROS) and activation of the protein hypoxia-inducible factor 1a (HIF-1a) (Metheni *et al.,* 2015). Cells can survive metabolic stress by the induction of autophagy that will result in the clearance of *Theileria* parasites from the host cytosol, because defects in autophagy have been associated with increased tumorigenesis in some cancers, determining how *Theileria* parasites avoid autophagic clearance, perhaps by directly blocking autophagy, without inducing metabolic stress-induced cell death could provide key insights into the coregulation of autophagy and metabolism (Duszenko *et al.,* 2011).

Metastasis by *Theileria* spp.

Sporozoite micronemes secretions causes hydrolysis of lipids in plasma membrane and the sporozoite surface firmly attaches lymphocyte plasma membrane. Lymphocyte plasma membrane lipids and sporozoite rhoptries derived lipids helps in formation of parasitophorous vesicle around internalized sporozoite and sporozoites escape from vesicle by secretion of phospholipases by sporozoites. *T. annulata*-infected macrophages invade tissues via an amoeboid invasion mechanism, for which matrix metalloproteinase 9 (MMP-9), transforming growth factor b (TGF-b), and TNF-a are essential (Ma and Baumgartner, 2014). *Theileria*-transformed host cells proliferate and disseminate into various organs causing lymph node swelling and *Theileria* parasites have been shown to have a close association with host microtubules which play a crucial role in metastasis. *T. annulata* recruits endbinding protein 1 to its cell surface by interactions with *T. annulata* polymorphic piroplasm antigen p104 and TaSE (Woods *et al.,* 2013).

Differences between theileria-infected cells and cancer cells

Theileria transformed bovine cell proliferation may lack some of the characteristics of cancer cells and two such phenotypes are the evasion of growth suppression and a breakdown in genomic integrity. Most somatic cells stop proliferating at a specific density as a result of interactions with other cells known as contact inhibition. Cancer cells must overcome this barrier for proliferation and to grow. *Theileria* transformed lymphocytes require contact with other infected or uninfected cells to proliferate.When cells are cured from parasite infection they die (by apoptosis -- this suicide response is usually suppressed in cancer cells) Seluanov *et al.,* 2009; Dobbelaere *et al.,* (1991). Although there are no reports of genomic instability in *Theileria*-transformed cells, there is evidence that *Theileria* parasites and other Apicomplexans such as *Cryptosporidium* and *Toxoplasma* affect host DNA integrity (Haller *et al.,* 2010). *Theileria* parasites have been shown to sequester p53 on their surface, in the host cytosol, presumably preventing it from executing its role in maintaining genomic stability.With all of the hallmarks that *Theileria* transformation has in common with many kinds of cancers. Cancer is a genetic disease induce genomic mutations in their host and is irreversible, but there are no reports of genomic instability in *Theileria*-transformed cells as treatment reverses the effect of transformed cells and these cells dies by apoptosis (Vogelstein *et al.,* 2013; Weitzman, 2014).

Parasite molecules required for the cancer-like phenotypes

A question persists in the study of *Theileria*–host interactions: which parasite molecules (proteins, lipids, RNA, other) are required for, or contribute to, these cancer-like phenotypes? Interactions with host signaling play a crucial role in transformation such as NF-kB. Size was a primary reason why this matter was unresolved. *Theileria* genomes contain about 4000 genes half of which still have no predicted function. According to Weir *et al.,* (2010) two secreted multigene families SVSP (sub-telomere-encoded variable secreted protein) and TashAT (*Theileria annulata* schizont AT-hook protein) play a role in host–parasite interactions. The SVSP family is the largest gene family in both *T. annulata* and *T. parva* has been suspected of playing a role in immune evasion. TashAT-family proteins are secreted by the parasite which localize to the host nucleus and has been found to alter the expression and interfered DNA-binding domains in *T. annulata*. *Theileria* infection of bovine leukocytes modulates oncogenic signalling pathways such as JNK and AP-12. Peptidyl Prolyl Isomerase Pin1 (designated TaPin1) in *T. annulata* which is secreted into the host cell and modulates oncogenic signalling pathways. The TaPin1 is a prolyl isomerase and it interacts with the host ubiquitin ligase FBW7 leading to its degradation and subsequent stabilization of c-Jun which promotes transformation. Marsolier *et al.,* (2015) conducted an *in silico* screening of parasite genome to identify proteins secreted by *Theileria* into the host cell and found that *Theileria* parasites secrete a prolyl isomerase to maintain host cell transformation. *Theileria*-induced replicative immortality, a very distinctive and enigmatic phenotype of *Theileria* transformed cells is that they can be cultured in vitro indefinitely, exactly as any standard established cell line. *Theileria* parasites invade lumphocytes using zippering mechanism and PV is lysed using roptries secretion. Parasite interferes with the NF B growth control by activating IKK complex. Moreover, *Theileria* parasite secretes prolyl isomerase to maintain host cell transformation. The parasite resides in the cytoplasm and associates with the host cells microtubules and centrosomes. When the host cell divides the parasite divides and seggregates alongside using host cells mitotic machenary, maintain an approximately 1:1 host-to-parasite ratio and the proliferation of infected cells is rapid and unchecked. *Theileria* schizont cause cancer like growth and transformation is parasite dependent but reversible. Moreover, many *Theileria* parasites do not seem to induce cancer hallmarks in their host cells and hence provide an excellent opportunity for comparative investigation. In the laboratory they provide a unique model to investigate how a simple eukaryotic cell can direct gene expression and induce proliferation of another complex eukaryotic host cell.

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How to cite this article:

Aquil Mohmad, Bilal Ahmad Malla, Ishfaq Maqbool, Zahoor Ahmad War, Irshad Ahmad Para and Sheikh Firdous Ahmad. 2018. *Theileria*-Induced Leukocyte Transformation: A Parasite Model for the Study of Lympho Proliferation. *Int.J.Curr.Microbiol.App.Sci.* 7(11): 2814-2821. doi: https://doi.org/10.20546/ijcmas.2018.711.324