Received: 30 September 2020 | Revised: 7 November 2020 | Accepted: 8 November 2020

DOI: 10.1111/mmi.14642

MICROREVIEW

WILEY

Interaction between transforming *Theileria* parasites and their host bovine leukocytes

Shahin Tajeri [1,2] | Malak Haidar [1,3,4] | Takaya Sakura [1,5,6] | Gordon Langsley [1]

[1]Université de Paris, Institut Cochin, INSERM, CNRS, Paris, France

[2]Sorbonne Université, INSERM, CNRS, Centre d'Immunologie et des Maladies Infectieuses, CIMI, Paris, France

[3]Pathogen Genomics Laboratory, BESE Division, King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia

[4]de Duve Institute, Université Catholique de Louvain, Brussels, Belgium

[5]Department of Molecular Infection Dynamics, Institute of Tropical Medicine (NEKKEN), Nagasaki University, Nagasaki, Japan

[6]School of Tropical Medicine and Global Health, Nagasaki University, Nagasaki, Japan

Correspondence
Gordon Langsley, Université de Paris, Institut Cochin, INSERM, CNRS, 75014 Paris, France.
Email: gordon.langsley@inserm.fr

Funding information
Agence Nationale de Recherche, Grant/Award Number: ANR-11-LABX-0024; King Abdullah University of Science and Technology

Abstract

Theileria are tick-transmitted parasites that cause often fatal leuko-proliferative diseases in cattle called tropical theileriosis (*T. annulata*) and East Coast fever (*T. parva*). However, upon treatment with anti-theilerial drug-transformed leukocytes die of apoptosis indicating that *Theileria*-induced transformation is reversible making infected leukocytes a powerful example of how intracellular parasites interact with their hosts. *Theileria*-transformed leukocytes disseminate throughout infected cattle causing a cancer-like disease and here, we discuss how cytokines, noncoding RNAs and oncometabolites can contribute to the transformed phenotype and disease pathology.

KEYWORDS

cytokines, dissemination, miRNA, oncometabolite, *Theileria*, transformation

1 | INTRODUCTION

Theileria annulata is a tick-transmitted protozoan parasite that causes a fatal leuko-proliferative diseases in cattle called tropical theileriosis (Dobbelaere & Heussler, 1999). *T. annulata* infects and transforms bovine B lymphocytes and myeloid cells into cancer-like leukomas characterized by immortalization, hyper proliferation, and dissemination (see life cycle in Figure 1). In contrast, *T. parva* preferentially infects and transforms T cells (and less frequently B cells) that cause East Coast fever (Dobbelaere & Heussler, 1999). *Theileria*-induced leukocyte transformation is associated with extensive reprogramming of host cell signaling, however transformed leukocytes revert to a non-transformed phenotype upon treatment with anti-theilerial drug called buparvaquone (Dobbelaere & Heussler, 1999). However, microarray-based transcription profiling of buparvaquone-treated *T. annulata*-infected B cells showed that they do not fully revert to a transcription profile of non-transformed B cells (Kinnaird et al., 2013). Interestingly, the virulence of *T. annulata*-infected and transformed myeloid cells can be attenuated by prolonged multiple passages in vitro and this process allows the generation of effective live vaccines against tropical theileriosis (Somerville et al., 1998). *Theileria*-induced transformation, therefore, provides a good example of how intracellular parasites communicate with their host cells to change the infected host cell phenotype into a pathogenic one. We have recently reviewed what is known about parasite proteins secreted into the infected leukocyte cytosol (Tajeri & Langsley, 2020), so we have not discussed them here, and have instead highlighted

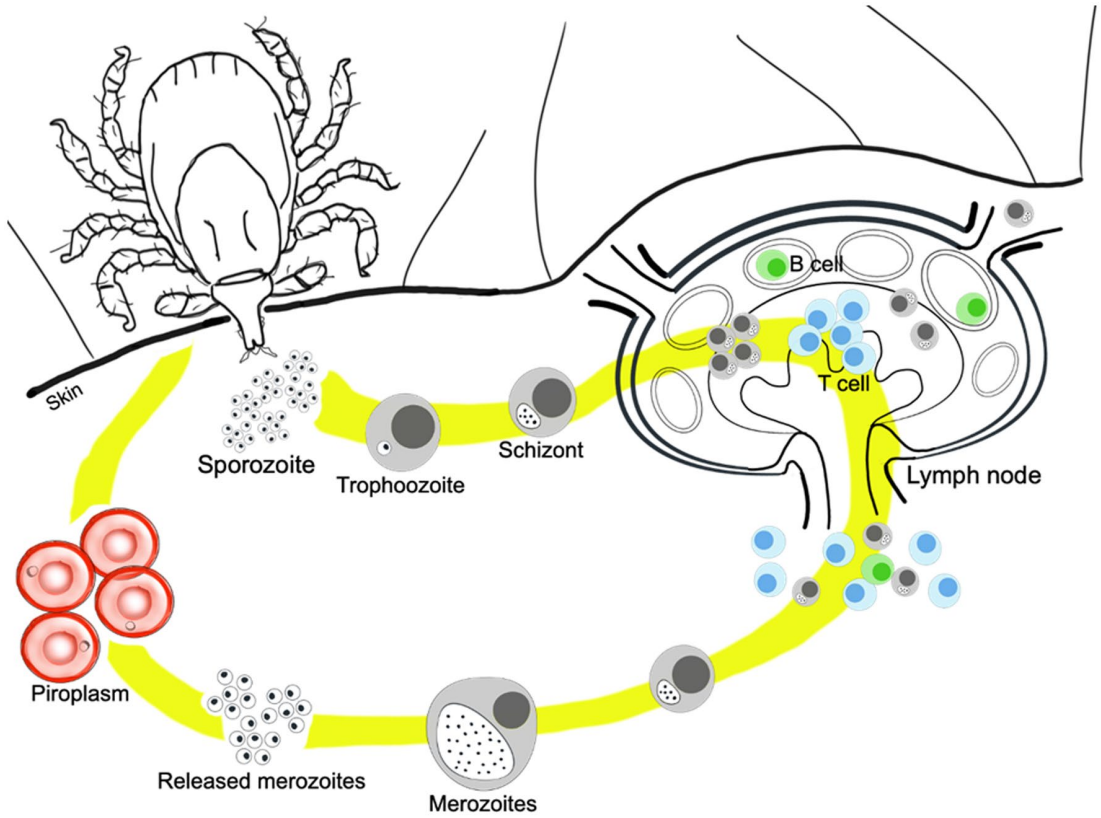

FIGURE 1 The life cycle of *Theileria* and early steps of disease pathogenesis in cattle. A single infected tick can inoculate thousands of sporozoites into the attachment site and transmit the disease. *Theileria* sporozoites, unlike *Plasmodium*, are not motile and immediately invade macrophages or lymphocytes resident at the site of inoculation. The intracellular sporozoite develops into a multinucleated schizont (also known as a macroschizont) in 4 days. The schizont is not surrounded by a parasitophorous vacuole and in the host cell cytoplasm is attached to the microtubule organizing center (MTOC). The schizont has the unique ability to transform its host leukocyte into a constantly proliferating tumor-like cell with hyper-dissemination potential. Transformed leukocytes secrete abundant levels of cytokines (see main text) and are drained into the closest lymph node, where they induce activation and proliferation of noninfected T cells leading to an enlargement of superficial lymph nodes. In fact, the schizont-infected leukocyte and the induced inflammatory changes in tissues form the pathologic basis of theileriosis. During each host cell division, the intracellular schizont due to being tightly associates with the MTOC is partitioned into both daughter leukocytes. The infected and noninfected cytokine expressing blasting leukocytes exit the node and transformed leukocytes disseminate to distant organs. At some point during infection, a sub-population of schizonts develop into merozoites that invade red blood cells and anemia contributes to the pathology of tropical theileriosis. The parasites circulate in blood as piroplasms that are taken up by feeding ticks and *Theileria* follows a complex life cycle in the tick that ends with production of infectious sporozoites inside tick salivary glands ready to infect the next cow

several examples of host-parasite interplay in terms of subversion of signaling pathways, cell metabolism, and epigenetic regulation describing how they potentially contribute to the virulent phenotype of infected host leukocytes.

Theileria-transformed leukocytes constantly proliferate both in vitro and in vivo and their dissemination throughout infected animals is largely due to the parasite's capacity to subvert host cell gene expression (Chaussepied & Langsley, 1996; Dobbelaere & Heussler, 1999; Tretina et al., 2015; Weir et al., 2009). One consequence of subversion is the secretion of a panoply of cytokines by infected and transformed leukocytes. These can act in an autocrine or paracrine manner to favor infected leukocyte propagation and dissemination due (Kinnaird et al., 2013) to their capacity to modulate host cell signal transduction pathways. In addition, by infecting and transforming immune effector cells *Theileria* parasites compromise the host's immune response and host ability to control infection.

When infectious *Theileria* sporozoites are inoculated into cattle by natural tick feeding or experimental needle injection, schizont-infected leukocytes are readily detected in lymph nodes closest to the site of inoculum. In primary infections of naive animals noninfected T cells become activated in a paracrine manner due to secretion of cytokines by infected leukocytes and blasting noninfected T cells lose their peptide-specific anti-parasite responses and ability to control infection (Houston et al., 2008). For example, 20%–30% of T cells exciting the lymph nodes that drain the site of infection are lymphoblasts (Emery, 1981) and hyperplasia and lymphadenomegaly are obvious clinical signs (Glass & Jensen, 2007). *Theileria*-transformed leukocytes disseminate throughout the body and accumulate in both distant lymphoid and nonlymphoid organs. Parasite-induced cytokines also probably signal endocrine functions to prime distant microenvironments for further tissue establishment of the *Theileria*-transformed leukocytes. In vivo, emergence of *Theileria*-transformed

leukocytes and blasting noninfected lymphocytes from draining lymph nodes triggers vicious cycles of cytokine secretion and immune cell activation leading to "cytokine storms" that non-specifically destroy tissue parenchyma and this contributes to pathology and death (Glass et al., 2012). For example, in East Coast Fever tissue infiltration by numerous CD163+ macrophages expressing abundant levels of IL-17 (a pro-inflammatory cytokine) have been linked to severe vasculitis and tissue destruction (Fry et al., 2016). Since *T. parva* does not infect macrophages these are recruited to different organs following dissemination of parasite-infected T cells (Fry et al., 2016). Thus, in infected cattle the pathologic unit is the transformed leukocyte and from a clinical point of view, theileriosis and East Coast Fever caused by transforming *Theileria* species are a disease of the immune system, where the outcome is highly dependent on the balance between pro- and anti-inflammatory cytokines. For example, *T. annulata*-infected and transformed myeloid cells that express the high levels of pro-inflammatory cytokines led to more severe disease in cattle (Graham et al., 2001). Below, we discuss some specific cytokines induced by *Theileria* infection and describe how they can contribute to disease progression (resumed in Table 1).

1.1 | Transforming growth factor-beta 2 (TGF-β2)

Transforming growth factor-β (TGF-β) is a family of cytokines known to regulate cell growth, differentiation, and motility (Massagué, 1998). In normal cells and early stages of cancer, TGF-β inhibits cell growth, while in late stages of cancer it plays a contrasting role, which favors metastasis and invasion. The mechanism underlying this contradiction is still not well defined. There are three isoforms of TGF-β: TGF-β1, 2, and 3 and in cancer, TGF-β2 is strongly expressed in highly aggressive and malignant tumors (Gomes et al., 2012; Gold et al., 2000). We have published that TGF-β2 production is higher in disease-susceptible *Theileria*-infected and transformed Holstein-Friesian (HF) myeloid cells than in disease-resistant Sahiwal-infected and transformed myeloid cells, and this correlates with the higher Matrigel traversal capacity (a surrogate of dissemination capacity) of HF-infected and transformed myeloid cells. Upon attenuation in vitro, the attenuated-infected and transformed myeloid cells displayed both reduced TGF-β2 expression and dissemination, which was re-established by stimulation with exogenous TGF-β (Chaussepied et al., 2010). Also, concomitant with the loss of TGF-β2 production, expression of COX2 and EP4 is ablated that leads to a drop in cyclic AMP (cAMP) levels, and consequently, reduced activation of protein kinase A (PKA) and EPAC. This ablated phenotype can be rescued in attenuated myeloid cells by the addition of exogenous TGF-β2 that reactivates the expression of COX2 and EP4, while repressing that of protein kinase inhibitor gamma (PKIG) to the levels in virulent myeloid cells. TGF-β2 therefore, promotes dissemination of virulent myeloid cells by modulating COX2, EP4, and PKIG transcription to initiate a prostaglandin E2 (PGE2)-driven auto-stimulatory loop that augments PKA and EPAC activities. A virulence phenotype stemming from the double activation of PKA and EPAC is the induction of a CREB-mediated transcriptional programme and the upregulation of JAM-L- and integrin 4αβ-mediated adhesion of *Theileria*-infected and transformed myeloid cells (Haidar, Echebli et al., 2015a). Also, TGF-β2 induced CREB drives catalase transcription leading to more catalase activity to detoxify the excess H_2O_2 and enhance the virulence of *Theileria*-infected and transformed myeloid cells (Haidar et al., 2019). Another way TGF-β2 contributes to virulence of *Theileria*-infected and transformed myeloid cells is by promoting podosomal adhesion structures and ROCK-mediated cortical actin-rich membrane blebs (Chaussepied et al., 2010). TGF-β-induced membrane bleb formation was blocked by the Rho-kinase (ROCK) inhibitor H-1152 and in the presence of serum the formation of actin-rich membrane blebs was significantly reduced after treatment with

TABLE 1 Major role-playing cytokines in *Theileria* infection

Cytokine type	Subcellular effect in transformed cells	Contribution to transformation	Cell type
TNF-α	-JNK/ATF2 activation -MAP4K4 expression -NF-kB activation	-Dissemination -Proliferation	TpB TaM
TGF-β	-Gene regulation -Activation of Rho kinase -JNK/AP-1 signaling	-Dissemination	TaM
GM-SCF	-NF-kB activation -AP-1 activation	-Proliferation	TpB
IL-2	nd	-Proliferation?	TpT, TpB
IL-10	nd	-Survival? -Immmunomodulation?	TpB, TpT, TaB, TaM

Note: Nd, not determined; TpB, *T. parva*-transformed B lymphocytes; TpT, *T. parva*-transformed T lymphocytes; TaB, *T. annulata*-transformed B lymphocytes; TaM, *T. annulata*-transformed myeloid cells.

the TGF-R inhibitor, but completely blocked in the presence of H-1152. Thus, one consequence of increased TGF- β2 production is increased cortical actin dynamics, which gives rise to enhanced podosomal adhesion structures (invadosomes) and membrane blebs in *Theileria*-infected and transformed myeloid cells derived from disease-susceptible HF cattle. In addition, TGF-receptor II and the p85 subunit of PI3-K were identified as Grb2 (growth factor receptor-bound protein 2) partners in virulent infected myeloid. Ablation of Grb2 interactions reduced PI3-K recruitment to TGF-RII and decreased PIP3 production, and dampened both JNK phosphorylation and AP-1 (activator protein 1)-driven transcription down to levels characteristic of attenuated myeloid cells (Haidar, Whitworth, et al., 2015b).

1.2 | Tumor necrosis factor-alpha (TNF-α)

TNF-α is a major cytokine driving high fever, a general clinical sign of *Theileria* infection (Glass & Jensen, 2007). Similar to TGF-β2, TNF-α is a well-characterized cytokine in theileriosis. In *T. parva*-transformed B cells, TNF-α is expressed and once secreted binds to its receptors on infected lymphocytes (i.e., autocrine mode of signaling). Consequent to TNF-α binding to its receptors (TNFR1 or TNFR2) there is recruitment of TRAF and activation NF-κB- and AP-1-driven transcription (Guergnon et al., 2003). NF-κB is non-canonically activated in a *Theileria*-dependent manner (Heussler et al., 2002) and is responsible for the regulation of TNF-α expression, since pharmacological blocking of NF-κB led to a decrease in TNF-α levels in *T. annulata*-infected and transformed myeloid cells (Ma & Baumgartner, 2014). Inhibition of TNF-α/TNFR/TRAF signaling via treatment of infected leukocytes with an inhibitor of TNF-α synthesis, or transfection of dominant-negative TNF receptor-associated factor 2 (TRAF2) reduced significantly the transcriptional activity of NF-κB (Guergnon et al., 2003). Finally, co-incubation with an anti-TNF-α antibody or pharmacological blockade of TNF-α synthesis reduced the proliferation of *T. parva*-transformed B cells. It should be noted that in *T. parva*-infected T cells TNF-α expression is low (Sager et al., 1999) and subsequently there is reduced receptor engagement and transfection of a dominant negative form of TRAF2 did not alter NF-κB activation (Heussler et al., 2002).

In *T. annulata*-infected and transformed myeloid cells induction of TNF-α contributes to invasion of extracellular matrix by induction of host cell serine/threonine kinase MAP4K4 expression. MAP4K4 increases accumulation of proteins of the Ezrin, Radixin, Moesin (ERM) family at the leading edge of moving macrophage lamellipodia and is partially responsible for their phosphorylation. Activated ERM proteins control host cell actin dynamics and increase cell mobilization and Matrigel traversal of *Theileria*-infected and transformed myeloid cells. Furthermore, MAP4K4 contributes to phosphorylation of JNK and JNK activation was found to be necessary for TNF-α-mediated expression of MAP4K4 (Ma & Baumgartner, 2014). Interestingly, an old study on experimentally infected cattle found parasitized TNF-α-positive cells in organs displaying histopathology providing in vivo evidence for its role in dissemination of *Theileria*-transformed myeloid cells (Forsyth et al., 1999). TNF-α, therefore, plays a role in two important aspects of *Theileria*-induced leukocyte transformation, proliferation, and cell motility.

1.3 | Granulocyte macrophage-colony stimulating factor (GM-CSF)

Proliferation of *Theileria*-transformed leukocytes depends on constitutive activation of membrane-associated phosphoinositide 3-kinase (PI3-K) that characterizes *Theileria*-induced leukocyte transformation (Chaussepied & Langsley, 2011). In *T. parva*-transformed B lymphocytes PI3-K induces a GM-SCF autocrine loop to promote proliferation and suppress apoptosis. GM-CSF mainly modulates cell division by indirect and direct induction of NF-κB-driven and directly AP-1-driven transcription (Baumgartner et al., 2000). The GM-SCF autocrine loop also elevates the transcripts of c-Myc through activation of JAK2, a receptor-associated kinase that mediates the phosphorylation of STAT3. Activated STAT3 then drives the expression c-Myc to promote survival of *Theileria*-transformed leukocytes, but unexpectedly, ablation of GM-CSF only reduced the proliferation without affecting apoptosis (Dessauge et al., 2005).

1.4 | IL-10

IL-10 can be considered as the only cytokine expressed by all the different types of *Theileria*-transformed leukocytes (McKeever et al., 1997). Its gene expression is regulated by mir-106a (Sharma et al., 2009), an oncomiR known to be significantly elevated in *T. annulata*-transformed B cells and myeloid cells (Marsolier et al., 2013). NF-κB also strongly upregulates the expression of this cytokine (Guergnon et al., 2003). IL-10 is generally considered as an anti-inflammatory cytokine known to suppress the activation of CD4+ T cells and recombinant human IL-10 can inhibit CD8+ T cell clones (de Waal Malefyt et al., 1991). Considering the importance of CD4+ and CD8+ lymphocyte responses in controlling *T. annulata* and *T. parva* infection (Morrison et al., 2015), IL-10 secretion is predicted to impact on the clinical pathogenesis of theileriosis and East Coast Fever. *Theileria*-transformed leukocytes display pro-inflammatory profiles, so their secretion of a negative regulator of inflammation is intriguing. One possibility is that IL-10 secretion dampens the activation status of noninfected myeloid cells, CD4+ and CD8+ T cells, as a way of countering an efficacious host immune response to *Theileria* infection. To play its anti-inflammatory role target cells have to express IL-10 receptor and receptor expression by *Theileria*- transformed leukocytes has not been studied. Indeed, there is only a single report showing *T. parva*-infected and transformed B cells can be stimulated by exogenous IL-10 to upregulate NF-κB-driven transcription implying that they express IL-10 receptor (Chaussepied et al., 2006). Whether other types of *Theileria*-transformed leukocytes express IL-10

receptor and whether IL-10 secretion reduces the adverse effects of cumulative inflammation on infected cattle awaits further study.

1.5 | IL-2

There is a debate on the actual importance of IL-2 in the biology of *Theileria*-transformed leukocytes. While it is expressed by all tested *T. parva*-transformed T cells and B cells, it's presence was only essential for proliferation of some transformed lines (Heussler et al., 1992). One study claimed that IL-2 is not necessary for the division of either *T. parva*- or *T. annulata*-transformed leukocytes, since antibody-mediated inhibition of IL-2 did not affect their proliferation (Shayan et al., 1999).

1.6 | Interferons (IFNs)

Leukocytes transformed by *T. annulata* and *T. parva* express distinct types of IFN (Entrican et al., 1991). IFN-β is the main cytokine secreted by *T. annulata*-infected and transformed myeloid cells in which IFN-α and IFN-ω are not expressed (Sager et al., 1998). *T. parva*-transformed lymphocytes express only IFN-γ and culture supernatants of *T. parva*-transformed leukocytes possess antiviral activity (Ahmed et al., 1993). It has not been established that *T. parva*-infected and transformed B cells expressed IFN-γ receptor and lack of receptor expression would render *T. parva*-infected leukocytes insensitive to IFN-γ. However, in *Toxoplasma gondii* infections IFN-γ has anti-parasite activity and consequently its expression is suppressed by a parasite secreted protein called TgIST (Gay et al., 2016). Surprisingly, TgIST appears unique to *Toxoplama* having no significant similarity with even its close relative *Neospora caninum* (Gay et al., 2016); (Olias et al., 2016). For the above reasons it seems unlikely that *Theileria* parasites express a TgIST-like protein to block IFN-γ expression.

1.7 | IL-6

IL-6 and GM-SCF belong to a same family of cytokines and share a common membrane receptor glycoprotein 130 (gp130) chain. Because infection with *T. annulata*-induced IL-6 expression in macrophages (Brown et al., 1995; McGuire et al., 2004), where it could act synergistically with GM-SCF. High levels of IL-6 in the serum of *T. lestoquardi*-infected sheep has been reported (Razmi et al., 2019). IL-6 is a pleiotropic cytokine and can induce production of acute phase proteins (APP) by hepatocytes (Tanaka et al., 2014). An APP response is increased during *T. annulata* infection and correlates well with the severity of the disease (Glass et al., 2003). Intraperitoneal injection of IL-6 into rats-induced anemia through edema and bleeding in the intestines (Jongen-Lavrencic et al., 1996). Hemorrhagy and ulcers of the digestive system are a typical pathology seen in tropical theileriosis and anemia is frequently observed in infected animals (Oryan et al., 2013). Therefore, IL-6 secretion could be associated these pathologic changes in the host and its expression is known to be regulated by NF-κB and activator protein 1 (AP-1) (Tanaka et al., 2014) both constitutively activated in *Theileria*-infected and transformed leukocytes.

In conclusion, infection of bovine leukocytes with transforming *Theileria* species deregulates the expression of several different types of host leukocyte cytokine and generally virulent, disseminating, transformed leukocytes have a pro-inflammatory cytokine profile. Unexpectedly, infected and transformed myeloid cells isolated from disease-susceptible and disease-resistant animals did not show any difference in IL-1β, IL-6, and TNF-α levels (McGuire et al., 2004). The exception was TGF-β2, and the contribution of other cytokines to pathogenesis of less virulent *Theileria*-infected and transformed leukocytes is unknown. Due to lack of development of techniques to knockout *Theileria* genes, it is not yet possible to test whether induction of host cell cytokine gene expression is caused by a parasite-secreted protein (see (Tajeri & Langsley, 2020), or if augmented cytokine expression results simply from infection-induced activation of host leukocyte transcription factors. Our current understanding is that inflammatory states in infected hosts enhance *Theileria*-transformed leukocyte survival, proliferation and dissemination. Cytokine expression profiles of leukocytes transformed by *T. parva* and *T. annulata* do not possess a common pattern partly due to the fact that T cells, B cells, and myeloid cells each have their own cell type-specific cytokine profiles.

1.8 | Epigenetic regulation

Many studies have shown that parasite infection induces extensive changes in epigenetic mechanisms that underlie cellular phenotypes. Not surprisingly, therefore, *Theileria* parasites promote leukocyte transformation by manipulating host cells epigenetic mechanisms such as noncoding RNAs. Noncoding RNAs (ncRNAs) are functional RNAs transcribed from DNA, but not translated into protein (Bayer-Santos et al., 2017). In general, ncRNAs are divided into two groups: the short ncRNAs (<30 nts) and the long ncRNAs (>200 nts). Short ncRNAs contain three major classes: microRNAs (miRNAs), short interfering RNAs (siRNAs), and piwi-interacting RNAs (piRNAs). Several studies have indicated miRNAs as key regulators of disease phenotype in *Theileria*-transformed leukocytes (Gillan et al., 2019; Haidar et al., 2018; Marsolier et al., 2013). The importance of miRNAs in *T. annulata* infection was first indicted by demonstrating a role for miR-155 in the virulence of transformed leukocytes. miR-155 was shown to be induced through c-Jun activation and AP-1-driven transcription. miR-155-mediated suppression of De-Etiolated Homolog 1 expression diminished c-Jun ubiquitination. The stability of c-Jun drove transcription of the BIC gene, which contains miR-155, explaining how a positive feedback-loop contributes to the growth and survival of *Theileria*-transformed leukocytes (Marsolier et al., 2013). Furthermore, we have identified a list of miRNAs differentially expressed following *T. annulata*-infection of B cells and in

addition, how their expression varies between virulent and attenuated myeloid cells (Haidar et al., 2018). We focused on miR-126-5p to demonstrate that infected of myeloid cells had high levels of miR-126-5p that suppressed JIP-2 (JNK-Interacting Protein-2), so liberating JNK1 to enter the nucleus and phosphorylate c-Jun (Haidar et al., 2018). This activated AP-1-driven transcription of the gene (*mmp9*) coding for Matrix Metallo-Proteinase 9 to promote tumor dissemination (Haidar et al., 2018). In addition, variation in miR-126-5p levels depended on the tyrosine phosphorylation status of AGO2, which is regulated by Grb2-recruitment of PTP1B (Haidar, Whitworth, et al., 2015b).

Also, the role of miR-125b in the virulence of *Theileria*-infected and transformed myeloid cells was examined. RNA-sequencing of virulent and attenuated myeloid cells transfected either with a specific inhibitor or mimic of miR-125b identified many signaling pathways under its control. Gene ontology functional enrichment analysis showed that genes whose expression was altered by miR-125b were significantly enriched in metabolic process, cell cycle, and DNA replication (Figure 2). miR-125b expression is significantly altered between virulent and attenuated myeloid cells, consistent with the greater propensity of attenuate cells to perform Warburg glycolysis (Metheni et al., 2015).

Lastly, a potential role for extracellular vesicles (EV) and their cargo in the pathobiology of *T. annulata* infection has been described. EV were purified from a noninfected bovine lymphosarcoma cell line (BL20) and from BL20 infected with *T. annulata* (TBL20) and were characterized by comparative mass spectrometry and miRNA profiling. This revealed an enrichment in EV from TBL20 cells of proteins involved in migration and extracellular matrix digestion (Gillan et al., 2019). Also, an altered repertoire was also observed of host miRNA many with known roles in tumor and/or infection biology (Gillan et al., 2019). Much evidence indicates that parasite-derived EVs can act as signaling vehicles in parasite-host interactions. EVs can mediate pathogenic process by regulating host gene expression, and immune responses. For instance, an increased level of circulating EVs is associated with malaria severity (Combes et al., 2004). In addition, microparticles derived from *Plasmodium berghei*-infected serum were found to contain parasite antigens, which can stimulate

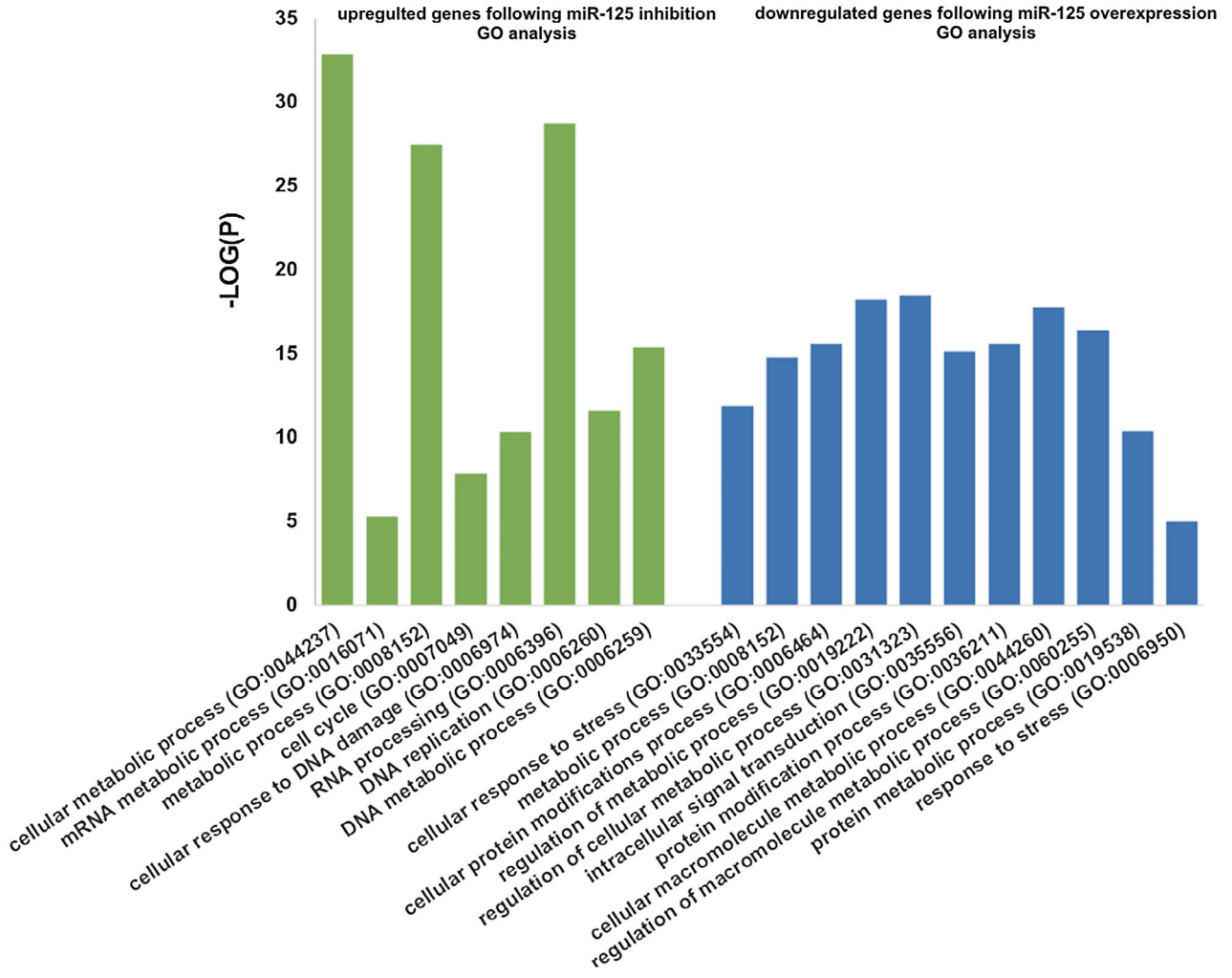

FIGURE 2 GO functional enrichment analysis of list of genes potentially regulated by miR-125b.GO analysis of DEGs following inhibition or stimulation of miR-125b. GO, gene ontology; DEGs, differentially expressed genes. Analysis was performed using the g:Profiler web server (http://biit.cs.ut.ee/gprofiler/)

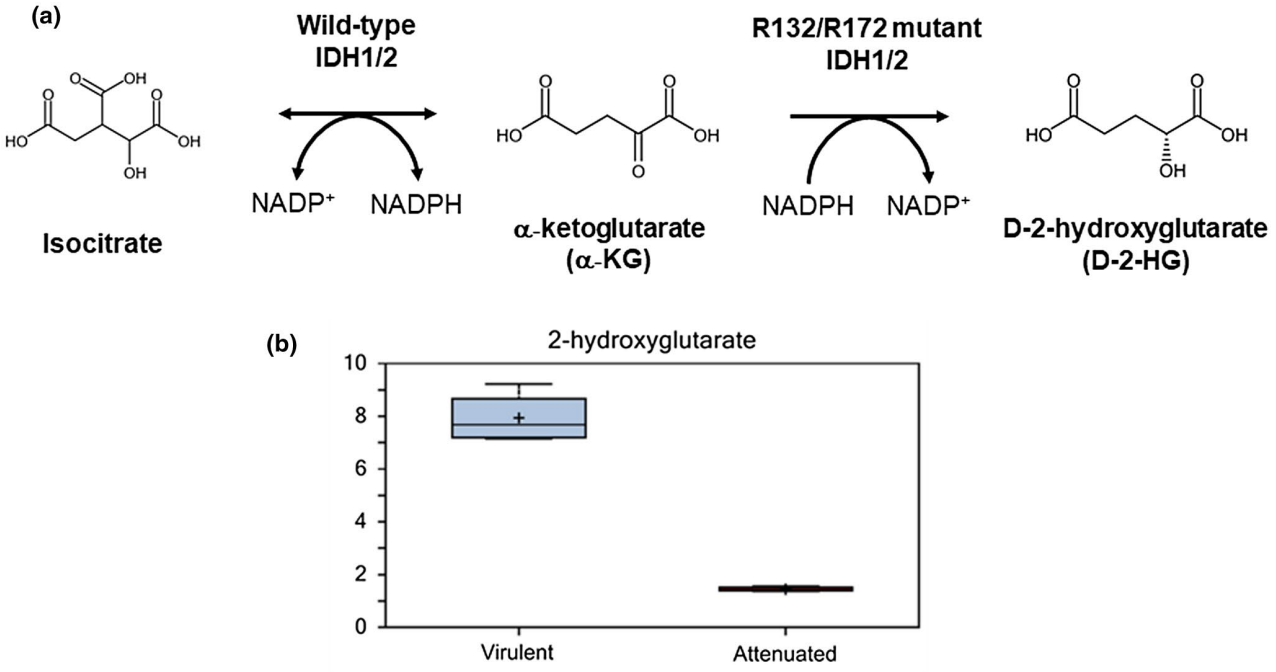

FIGURE 3 2-HG production in *T. annulata*-infected and transformed myeloid cells. Catalytic reaction of IDHs producing the oncometabolite, D-2-HG (A). Abundant 2-HG was detected in virulent myeloid cells compared to attenuated myeloid cells (B)

macrophage CD40 surface expression, TNF release, and induce immune responses via macrophage activation (Couper et al., 2010). Moreover, exosome-like vesicles secreted by *P. falciparum*-infected red blood cells (iRBCs) can increase the number of *P. falciparum* gametocytes and promote transmission to mosquitoes (Mantel et al., 2013; Regev-Rudzki et al., 2013). Furthermore, injection of exosomes released by *Toxoplasma gondii* antigen-stimulated DCs induced a strong systemic Th1-modulated *Toxoplasma*-specific immune response in vivo, and conferred good protection against toxoplasmosis (Aline et al., 2004).

1.9 | An oncometabolite in *Theileria*-infected and transformed myeloid cells

Research on cancer has demonstrated that the oncometabolite, D-2-hydroxyglutarate (D-2-HG) alters cellular metabolism leading to tumorigenesis (Dang et al., 2009; Mardis et al., 2009). The catalytic enzyme responsible for producing of D-2-HG is isocitrate dehydrogenase (IDH) that converts isocitrate to α-ketoglutarate α-KG) and simultaneously produces NADH or NADPH as its normal function (oxidative decarboxylation). Both humans and cows have three IDHs, cytosolic IDH1 and mitochondrial IDH2 and IDH3 (Losman & Kaelin, 2013). IDH1 and IDH2 catalyze a $NADP^+$-dependent reaction in the cytoplasm and mitochondria, respectively, and IDH3 is the NAD^+-dependent enzyme in the tricarboxylic acid cycle (TCA cycle). An association of IDH3 with human cancer has not been described, while mutated IDH1/2 gain an additional function that converts α-KG to D-2-HG in a NADPH-dependent manner that perturbs cellular metabolism via production of D-2-HG (Figure 3a). An arginine substitution in IDH1/2 (R132/R172) has been frequently identified in human cancers such as gliomas and acute myeloid leukemias (AML) (Gross et al., 2010; Johannessen et al., 2016; Losman et al., 2013). Targeting IDH mutations is one of the therapeutic strategies for these tumors (Dang et al., 2016; Waitkus & Yan, 2020). Accumulated D-2-HG alters the epigenetic landscape of cells by inhibiting histone lysine demethylases (KDMs) and the Ten-Eleven Translocation (TET) family of enzymes involved in DNA methylation (Lu et al., 2012; Sulkowski et al., 2020; Xu et al., 2011). These epigenetic regulations are also involved in *Theileria*-induced leukocyte transformation, as for instance, the high expression of matrix metalloproteinase-9 (MMP-9) that promotes to tumor dissemination is induced by the SET- and MYND-domain containing protein 3 (SMYD3) via trimethylation of histone H3 lysine 4 (H3K4me3) (Cock-Rada et al., 2012; Cheeseman & Weitzman, 2015).

Apicomplexa parasites have no homolog of NAD^+-dependent IDH that functions in the TCA cycle of mammalian mitochondria and *Theileria* spp. possess only one $NADP^+$-dependent IDH (Gardner et al., 2005; MacRae et al., 2012; Pain et al., 2005; Wrenger & Müller, 2003). Our metabolome analyses demonstrated that virulent myeloid cells produce abundant amounts of 2-HG compared to attenuated myeloid cells (Figure 3b). Surprisingly however, RNAseq analyses (Rchiad et al., 2020) demonstrated that neither *Bos taurus*-transformed myeloid cell IDH1/2 (BtIDH1/2), nor *T. annulata* IDH (TaIDH) harbor mutations of arginine residues normally responsible for the 2-HG production in human tumors. This suggests two possibilities, (1) parasite TaIDH can convert 2-HG without having an arginine mutation; (2) 2-HG is produced in an IDH-independent manner in *Theileria*-infected and transformed myeloid cells. The first possibility arises from a simple hypothesis that *Theileria* parasites intrinsically

produces 2-HG to drive host cell transformation. Biochemical characterization of TaIDH will be required to determine whether TaIDH can generate 2-HG that is released into infected host leukocytes. The second hypothesis resides on the observation that while most cancer research has investigated production of 2-HG from the point of view of IDH mutations, very few studies have focused on 2-HG accumulation in cells lacking IDH mutations. However, it is known that intracellular hypoxic conditions induce promiscuous reactions of several enzymes that can produce both D-2-HG and L-2-HG, the enantiomer of D-2-HG (Intlekofer et al., 2015; Struys et al., 2007; Ye et al., 2018). Although L-2-HG inhibits KDM and causes L-2-HG aciduria and renal cell carcinoma, an association of L-2-HG and leukemia has not yet been described (Kranendijk et al., 2012; Yong et al., 2020). The main function of L-2-HG is to control cellular redox homeostasis (Oldham et al., 2015). As the intracellular environment of attenuated myeloid cells is more hypoxic than that of virulent myeloid cells (Metheni et al., 2015), a basal level of L-2-HG is likely produced as part of a redox response in attenuated myeloid cells (our metabolome analysis did not distinguish 2-HG enantiomers). In contrast, abundant 2-HG detected in less hypoxic virulent myeloid cells is presumably the oncometabolite D-2-HG (Figure b). Future metabolome analyses need to differentiate between the two enantiomers, so we can determine the different roles of L-2-HG versus D-2-HG in *Theileria*-induced leukocyte transformation, as this may be an example of communication between parasite and host that modulates metabolism and phenotype of the infected cell.

2 | CONCLUSION

We have concentrated on how infection impacts on specific host cell processes and how these can underpin the pathology of disease. We have highlighted how cytokines, ncRNAs, and oncometabolites can contribute to the transformed phenotype and the virulent, lethal dissemination of infected leukocytes. What's clear is that all these processes depend on leukocytes being infected with live parasites, since upon drug-induced parasite death the once infected leukocyte dies if not further stimulated. This differentiates *Theileria*-induced leukocyte transformation from that of virus-induced cellular transformation making homology searches for putative *Theileria* oncogenes a thankless task. *Theileria* parasites are the only eukaryotes known to transform another eukaryote (host leukocytes) and we are thankful that this unique capacity makes them fascinating to study.

ACKNOWLEDGMENTS

ST and TS acknowledge postdoctoral fellowships from ParaFrap (ANR-11-LABX-0024) and MH postdoctoral funding from a Competitive Research Grant from the Office for Sponsored Research (OSR-2015-CRG4-2610) at King Abdullah University of Science and Technology (KAUST) awarded to Arnab Pain and GL. The RNAseq data in Figure 2 were generated by MH, while a post-doc in Arnab Pain's laboratory. GL also acknowledges ANR-11-LABX-0024 and core support from INSERM and the CNRS.

CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no data sets were generated or analyzed during the current study.

ORCID

Shahin Tajeri https://orcid.org/0000-0002-0482-1194
Malak Haidar https://orcid.org/0000-0003-1499-6489
Takaya Sakura https://orcid.org/0000-0002-8320-8485
Gordon Langsley https://orcid.org/0000-0001-6600-6286

REFERENCES

Ahmed, J.S., Wiegers, P., Steuber, S., Schein, E., Williams, R.O. and Dobbelaere, D. (1993) Production of interferon by*Theileria annulata*- and *T. parva*-infected bovine lymphoid cell lines. *Parasitology Research*, *79*, 178–182.

Aline, F., Bout, D., Amigorena, S., Roingeard, P. and Dimier-Poisson, I. (2004) *Toxoplasma gondii* antigen-pulsed-dendritic ell-derived exosomes induce a protective immune response against *T. gondii* infection. *Infection and Immunity*, *72*, 4127–4137.

Baumgartner, M., Chaussepied, M., Moreau, M.-F., Werling, D., Davis, W.C., Garcia, A. et al (2000) Constitutive PI3-K activity is essential for proliferation, but not survival, of *Theileria parva*-transformed B cells. *Cellular Microbiology*, *2*, 329–339.

Bayer-Santos, E., Marini, M.M. and da Silveira, J.F. (2017) Non-coding RNAs in host–pathogen interactions: subversion of mammalian cell functions by protozoan parasites. *Frontiers in Microbiology*, *8*, 1–8.

Brown, D.J., Campbell, J.D.M., Russell, G.C., Hopkins, J. and Glass, E.J. (1995) T cell activation by *Theileria annulata*-infected macrophages correlates with cytokine production. *Clinical and Experimental Immunology*, *102*, 507–514.

Chaussepied, M., Janski, N., Baumgartner, M., Lizundia, R., Jensen, K., Weir, W. et al (2010) TGF-b2 induction regulates invasiveness of *Theileria*-transformed leukocytes and disease susceptibility. *PLOS Pathogens*, *6*, e1001197.

Chaussepied, M. and Langsley, G. (1996) *Theileria* transformation of bovine leukocytes: a parasite model for the study of lymphoproliferation. *Research in Immunology*, *147*, 127–138.

Chaussepied, M., Michie, A.M., Moreau, M.F., Harnett, M.M., Harnett, W. and Langsley, G. (2006) Notch is constitutively active in *Theileria*-transformed B cells and can be further stimulated by the filarial nematode-secreted product, ES-62. *Microbes and Infection*, *8*, 1189–1191.

Chaussepied, M. & Langsley, G. (2011) Theileria-Induced Leukocyte Transformation: an Example of Oncogene Addiction? In Kaufmann, S.H.E., Rouse, B.T. & Sacks, D.L. (Eds.), *The Immune Response to Infection* (pp. 537–546). Washington, DC: ASM Press.

Cheeseman, K. and Weitzman, J.B. (2015) Host–parasite interactions: an intimate epigenetic relationship. *Cellular Microbiology*, *17*, 1121–1132.

Cock-Rada, A.M., Medjkane, S., Janski, N., Yousfi, N., Perichon, M., Chaussepied, M. et al (2012) SMYD3 promotes cancer invasion by epigenetic upregulation of the metalloproteinase MMP-9. *Cancer Research*, *72*, 810–820.

Combes, V., Taylor, T.E., Juhan-Vague, I., Mège, J.-L., Mwenechanya, J., Tembo, M. et al (2004) Circulating endothelial microparticles in Malawian children with severe falciparum malaria complicated with coma. *JAMA*, *291*, 2542–2544.

Couper, K.N., Barnes, T., Hafalla, J.C.R., Combes, V., Ryffel, B., Secher, T. et al (2010) Parasite-derived plasma microparticles contribute

significantly to malaria infection-induced inflammation through potent macrophage stimulation. *PLOS Pathogens*, *6*, e1000744.

Dang, L., White, D.W., Gross, S., Bennett, B.D., Bittinger, M.A., Driggers, E.M. et al (2009) Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature*, *462*, 739–744.

Dang, L., Yen, K. and Attar, E.C. (2016) IDH mutations in cancer and progress toward development of targeted therapeutics. *Annals of Oncology*, *27*, 599–608.

de Waal Malefyt, R., Haanen, J., Spits, H., Roncarolo, M.G., te Velde, A., Figdor, C. et al (1991) Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. *Journal of Experimental Medicine*, *174*, 915–924.

Dessauge, F., Hilaly, S., Baumgartner, M., Blumen, B., Werling, D. and Langsley, G. (2005) c-Myc activation by *Theileria* parasites promotes survival of infected B-lymphocytes. *Oncogene*, *24*, 1075–1083.

Dobbelaere, D. and Heussler, V. (1999) Transformation of leukocytes by *Theileria parva* and *T. annulata*. *Annual Review of Microbiology*, *53*, 1–42.

Emery, D.L. (1981) Kinetics of infection with *Theileria parva* (East Coast fever) in the central lymph of cattle. *Veterinary Parasitology*, *9*, 1–16.

Entrican, G., Mcinnes, C.J., Logan, M., Preston, P.M., Martinods, S. and Brown, C.G.D. (1991) Production of interferons by bovine and ovine cell lines infected with *Theileria annulata* or *Theileria parva*. *Parasite Immunology*, *13*, 339–343.

Forsyth, L.M.G., Minns, F.C., Kirvar, E., Adamson, R.E., Hall, F.R., McOrist, S. et al (1999) Tissue damage in cattle infected with *Theileria annulata* accompanied by metastasis of cytokine-producing, schizont-infected mononuclear phagocytes. *Journal of Comparative Pathology*, *120*, 39–57.

Fry, L.M., Schneider, D.A., Frevert, C.W., Nelson, D.D., Morrison, W.I. and Knowles, D.P. (2016) East Coast Fever caused by *Theileria parva* is characterized by macrophage activation associated with vasculitis and respiratory failure. *PLoS ONE*, *11*, e0156004.

Gardner, M.J., Bishop, R., Shah, T., de Villiers, E.P., Carlton, J.M., Hall, N. et al (2005) Genome sequence of *Theileria parva*, a bovine pathogen that transforms lymphocytes. *Science*, *309*, 134–137.

Gay, G., Braun, L., Brenier-Pinchart, M.-P., Vollaire, J., Josserand, V., Bertini, R.-L. et al (2016) *Toxoplasma gondii* TgIST co-opts host chromatin repressors dampening STAT1-dependent gene regulation and IFN-γ–mediated host defenses. *Journal of Experimental Medicine*, *213*, 1779–1798.

Gillan, V., Simpson, D.M., Kinnaird, J., Maitland, K., Shiels, B. and Devaney, E. (2019) Characterisation of infection associated microRNA and protein cargo in extracellular vesicles of *Theileria annulata* infected leukocytes. *Cellular Microbiology*, *21*, e12969.

Glass, E.J., Craigmile, S.C., Springbett, A., Preston, P.M., Kirvar, E., Wilkie, G.M. et al (2003) The protozoan parasite, *Theileria annulata*, induces a distinct acute phase protein response in cattle that is associated with pathology. *International Journal for Parasitology*, *33*, 1409–1418.

Glass, E.J., Crutchley, S. and Jensen, K. (2012) Living with the enemy or uninvited guests: functional genomics approaches to investigating host resistance or tolerance traits to a protozoan parasite, *Theileria annulata*, in cattle. *Veterinary Immunology and Immunopathology*, *148*, 178–189.

Glass, E.J. and Jensen, K. (2007) Resistance and susceptibility to a protozoan parasite of cattle—gene expression differences in macrophages from different breeds of cattle. *Veterinary Immunology and Immunopathology*, *120*, 20–30.

Gold, L.I., Jussila, T., Fusenig, N.E. and Stenbäck, F. (2000) TGF-β isoforms are differentially expressed in increasing malignant grades of HaCaT keratinocytes, suggesting separate roles in skin carcinogenesis. *The Journal of Pathology*, *190*, 579–588.

Gomes, L.R., Terra, L.F., Wailemann, R.A.M., Labriola, L. and Sogayar, M.C. (2012) TGF-β1 modulates the homeostasis between MMPs and MMP inhibitors through p38 MAPK and ERK1/2 in highly invasive breast cancer cells. *BMC Cancer*, *12*, 26.

Graham, S.P., Brown, D.J., Vatansever, Z., Waddington, D., Taylor, L.H., Nichani, A.K. et al (2001) Proinflammatory cytokine expression by *Theileria annulata* infected cell lines correlates with the pathology they cause *in vivo*. *Vaccine*, *19*, 2932–2944.

Gross, S., Cairns, R.A., Minden, M.D., Driggers, E.M., Bittinger, M.A., Jang, H.G. et al (2010) Cancer-associated metabolite 2-hydroxyglutarate accumulates in acute myelogenous leukemia with isocitrate dehydrogenase 1 and 2 mutations. *Journal of Experimental Medicine*, *207*, 339–344.

Guergnon, J., Chaussepied, M., Sopp, P., Lizundia, R., Moreau, M.-F., Blumen, B. et al (2003) A tumour necrosis factor alpha autocrine loop contributes to proliferation and nuclear factor-κB activation of *Theileria parva*-transformed B cells. *Cellular Microbiology*, *5*, 709–716.

Haidar, M., Echebli, N., Ding, Y., Kamau, E. and Langsley, G. (2015a) Transforming growth factor β2 promotes transcription of COX2 and EP4, leading to a prostaglandin E2-driven autostimulatory loop that enhances virulence of *Theileria annulata*-transformed macrophages. *Infection and Immunity*, *83*, 1869–1880.

Haidar, M., Metheni, M., Batteux, F. and Langsley, G. (2019) TGF-β2, catalase activity, H_2O_2 output and metastatic potential of diverse types of tumour. *Free Radical Biology and Medicine*, *134*, 282–287.

Haidar, M., Rchiad, Z., Ansari, H.R., Ben-Rached, F., Tajeri, S., Latre De Late, P. et al (2018) miR-126-5p by direct targeting of JNK-interacting protein-2 (JIP-2) plays a key role in Theileria-infected macrophage virulence. *PLoS Pathogens*, *14*, e1006942.

Haidar, M., Whitworth, J., Noé, G., Liu, W.Q., Vidal, M. and Langsley, G. (2015b) TGF-β2 induces Grb2 to recruit PI3-K to TGF-RII that activates JNK/AP-1-signaling and augments invasiveness of *Theileria*-transformed macrophages. *Scientific Reports*, *5*, 15688.

Heussler, V.T., Eichhorn, M., Reeves, R., Magnuson, N.S., Williams, R.O. and Dobbelaere, D.A. (1992) Constitutive IL-2 mRNA expression in lymphocytes, infected with the intracellular parasite *Theileria parva*. *The Journal of Immunology*, *149*, 562–567.

Heussler, V.T., Rottenberg, S., Schwab, R., Küenzi, P., Fernandez, P.C., McKellar, S. et al (2002) Hijacking of host cell IKK signalosomes by the transforming parasite *Theileria*. *Science*, *298*, 1033–1036.

Houston, E.F., Taracha, E.L., Brackenbury, L., MacHugh, N.D., McKeever, D.J., Charleston, B. et al (2008) Infection of cattle with *Theileria parva* induces an early CD8 T cell response lacking appropriate effector function. *International Journal for Parasitology*, *38*, 1693–1704.

Intlekofer, A.M., Dematteo, R.G., Venneti, S., Finley, L.W.S., Lu, C., Judkins, A.R. et al (2015) Hypoxia induces production of L-2-hydroxyglutarate. *Cell Metabolism*, *22*, 304–311.

Johannessen, T.-C.-A., Mukherjee, J., Viswanath, P., Ohba, S., Ronen, S.M., Bjerkvig, R. et al (2016) Rapid conversion of mutant IDH1 from driver to passenger in a model of human gliomagenesis. *Molecular Cancer Research*, *14*, 976–983.

Jongen-Lavrencic, M., Peeters, H.R.M., Rozemuller, H., Rombouts, W.J.C., Martens, A.C.M., Vreugdenhil, G. et al (1996) IL-6-induced anaemia in rats: possible pathogenetic implications for anaemia observed in chronic inflammations. *Clinical and Experimental Immunology*, *103*, 328–334.

Kinnaird, J.H., Weir, W., Durrani, Z., Pillai, S.S., Baird, M. and Shiels, B.R. (2013) A bovine lymphosarcoma cell line infected with *Theileria annulata* exhibits an irreversible reconfiguration of host cell gene expression. *PLoS ONE*, *8*, e66833.

Kranendijk, M., Struys, E.A., Salomons, G.S., Van der Knaap, M.S. and Jakobs, C. (2012) Progress in understanding 2-hydroxyglutaric acidurias. *Journal of Inherited Metabolic Disease*, *35*, 571–587.

Losman, J.-A. and Kaelin, W.G. (2013) What a difference a hydroxyl makes: mutant IDH, (R)-2-hydroxyglutarate, and cancer. *Genes and Development*, *27*, 836–852.

Losman, J.-A., Looper, R.E., Koivunen, P., Lee, S., Schneider, R.K., McMahon, C. et al (2013) (R)-2-hydroxyglutarate is sufficient to promote leukemogenesis and its effects are reversible. *Science*, *339*, 1621–1625.

Lu, C., Ward, P.S., Kapoor, G.S., Rohle, D., Turcan, S., Abdel-Wahab, O. et al (2012) IDH mutation impairs histone demethylation and results in a block to cell differentiation. *Nature*, *483*, 474–478.

Ma, M. and Baumgartner, M. (2014) Intracellular *Theileria annulata* promote invasive cell motility through kinase regulation of the host actin cytoskeleton. *PLOS Pathogens*, *10*, e1004003.

MacRae, J.I., Sheiner, L., Nahid, A., Tonkin, C., Striepen, B. and McConville, M.J. (2012) Mitochondrial metabolism of glucose and glutamine Is required for intracellular growth of *Toxoplasma gondii*. *Cell Host and Microbe*, *12*, 682–692.

Mantel, P.-Y., Hoang, A.N., Goldowitz, I., Potashnikova, D., Hamza, B., Vorobjev, I. et al (2013) Malaria-infected erythrocyte-derived microvesicles mediate cellular communication within the parasite population and with the host immune system. *Cell Host and Microbe*, *13*, 521–534.

Mardis, E.R., Ding, L., Dooling, D.J., Larson, D.E., McLellan, M.D., Chen, K. et al (2009) Recurring mutations found by sequencing an acute myeloid leukemia genome. *New England Journal of Medicine*, *361*, 1058–1066.

Marsolier, J., Pineau, S., Medjkane, S., Perichon, M., Yin, Q., Flemington, E. et al (2013) OncomiR addiction is generated by a miR-155 feedback loop in *Theileria*-transformed leukocytes. *PLOS Pathogens*, *9*, e1003222.

Massagué, J. (1998) TGF-β signal transduction. *Annual Review of Biochemistry*, *67*, 753–791.

McGuire, K., Manuja, A., Russell, G.C., Springbett, A., Craigmile, S.C., Nichani, A.K. et al (2004) Quantitative analysis of pro-inflammatory cytokine mRNA expression in *Theileria annulata*-infected cell lines derived from resistant and susceptible cattle. *Veterinary Immunology and Immunopathology*, *99*, 87–98.

McKeever, D.J., Nyanjui, J.K. and Ballingall, K.T. (1997) *In vitro* infection with *Theileria parva* is associated with IL10 expression in all bovine lymphocyte lineages. *Parasite Immunology*, *19*, 319–324.

Metheni, M., Lombès, A., Bouillaud, F., Batteux, F. and Langsley, G. (2015) HIF-1α induction, proliferation and glycolysis of *Theileria*-infected leukocytes. *Cellular Microbiology*, *17*, 467–472.

Morrison, W.I., Connelley, T., Hemmink, J.D. and MacHugh, N.D. (2015) Understanding the basis of parasite strain-restricted immunity to *Theileria parva*. *Annual Review of Animal Biosciences*, *3*, 397–418.

Oldham, W.M., Clish, C.B., Yang, Y. and Loscalzo, J. (2015) Hypoxia-mediated increases in l-2-hydroxyglutarate coordinate the metabolic response to reductive stress. *Cell Metabolism*, *22*, 291–303.

Olias, P., Etheridge, R.D., Zhang, Y., Holtzman, M.J. and Sibley, L.D. (2016) *Toxoplasma* effector recruits the Mi-2/NuRD complex to repress STAT1 transcription and block IFN-γ-dependent gene expression. *Cell Host and Microbe*, *20*, 72–82.

Oryan, A., Namazi, F., Sharifiyazdi, H., Razavi, M. and Shahriari, R. (2013) Clinicopathological findings of a natural outbreak of *Theileria annulata* in cattle: an emerging disease in southern Iran. *Parasitology Research*, *112*, 123–127.

Pain, A., Renauld, H., Berriman, M., Murphy, L., Yeats, C.A., Weir, W. et al (2005) Genome of the host-cell transforming parasite *Theileria annulata* compared with *T. parva*. *Science*, *309*, 131–133.

Razmi, G., Yaghfoori, S., Mohri, M., Haghparast, A. and Tajeri, S. (2019) The haematological, proinflammatory cytokines and IgG changes during an ovine experimental theileriosis. *Onderstepoort Journal of Veterinary Research*, *86*(1), 1–6. http://dx.doi.org/10.4102/ojvr.v86i1.1629

Rchiad, Z., Haidar, M., Ansari, H.R., Tajeri, S., Mfarrej, S., Ben Rached, F. et al (2020) Novel tumour suppressor roles for GZMA and RASGRP1 in *Theileria annulata*-transformed macrophages and human B-lymphoma cells. *Cellular Microbiology*, e13255.

Regev-Rudzki, N., Wilson, D.W., Carvalho, T.G., Sisquella, X., Coleman, B.M., Rug, M. et al (2013) Cell-cell communication between malaria-infected red blood cells via exosome-like vesicles. *Cell*, *153*, 1120–1133.

Sager, H., Brunschwiler, C. and Jungi, T.W. (1998) Interferon production by *Theileria annulata*-transformed cell lines is restricted to the beta family. *Parasite Immunology*, *20*, 175–182.

Sager, H., Davis, W.C. and Jungi, T.W. (1999) Bovine monocytoid cells transformed to proliferate cease to exhibit lineage-specific functions. *Veterinary Immunology and Immunopathology*, *68*, 113–130.

Sharma, A., Kumar, M., Aich, J., Hariharan, M., Brahmachari, S.K., Agrawal, A. et al (2009) Posttranscriptional regulation of interleukin-10 expression by hsa-miR-106a. *Proceedings of the National Academy of Sciences*, *106*, 5761–5766.

Shayan, P., Schop, B., Conze, G., Schein, E. and Ahmed, J.S. (1999) Is interleukin 2 necessary for the autocrine proliferation of *Theileria* -infected bovine cells? *Parasitology Research*, *85*, 409–412.

Somerville, R.P.T., Littlebury, P., Pipano, E., Duncan Brown, C.G., Shkap, V., Adamson, R.E. et al (1998) Phenotypic and genotypic alterations associated with the attenuation of a *Theileria annulata* vaccine cell line from Turkey. *Vaccine*, *16*, 569–575.

Struys, E.A., Gibson, K.M. and Jakobs, C. (2007) Novel insights into L-2-hydroxyglutaric aciduria: Mass isotopomer studies reveal 2-oxoglutaric acid as the metabolic precursor of L-2-hydroxyglutaric acid. *Journal of Inherited Metabolic Disease*, *30*, 690.

Sulkowski, P.L., Oeck, S., Dow, J., Economos, N.G., Mirfakhraie, L., Liu, Y. et al (2020) Oncometabolites suppress DNA repair by disrupting local chromatin signalling. *Nature*, *582*, 586–591.

Tajeri, S.andLangsley, G. (2020) *Theileria* secretes proteins to subvert its host leukocyte. *Biology of the Cell* (in press).

Tanaka, T., Narazaki, M. and Kishimoto, T. (2014) IL-6 in inflammation, immunity, and disease. *Cold Spring Harbor Perspectives in Biology*, *6*, 1–16.

Tretina, K., Gotia, H.T., Mann, D.J. and Silva, J.C. (2015) *Theileria*-transformed bovine leukocytes have cancer hallmarks. *Trends in Parasitology*, *31*, 306–314.

Waitkus, M.S. and Yan, H. (2020) Targeting isocitrate dehydrogenase mutations in cancer: emerging evidence and diverging strategies. *Clinical Cancer Research*, clincanres.1827.2020.

Weir, W., Sunter, J., Chaussepied, M., Skilton, R., Tait, A., de Villiers, E.P. et al (2009) Highly syntenic and yet divergent: a tale of two Theilerias. *Infection, Genetics and Evolution*, *9*, 453–461.

Wrenger, C. and Müller, S. (2003) Isocitrate dehydrogenase of *Plasmodium falciparum*. *European Journal of Biochemistry*, *270*, 1775–1783.

Xu, W., Yang, H., Liu, Y., Yang, Y., Wang, P., Kim, S.-H. et al (2011) Oncometabolite 2-hydroxyglutarate Is a competitive inhibitor of α-ketoglutarate-dependent dioxygenases. *Cancer Cell*, *19*, 17–30.

Ye, D., Guan, K.-L. and Xiong, Y. (2018) Metabolism, cctivity, and targeting of D- and L-2-hydroxyglutarates. *Trends in Cancer*, *4*, 151–165.

Yong, C., Stewart, G.D. and Frezza, C. (2020) Oncometabolites in renal cancer. *Nature Reviews Nephrology*, *16*, 156–172.

How to cite this article: Tajeri S, Haidar M, Sakura T, Langsley G. Interaction between transforming *Theileria* parasites and their host bovine leukocytes. *Mol Microbiol*. 2021;115:860–869. https://doi.org/10.1111/mmi.14642