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The kinetics of *Theileria parva* infection and lymphocyte transformation in vitro

Mara S.L. Rocchi [a,*], Keith T. Ballingall [a], Niall D. MacHugh [b], Declan J. McKeever [a,c]

[a] *Clinical Division, Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik, Scotland, UK*
[b] *Division of Animal Health and Welfare, College of Medicine and Veterinary Medicine, University of Edinburgh, Scotland, UK*
[c] *Division of Veterinary Clinical Studies, College of Medicine and Veterinary Medicine, University of Edinburgh, Scotland, UK*

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Abstract

Theileria parva is an intracellular protozoan parasite that causes a fatal lymphoproliferative disease of cattle known as East Coast Fever. The parasite infects host lymphocytes causing their transformation and uncontrolled proliferation. Infiltration of major organs with parasitized lymphoblasts results in most cases in death within 3 weeks. Although both T and B lymphocytes are susceptible to infection, the majority of cell lines arising from infection of peripheral blood mononuclear cells in vitro are of T cell lineage. To explore the basis of this phenotypic bias we have followed the very early stages of parasite development in vitro at the single cell level. Peripheral blood mononuclear cells were infected and stained for both surface phenotype and intracellular parasite antigen and analysed by flow cytometry. Although the parasite antigen was detected intracellularly as early as 6 h p.i., our data indicate that parasite infection does not lead to cell transformation in all instances. Rather, specific cell types appear to undergo selection very early after infection and expansion of particular cell subsets results in survival and growth of only a small proportion of the cells originally parasitized.

Keywords: *Theileria parva*; *Theileria parva*-infected lymphocytes (TpL); Polymorphic immunodominant molecule (PIM); Flow cytometry; Infection kinetics

1. Introduction

Theileria parva is an intracellular protozoan parasite of cattle that causes a fatal lymphoproliferative disease known as East Coast fever (ECF). With 25 million cattle at risk in Eastern, Central and Southern Africa, the economic impact of ECF on the livestock sector in the region has been estimated at $USD169 million per year (Mukhebi et al., 1992).

The life cycle of the parasite in its ruminant host begins with the inoculation of sporozoites during feeding of infected ticks. Within minutes sporozoites invade host lymphocytes where they rapidly differentiate into schizonts (Fawcett et al., 1982). This process is associated with transformation of the infected cells to a state of uncontrolled proliferation. By associating with the mitotic spindle, the parasite divides in synchrony with the host cell, resulting in each daughter cell inheriting the infection (Hullinger et al., 1964). In a proportion of parasitized cells, schizonts undergo further differentiation to a merozoite stage which, after rupture of the cell, invade erythrocytes giving rise to piroplasms. Infected erythrocytes are ingested by feeding ticks where the sexual stage of the parasite life cycle occurs.

Theileria-infected cells can be cultured directly from infected cattle or generated in vitro by infection of peripheral blood mononuclear cells (PBMC) with sporozoites (Malmquist et al., 1970; Brown et al., 1973). Infected cells can be propagated indefinitely as continuously growing cell lines (Dobbelaere et al., 1988). Virtually all infected cell lines obtained from cattle belong to the T cell lineage, although both T and B lymphocytes as well as macrophages and dendritic cells have been described as potential targets for sporozoite invasion in vitro (Moulton et al., 1984; Baldwin et al., 1988; Wells et al., 2000. *Theileria parva* sporozoite invasion and development within bovine dendritic cells. Microscopy Society of Southern Africa, Proceedings 29, 61; Stephens and Howard, 2002). Similarly, the majority of the cell lines that develop after in vitro infection of PBMC are of the T cell lineage (Morrison et al., 1996). Morrison et al. (1996) reported that in vitro infection gave rise initially to both infected B and T

* Corresponding author. Tel.: +44 131 4455111; fax: +44 131 4456111.
E-mail address: mara.rocchi@moredun.ac.uk (M.S.L. Rocchi).

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cells but that the B cells were eventually overgrown by T cells. They suggested that a selection process that preferentially supports growth of infected T cells may operate both in vivo and in vitro. We have investigated this further by monitoring the expansion of parasitized lymphocyte subsets in the period immediately following in vitro infection. Using a highly sensitive assay based on intracellular immunostaining and flow cytometry, we have obtained data on the establishment of infection in individual lymphocyte subpopulations and the kinetics of their growth over time. We used a mAb directed against a conserved region of the polymorphic immunodominant molecule (PIM) of *T. parva,* which is strongly expressed on the surface of the schizont, for detection of intracellular parasites. Previous work (Honda et al., 1998) has demonstrated the utility of this mAb for the analysis of infection kinetics in vitro.

2. Materials and methods

2.1. Source of animals and cells

Blood was collected in Alsever's solution (Alsever and Ainslie, 1941) by jugular venipuncture from healthy Boran cattle maintained under tick control at the International Livestock Research Institute (ILRI), Nairobi, Kenya. PBMC were isolated by flotation on Ficoll (Histopaque 1.077 g/ml, Sigma, UK) according to standard protocols. PBMC were resuspended in tissue culture media (RPMI 1640 supplemented with 10% heat inactivated FCS, 2 mM L-glutamine, 100 units/ml penicillin, 50 μg/ml streptomycin and 5×10^{-5} M 2-mercaptoethanol) at a density of 2×10^{6}/ml.

2.2. Sporozoites

Theileria parva sporozoites were prepared by trituration of salivary glands dissected from infected ticks under sterile conditions as described previously (Morrison et al., 1996). Sporozoite numbers were estimated assuming an average of 10^{4} sporozoite per infected acinus. The infection dose employed is the one that in previous studies (Morrison et al., 1996) has been shown to give saturating levels of binding to PBMCs. Sporozoites were resuspended in tissue culture media at a density of 2×10^{6}/ml and kept on ice until used for infection of PBMC.

2.3. In vitro infection of PBMC

For in vitro infection of PBMC, 1 ml aliquots of cell suspension were incubated with an equal volume of *T. parva* sporozoite suspension at 37 °C for 1 h. Cells were washed by centrifugation, resuspended in 2 ml of culture media and each aliquot was transferred to a single well of a 24 well plate. Cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air until harvested and stained for flow cytometric analysis. Once infection was established, *T. parva*-infected lymphocyte (TpL) lines were maintained by passage (1:5) every 3–4 days. Uninfected control cells were prepared by adding an equal volume of tissue culture media without sporozoites to PBMC followed by culture under identical conditions.

2.4. Immunofluorescent staining

Antigens present on the cell surface and within the cytosol were detected by sequential surface and intracytoplasmic immunofluorescence staining with the mAb outlined in Table 1. The optimal staining concentration of each mAb was determined by titration.

Cells (5×10^{5}) were first stained with a mAb specific for a surface determinant diluted in FACS buffer (phenol red free L15 media (Gibco BRL), 10% FCS, 0.1% NaN_3) for 30 min at 4 °C. After two washes, cells were incubated with a goat anti-mouse (GAM) IgG1 phycoerythrin (PE)-conjugated secondary antibody (SouthernBiotech, AL, USA) for a further 30 min at 4 °C. After a further two washes, cells were fixed in PBS-4% paraformaldehyde (PFA) for 10 min at room temperature (RT) and maintained at 4 °C in the dark up to 96 h pending analysis or further processed for cytoplasmic staining.

For cytoplasmic staining, fixed surface-stained cells were washed once in PBS and incubated for 30 min at RT in permeabilisation buffer (FACS buffer, 0.1% saponin)

Table 1
Primary (top) and secondary (bottom) antibodies to surface and cytoplasmic antigens

Antibody	Specificity	Isotype	Reference
IL-S 40	PIM	mouse IgG_{2a}	Honda et al. (1998)
Mouse Ig control	—	mouse IgG_1,	Commercial (Becton Dickinson)
IL-A 30	Bovine IgM	mouse IgG_1	Naessens et al. (1988)
MM1A	CD3-ε chain	mouse IgG_1	MacHugh et al. (1998)
CC30	CD4	mouse IgG_1	Bensaid and Hadam (1991)
IL-A 51	CD8-α chain	mouse IgG_1	MacHugh et al. (1991)
gb21A	δ chain of γ/δ TCR	mouse IgG_1	MacHugh et al. (1997)
IL-A 24	CD172a (monocytes)	mouse IgG_1	Ellis et al. (1988)
IL-A 111	CD25 (IL-2Rα)	mouse IgG_1	Naessens et al. (1992)
J11	BoLA Class II DR	mouse IgG1	Russell et al. (2000)
GAM IgG2a FITC conjugated	Mouse IgG2a	goat polyclonal	Commercial (SouthernBiotech)
GAM IgG1 PE-conjugated	Mouse IgG1	goat polyclonal	Commercial (SouthernBiotech)

Specification of antibodies used for surface and intracellular staining. PIM, polymorphic immunodominant molecule of *Theileria parva*; CD, HLDA (Human Leukocyte Differentiation Antigens) Cluster of Differentiation number; GAM, goat anti-mouse.