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## Bovine T Cells, B Cells, and Null Cells Are Transformed by the Protozoan Parasite *Theileria parva*†

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The target cells for infection and transformation by *Theileria parva* were investigated. Peripheral blood mononuclear cells were reacted with monoclonal antibodies specific for bovine leukocyte differentiation antigens, sorted into subpopulations with a fluorescence-activated cell sorter, and infected in vitro with *T. parva* sporozoites. Infected cells were cultured at limiting dilution, and transformed clones were screened with monoclonal antibodies. The results indicated that B cells, T cells (including BoT4<sup>+</sup> and BoT8<sup>+</sup> cells), and null cells but not monocytes or neutrophils were transformed in vitro after infection with *T. parva*. After transformation, peripheral blood T cells and T-cell clones retained expression of most or all of the T-cell differentiation antigens including the mature T-cell marker recognized by monoclonal antibody IL-A27, BoT2, and BoT4 or BoT8, and some cells acquired a low level of expression of BoT4, BoT8, or the null cell marker recognized by monoclonal antibody IL-A29. *T. parva*-transformed null cells retained expression of the IL-A29 determinant and acquired expression of BoT2 and BoT8 but not the IL-A27 determinant or BoT4. *T. parva*-transformed B cells in most instances lost expression of surface immunoglobulin and never acquired expression of the IL-A27 determinant, BoT2, BoT4, or BoT8, although some cells acquired a low level of expression of the null cell marker recognized by monoclonal antibody IL-A29. Further studies on cell lines and clones grown in vitro from populations isolated from *T. parva*-infected cattle suggested that the majority of the in vivo *T. parva*-transformed cells were of T-cell origin.

Theileriosis is a lymphoproliferative disease of cattle caused by the tick-transmitted protozoan *Theileria parva* (28). The parasites mature to sporozoites in the salivary glands of *Rhipicephalus appendiculatus*; after deposition with tick saliva into the mammalian host, they bind to and enter leukocytes, in which they differentiate to multinucleated bodies termed schizonts. The schizont can induce and maintain host cell blastogenesis and thereby clonal expansion of the infected cells (5, 18). Some infected cells proliferate in vivo (19), leading to rapid death of the host (28). In a proportion of infected cells, merogony occurs (26); released merozoites invade erythrocytes, differentiate to piroplasms, and reinitiate the infective cycle upon ingestion by ticks.

A number of investigators have sought to identify the bovine cell types which become infected with *T. parva* (10, 16, 21, 25). The studies suggest that in vitro *T. parva* can infect and transform bovine peripheral blood T cells, B cells (16), and perhaps monocytes (20), but that all transformed cells acquire some surface differentiation antigens characteristic of a subpopulation of proliferating T cells (21, 25). We have continued these investigations by using lineage-specific monoclonal antibodies (MAbs), which react with differentiation antigens on bovine peripheral blood B cells (24), monocytes (17), T cells (C. L. Baldwin, N. D. MacHugh, J. Naessens, J. Newson, and W. I. Morrison, *Immunology*, in press), and subsets of T cells (BoT4<sup>+</sup> and BoT8<sup>+</sup>) (3, 11, 27), and a subpopulation of null cells (unpublished data). In this paper we describe the precursor cells for *T. parva*-mediated transformation and the MAb phenotypes before and after transformation.

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### MATERIALS AND METHODS

**MAbs and immunofluorescence.** A summary of the MAbs used in immunofluorescence assays (16) for phenotyping cells before and after infection with *T. parva* is presented in Table 1.

**Cells.** Bovine peripheral blood mononuclear cells (PBM) were obtained from Ficoll-Hypaque gradients of blood collected in Alsever solution or defibrinated with glass beads as described previously (17). Neutrophils (98% pure) were obtained from the erythrocyte layer of Ficoll-Hypaque gradients by hypotonic lysis of the erythrocytes (3). Lymph node cells were obtained by biopsy as described elsewhere (5). Other tissue samples including lung and bone marrow were collected postmortem into RPMI 1640 culture medium (GIBCO, Uxbridge, United Kingdom) containing 10% heat-inactivated fetal bovine serum (GIBCO) with 2 mM glutamine, 50 µg of gentamycin per ml, and  $5 \times 10^{-5}$  M 2-mercaptoethanol (complete culture medium).

By indirect immunofluorescence staining (17) and sorting with a fluorescence-activated cell sorter (FACS) (Becton Dickinson and Co., Sunnyvale, Calif.) (3), PBM were enriched for specific subpopulations including monocytes (P8), BoT4<sup>+</sup> T cells (IL-A12), BoT8<sup>+</sup> T cells (IL-A17), surface immunoglobulin M-positive cells (B5/4), or null cells, which are non-T, non-B, nonmonocytic cells in PBM which uniquely express a differentiation antigen, recognized by MAb IL-A29 (the bovine homolog of the sheep antigen SBU-T19) (18). (Table 1).

**T-cell clones.** T-cell clones, which were used for infections with *T. parva* parasites in vitro, were either T-cell growth factor (TCGF)-dependent cells (6) (657.G6, 648.E4, and T19.17) or T-cell helper clones with specificity for autologous *T. parva*-infected cells (1) (T17.27 and T16.13).

TABLE 1. Expression of differentiation antigens by uninfected cells and cells infected and transformed by *T. parva*

Cell surface antigen	Defined by MAb (reference):	Expression on normal bovine cells	Expression on <i>T. parva</i> -infected <sup>a</sup> :		
			T cells	B cells	Null cells
IgM	B5/4 (2, 24), IL-A30 <sup>b</sup>	B cells	-	-, P	-
IgG	IL-A2 (2)	B cells	-	-, P	-
BoT2	IL-A26 (2) <sup>c</sup>	T cells	+	-	+, P
Mature T-cell marker	IL-A27 (2) <sup>c</sup>	T cells	+, -	-	-
BoT4	IL-A11,12 (3)	Class II-restricted T cells	+, -, P	-	-
BoT8	IL-A17 (11)	Class I-restricted T cells	+, -, P	-	+, P
Null-cell marker	IL-A29 <sup>d</sup>	Proportion of null cells	-, P	-, P	+
Monocyte marker	P8 (16)	Monocytes and neutrophils	-	-	ND

<sup>a</sup> +, 100% of the cells within a cell line expressed the antigen identified by the MAb; -, 0% of cells expressed the antigen; P, both a positive and negative population were present. With some antigens, variation of expression may occur among cell lines; i.e., all members of some cell lines may express the antigen (+), whereas no members of others do (-), or a variable expression among cells of still others may occur (P). ND, Not done.

<sup>b</sup> J. Naessens, J. Newson, D. J. L. Williams, and V. Lutje, Immunology, in press.

<sup>c</sup> Baldwin et al., in press.

<sup>d</sup> W. I. Morrison and N. D. MacHugh, unpublished data.

**Infection of cells with *T. parva*.** Cells were infected in vitro with *T. parva* (5) by incubation with sporozoites derived from salivary glands of infected adult ticks (9). Infected cells were also obtained from cattle infected either naturally or experimentally. Experimental infections were achieved by inoculation of cattle with stabilates of sporozoites derived from infected ticks (7) or with allogeneic *T. parva*-infected cells (22). Different stocks of *T. parva* parasites were used for infections; the names of the stocks are indicated within parentheses below.

Cells incubated with sporozoites in vitro or cells from tissues infected in vivo were cultured in complete medium either with fibroblastic feeder layers (5, 15), with TCGF (6), or with  $5 \times 10^4$  irradiated autologous PBM as filler cells. Cell lines were established as bulk cultures or from limiting dilution cultures in microwells in which cells were distributed at concentrations ranging from 1 to 3,000 cells per well (23). Cell lines established from cell dilutions which gave rise to cell growth in less than 30% of wells had greater than 83% probability of being clones (14) and are herein referred to as such. The frequencies of precursor cells which were transformed was calculated by using the maximum likelihood solution of de St. Groth (8). The presence of intralymphocytic schizonts in infected cultures was ascertained by

staining cytopsin smears of the cells with Giemsa after acid hydrolysis (15).

**RESULTS**

**Bovine leukocyte populations which can be transformed by *T. parva* in vitro.** To determine which cell populations could be transformed by *T. parva*, subpopulations of bovine PBM were isolated by using a FACS and incubated with *T. parva* sporozoites, and transformed cells were cloned by limiting dilution. Populations enriched for B cells were defined by MAb B5/4 (surface immunoglobulin M positive), T-cell subsets were defined by MAb IL-A12 (BoT4<sup>+</sup>) and IL-A17 (BoT8<sup>+</sup>), monocytes were defined by MAb P8, and null cells were defined by MAb IL-A29. Bovine peripheral blood neutrophils were incubated with sporozoites in the same way. The cultures were examined for a period of 3 to 5 weeks, and the proportion of cultures at each cell dilution which gave rise to *T. parva*-transformed cell lines was scored; from this the relative frequencies of precursor cells which gave rise to transformed cells were determined (Table 2). The frequencies of cell line establishment (Table 2) could not be compared between experiments because they were performed at different times with sporozoite batches which

TABLE 2. Frequency of cells infected and transformed by *T. parva* (Muguga) in vitro within PBM populations stained with MAb and sorted by the FACS and within populations enriched for neutrophils

Expt no.(n) <sup>a</sup>	Cell population enriched for:	Reactivity with MAb used for sorting	% Purity	Frequency of cells transformed (no/total)	Culture conditions for establishment of clones
1 (8)	Unsorted	NA <sup>b</sup>	NA	1/52	Fibroblasts
	B cells	B5/4 <sup>+</sup>	96	1/47	
	non-B cells	B5/4 <sup>-</sup>	97	1/49	
2 (2)	Unsorted	NA	NA	1/370	Fibroblasts
	Monocytes	P8 <sup>+</sup>	95	1/4,326	
	Nonmonocytes	P8 <sup>-</sup>	99	1/128	
3 (3)	Unsorted	NA	NA	1/27	TCGF
	Null cells	IL-A29 <sup>+</sup>	98	1/155	
4 (2)	BoT4 <sup>+</sup> T cells	IL-A12 <sup>+</sup>	90	1/25	Filler cells
	BoT8 <sup>+</sup> T cells	IL-A17 <sup>+</sup>	86	1/198	
5 (2)	Neutrophils	NA	98	0	Fibroblasts

<sup>a</sup> n = number of times experiment was carried out.

<sup>b</sup> NA, Not applicable.

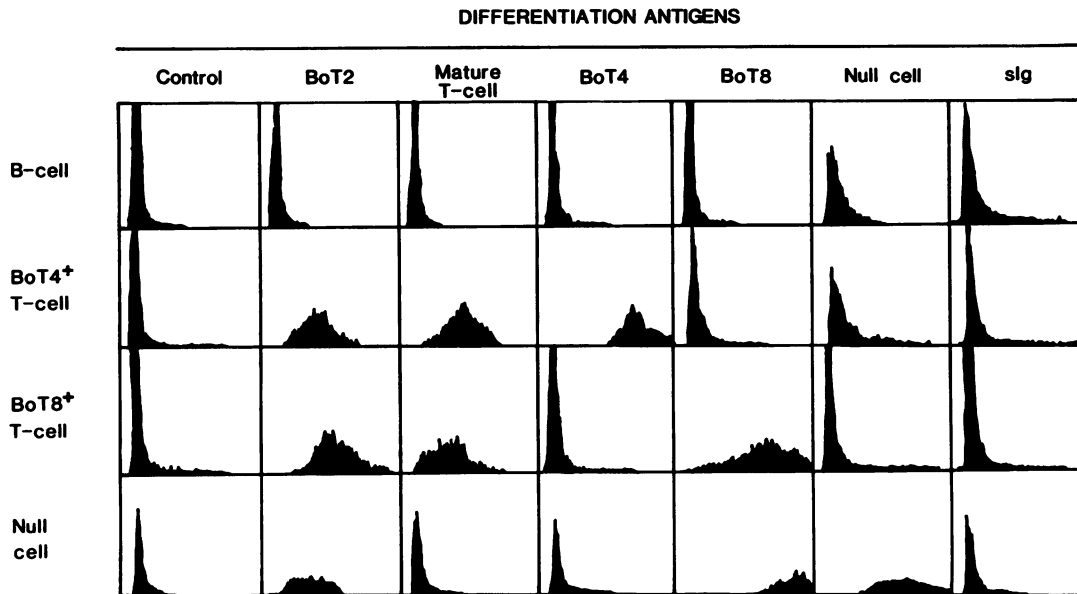


FIG. 1. FACS profiles of *T. parva*-transformed clones of an infected B cell, a BoT4<sup>+</sup> T cell, a BoT8<sup>+</sup> T cell, and a null cell established from PBM sorted by the FACS into the various subpopulations, infected with sporozoites, and cultured by limiting dilution. Reactivity is with MAbs to the differentiation antigens indicated (BoT2, the mature T-cell marker recognized by MAb IL-A27, BoT4, BoT8, the null cell marker recognized by MAb IL-A29, and surface immunoglobulin [sIg]). Horizontal axes are log relative fluorescence intensity, and vertical axes indicate relative cell numbers.

might have differed in their relative infectivity. Infection of cell populations enriched for and depleted of B cells indicated that B cells, as well as cells other than B cells in PBM, can be transformed by *T. parva* (Table 2, experiment 1). Bovine PBM contain T cells, monocytes, and null cells in addition to B cells (2). Further experiments showed that, although monocytes are not transformed, since the frequency of cells transformed in the enriched population was consistent with the proportion of contaminating cells (experiment 2), null cells were transformed (experiment 3). In addition, experiments were undertaken to infect PBM enriched for BoT4<sup>+</sup> and BoT8<sup>+</sup> T cells and to stain the resulting transformed clones with mAbs (experiment 4). For T cells sorted into a BoT4<sup>+</sup> enriched population, of the 12 clones tested, all were BoT4<sup>+</sup>; four were BoT4<sup>+</sup>/T8<sup>+</sup>, whereas the rest were BoT4<sup>+</sup>/T8<sup>-</sup>. In the BoT8<sup>+</sup> enriched population, 8 of 12 clones were BoT8<sup>+</sup>/T4<sup>-</sup>, whereas 4 were BoT4<sup>+</sup>/T8<sup>-</sup> and probably arose from the BoT8<sup>-</sup> contaminants. These results indicate that both major subsets of T cells are transformed. No cell lines were established from neutrophils (experiment 5), even though the sporozoites were shown to be infective for other cell populations (data not shown).

**Surface phenotypes of bovine B cells, T cells, and null cells after transformation by *T. parva*.** Examples of cell lines transformed by *T. parva* in vitro and derived from limiting dilution cultures, whose proportion of positive wells was highly consistent with giving rise to cloned cell lines, were stained with the MAbs described in Table 1 (Fig. 1). A summary of results of reactivity with MAbs following transformation, by the various types of cells categorized according to their pretransformation phenotype, is presented in Table 1. *T. parva*-transformed B cells never expressed the mature T-cell marker recognized by MAb IL-A27, BoT2, BoT4, or BoT8, and most clones (55 out of 60) ceased to express surface immunoglobulin M or G. Occasionally a

proportion of cells within some clones derived from *T. parva*-transformed B cells acquired a low level of expression of the peripheral blood null cell marker recognized by MAb IL-A29. In contrast, over 50 *T. parva*-transformed T cells examined expressed the mature T-cell marker recognized by MAb IL-A27, BoT2, and BoT4 and/or BoT8, and a proportion of cells in some clones acquired a low level of expression of the peripheral blood null cell marker recognized by MAb IL-A29 but never surface immunoglobulin. All 10 *T. parva*-transformed null cell clones examined expressed high levels of the IL-A29 determinant and acquired expression of BoT8 and BoT2 but not the IL-A27 determinant, BoT4, or surface immunoglobulin.

**Transformation of cloned T cells by *T. parva* in vitro.** To examine the influence of *T. parva*-induced transformation on the surface phenotypes of T cells, over 30 T-cell clones dependent on TCGF or on TCGF and antigen were infected and transformed with different stocks of *T. parva*. Transformed cells grew independently of further TCGF or antigenic stimulation and were screened for expression of the IL-A27 determinant, BoT2, BoT4, BoT8, surface immunoglobulin, and the null cell marker defined by MAb IL-A29. Most *T. parva*-transformed T-cell clones retained expression of the IL-A27 determinant, BoT2, and either BoT4 or BoT8 (Table 3). Expression, or lack thereof, of all four T-cell differentiation antigens by clones 639.B7 and 648.E4 remained the same after transformation. With some clones changes in the expression of differentiation antigens occurred (Table 3). Of particular significance was the complete loss of expression of the IL-A27 determinant by cells of clone T19.17 after transformation with *T. parva* (Muguga). This alteration, which occurred with only two of the transformed T-cell clones, resulted in a MAb phenotype which was similar to that of *T. parva*-transformed null cells (Table 1). The *T. parva*-transformed T19.17 cells also acquired an intermediate level of expression of the target epitope for

TABLE 3. Expression of differentiation antigens by T-cell clones before and after infection in vitro with different parasite stocks of *T. parva*

T-cell clone	<i>T. parva</i> stock used for infection	% Cells expressing the differentiation antigen:				
		BoT2	IL-A27	BoT4	BoT8	Surface immunoglobulin
639.B7	Uninfected	100	100	100	0	0
	Marikebuni	100	100	100	0	0
	Muguga	100	100	100	0	0
T17.27	Uninfected	100	100	100	0	0
	Uganda	100	100	100	10	ND <sup>a</sup>
	Muguga	100	100	100	66	ND
T16.13	Uninfected	100	100	100	0	ND
	Muguga	100	100	100	0	ND
	Marikebuni	100	100	22	0	ND
657.G6	Uninfected	100	100	0	100	0
	Muguga	100	100	0	100	0
	Marikebuni	100	100	0	100	0
	Mariakani	100	100	16	100	0
648.E4	Uninfected	100	100	52	100	0
	Muguga	100	100	43	100	0
T19.17	Uninfected	100	100	0	100	ND
	Marikebuni	100	80	0	75	0
	Muguga	100	0	0	100	0

<sup>a</sup> ND, Not done.

MAB IL-A29 and hence closely resembled transformed null cells. None of the T-cell clones acquired expression of surface immunoglobulin after transformation.

**Analyses of the surface phenotypes of bovine leukocytes infected with *T. parva* in vivo and propagated in vitro.** The previous studies showed that bovine peripheral blood T cells, B cells, and null cells could be infected and transformed in vitro with *T. parva* and that transformed B and T cells could be distinguished by their surface MAB phenotypes after transformation (Table 1). To ascertain which cell types were infected and transformed in vivo, we examined bulk cell lines and clones established from various organs of infected cattle.

All but one of the cell lines established as bulk cultures from lymph nodes of 20 infected cattle appeared to be of T-cell origin, since they expressed BoT2 and the IL-A27

TABLE 4. Expression of differentiation antigens by cells infected with *T. parva* in vivo and generated in bulk cultures from various organs of animal B662

Origin of cell line <sup>a</sup>	% Cells expressing the differentiation antigen:				Surface immunoglobulin
	BoT2	IL-A27	BoT4	BoT8	
Lymph node					
Draining	100	100	70	100	0
Contralateral	100	100	100	49	0
Prefemoral	100	100	100	42	0
Thymus	100	100	100	50	0
Bone marrow	100	100	100	60	0
Lung	100	100	100	45	0
Peripheral blood	100	100	100	35	0

<sup>a</sup> All bulk cultures were taken from the animal at 14 days postinfection and grown for at least 4 weeks in vitro before staining.

determinant plus BoT4 and/or BoT8. The cell lines included lines established from lethally or nonlethally infected cattle at different times after infection (days 8 through 18), with parasites belonging to all three subspecies (29) of *T. parva* (*parva*, *lawrencei*, or *bovis*). The one exception (B593) appeared to be of B-cell origin, since the cells did not express any of the T-cell differentiation antigens. None of the infected cell lines expressed surface immunoglobulin.

In one experiment, *T. parva*-transformed bulk cell lines and clones were established from various organs of a single infected animal (B662). All of the bulk cell lines appeared to be of T-cell origin (Table 4). In contrast, cloned cell lines established by limiting dilution from the lymph node draining the site of inoculation with sporozoites, but not from other organs, appeared to be of B-cell origin in that they were negative for T-cell markers (Table 5). In addition, one clone established from the prefemoral lymph node might have been of null cell origin, since it expressed BoT2 and BoT8 but not the IL-A27 determinant or BoT4 and had high levels of expression of the IL-A29 determinant. As mentioned above, *T. parva*-transformed null cells cannot be distinguished from all transformed T cells on the basis of the MABs used, and hence it remains possible that the above cell line was of T-cell origin.

## DISCUSSION

Bovine peripheral blood B cells, BoT8<sup>+</sup> and BoT4<sup>+</sup> T cells, and null cells, isolated with specific MABs by FACS separation, could be infected and transformed by *T. parva* in vitro. This confirms and extends studies conducted with a more limited battery of MABs (10, 16, 22, 25) and supersedes the idea that only a minor subset of T cells is transformed by the parasite (25). Although monocytes have been shown to bind and endocytose *T. parva* sporozoites (12) and have been speculated to be targets for *T. parva*-mediated trans-

TABLE 5. Expression of differentiation antigens by cells infected with *T. parva* in vivo and cloned by limiting dilution cultures from various organs of animal B662

Origin of cell line	Days p.i. <sup>a</sup>	Clone designation	% Cells expressing the differentiation antigens:					Surface immunoglobulin
			BoT2	IL-A27	BoT4	BoT8	Null	
Lymph node								
Draining	8	2	0	0	0	0	36	0
	8	3	0	0	0	0	0	0
	8	4	0	0	0	0	0	0
Prefemoral	14	2	100	0	0	100	100	0
Bone marrow	14	C10	100	100	100	0	0	0
Lung	14	B9	91	100	100	53	27	0
	14	F8	89	100	100	81	42	0
	14	G5	77	100	100	0	0	0
	14	H6	100	100	100	20	37	0
	14	H9	88	100	100	28	0	0

<sup>a</sup> Days postinfection (p.i.) indicates the day after infection on which the sample was obtained for cloning.

formation (20), we have shown in this study that neither monocytes nor neutrophils were transformed by the parasites.

B cells transformed by *T. parva* in vitro often lost expression of immunoglobulin but never acquired the expression of the T-cell differentiation antigens recognized by MAb IL-A27, BoT2, BoT4, or BoT8. Peripheral blood T cells or T-cell clones propagated in vitro, when transformed with *T. parva* and propagated in vitro, almost always retained expression of BoT2 and the IL-A27 determinant and generally retained expression of either BoT4 or BoT8. Additionally some clones also acquired expression of BoT4 or BoT8. Null cells transformed in vitro with *T. parva* retained expression of the IL-A29 determinant and acquired expression of BoT2 and BoT8 on a proportion of the cells. Thus although all *T. parva*-transformed cells acquire some markers characteristic of a subset of multiplying T cells as shown with other MAbs (21), cells of different precursor origin nonetheless retain other characteristic differences.

It was therefore reasonable, on the basis of MAb phenotypes of transformed cells, to speculate on the precursors of cells transformed in vivo. Analyses of bulk cell lines and clones derived from infected cattle suggested that most cells transformed in vivo by *T. parva* were T cells, consistent with results by Emery and co-workers, who demonstrated that the majority of parasitized cells in the efferent lymph from the node draining the site of sporozoite inoculation had T-cell differentiation antigens (D. L. Emery, N. D. MacHugh, and W. I. Morrison, *Parasite Immunol.*, in press). However, clones of *T. parva*-transformed cells established from the draining lymph node of one infected cow were most likely of B-cell origin. In contrast, the bulk cell lines derived from the same organ were clearly of T-cell origin, suggesting that *T. parva*-transformed T cells either predominate or overgrow *T. parva*-transformed B cells in bulk cultures. A high proportion of cells within the transformed T-cell bulk cultures expressed both BoT4 and BoT8. These cultures probably contain a mixture of T cells, including some which have acquired expression of either BoT4 or BoT8 after transformation, as has been shown for cloned T cells in vitro. Similar results with both in vitro and in vivo transformed cells have been found by Emery and co-workers (Emery et al., in press).

The demonstration that bovine B cells, T cells, and null cells can all be infected and transformed by *T. parva* raises several possibilities. Different transformed cells may elicit

different pathogenic processes, differ in their capacity to elicit protective immune responses, or differ in their capacity to support *T. parva* differentiation to merozoites. With respect to these questions, preliminary studies show that culture supernatants from infected T cells, but not B cells, can be suppressive in a number of in vitro cell-mediated immune assays including mixed lymphocyte responses (13) and concanavalin A- and phytohemagglutinin-stimulated proliferation (Baldwin, unpublished data). However all *T. parva*-transformed cells express both class I and class II major histocompatibility complex determinants (1, 4, 19) and hence, regardless of phenotype, may be able to present parasite-induced antigens to stimulate helper and cytotoxic T cells. Currently studies are being conducted to evaluate the pathological consequences of infections initiated in autologous hosts with cloned infected cells from different lymphocyte subpopulations.

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