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Pathogenicity of *Theileria parva* Is Influenced by the Host Cell Type Infected by the Parasite

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Theileria parva **has been shown to infect and transform B cells and T cells at similar frequencies in vitro. However, the majority of parasitized cells in the tissues of infected cattle are α/β T cells. The aim of this study was to determine whether the cell type infected with *T. parva* influenced the pathogenicity of the parasite. The initial approach, which involved inoculation of cattle with autologous cloned cell lines of different phenotypes, failed to resolve the issue, because the prolonged period of culture required to clone and characterize the cell lines resulted in attenuation of the cells. As an alternative approach, cattle were inoculated with purified populations of autologous cells that had been incubated in vitro with *T. parva* sporozoites for 48 h. As few as 3×10^4 peripheral blood mononuclear cells (PBMC) treated in this way were found to produce severe clinical reactions with high levels of parasitosis. Infections of similar severity were produced with purified populations of $CD2^+$, $CD4^+$, and $CD8^+$ T cells. By contrast, infected B cells gave rise to mild self-limiting infections even when administered at a 10-fold-higher dose. In animals that received infected $CD4^+$ or $CD8^+$ T cells, the parasitized cells in the lymph nodes on day 11 of infection were all within the $CD4^+$ and $CD8^+$ populations, respectively, indicating that there had been minimal transfer of the parasite between cell types. Phenotypic analyses of cultures of PBMC infected in vitro with saturating concentrations of sporozoites revealed that parasitized B cells were abundant in the cultures after 1 week but were subsequently overgrown by T cells. The results of these experiments indicate that the cell type infected by *T. parva* influences the pathogenicity of the parasite.**

Theileria parva is a tick-borne protozoan parasite of cattle that infects lymphocytes and causes an acute, usually fatal lymphoproliferative disease known as East Coast fever. The disease is characterized by the presence of large numbers of parasitized lymphoblasts throughout the lymphoid system, which eventually leads to lymphocyte destruction and leukopenia (reviewed in reference 15). A unique relationship of *T. parva* with host lymphocytes is central to its ability to undergo rapid replication in the animal. Following invasion of the lymphocyte, the sporozoite rapidly gains access to the cytosol and develops into the multinucleate schizont, an event that is associated with activation and proliferation of the infected cell. An association with the mitotic spindle enables the parasite to divide synchronously with the lymphocyte (14). Thus, multiplication of parasites is believed to occur mainly by clonal expansion of the lymphocytes initially infected with sporozoites. This characteristic of parasitized cells allows them to be propagated in vitro as continuously growing cell lines; such lines can be established with cells taken from infected cattle (20) or by infection of lymphocytes in vitro with sporozoites (4).

Limiting-dilution cultures of purified populations of lymphocytes infected in vitro with sporozoites have been employed to identify the cell types that support growth of the parasite. These experiments have demonstrated that T cells of CD4, CD8, or γ/δ lineages and B cells can all be infected at similar frequencies (1). Phenotypic analyses of the resultant cloned cell lines showed that each cell type retained a distinctive phenotype, although expression of immunoglobulin was down-regulated in infected B cells and many infected $CD4^+$ clones coexpressed CD8. By contrast, analyses of cells from lymphoid tissues of infected cattle showed that virtually all of the infected cells expressed T-cell markers (11). An important question raised by these findings is whether parasitized T and B cells differ in their growth characteristics in vivo and hence in their capacity to cause disease or whether there is preferential infection of T cells in vivo. The studies described herein set out to resolve these issues by examining the nature of infections established following inoculation of cattle with purified populations of autologous lymphocytes infected with *T. parva.*

MATERIALS AND METHODS

Animals. All the animals used in the study were female or castrated male Boran cattle (*Bos indicus*) between 6 and 18 months old. The animals were produced on the International Livestock Research Institute ranch, which is free of *T. parva*, and were reared indoors under parasite-free conditions from 4 to 5 days of age. All animals were seronegative for antibodies to *T. parva* schizonts (13) at the outset of the experiments.

Parasites. The Muguga stock of *T. parva* was used throughout. Sporozoites were harvested from salivary glands dissected from infected nymphal *Rhipicephalus appendiculatus* ticks that had been prefed on rabbits for 5 days as described previously (5). The level of infection in each batch of ticks was estimated by counting the number of infected acini in a sample of dissected salivary glands stained with methyl green pyronine.

Cell populations. Peripheral blood mononuclear cells (PBMC) were isolated from venous blood by density gradient centrifugation as described elsewhere (18). Purified populations of B cells and T cells were obtained by indirect immunofluorescence staining of PBMC with specific monoclonal antibodies (MAbs) and then by sorting of positive cells on a FACStar Plus cell sorter (Becton Dickinson, Mountain View, Calif.). In each experiment, a sample of sorted cells was checked for purity and if necessary the cells were subjected to a second round of sorting. All sorted cell populations used in the experiments were of 98% or greater purity. The following MAbs were employed: CH128A (immunoglobulin G1 [IgG1]), IL-A11 (IgG2a), and IL-A51 (IgG2a), specific for bovine CD2, CD4, and CD8, respectively (2, 8, 19); IL-A30 (IgG2a), specific for bovine IgM (27); and IL-A29 (IgG1), specific for a molecule termed WC1 whose expression is restricted to bovine γ/δ T cells (6).

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TABLE 1. Reactions of cattle inoculated with autologous *T. parva*-infected cell lines of different phenotypes

Animal	No. of cells	Source of cell line	Phenotype of cell line[a]				Parasitosis in regional lymph node		
			CD4	CD8	WC1	Ig	Onset (day)	Level[b]	Duration (days)
B944	5×10^4	PBMC	+	−	−	−	7	+++	6
C166	1×10^5	PBMC	+	±	−	−	5	+++	11
C428	1×10^5	PBMC	+	+	−	−	6	+	4
C244	1×10^5	PBMC	+	±	−	−	6	+	1
C190	1×10^5	PBMC	−	+	−	−	8	+	2
C197	1×10^5	PBMC	−	+	−	−		nd	
D673	1×10^5	$WC1^+$ cells	−	±	+	−	6	+	2
D647	1×10^5	sIg^+ cells	−	−	−	−		nd	
C672	1×10^5	sIg^+ cells	−	−	−	−		nd	
C193	1×10^5	sIg^+ cells	−	−	−	−		nd	
D648	1×10^5	sIg^+ cells	−	−	−	−	9	+	2

[a] +, all cells in the culture were positive for the marker; ±, only a portion of the cells were positive; −, none of the cells were positive.
[b] +++, ++, and +, levels of parasitosis of >10%, 1 to 10%, and <1%, respectively; nd, not detectable.

Infection of cells with *T. parva*. For infection with *T. parva*, PBMC and sorted lymphocytes were suspended at 2×10^7 cells per ml in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, L-glutamine, 5×10^{-5} M 2-mercaptoethanol, and gentamicin (culture medium). Infected tick salivary glands were suspended in the same medium and ground in a glass tissue grinder at room temperature. After centrifugation at $100 \times g$ for 5 min to remove coarse debris, the sporozoite suspension was adjusted to a concentration estimated to be the equivalent of 2,000 infected salivary gland acini per ml. Previous studies had shown that this concentration of sporozoites gave saturating levels of binding (i.e., maximal numbers of cells with sporozoites bound) to PBMC (17). Equal volumes of sporozoites and cells were mixed in 10-ml polycarbonate test tubes, and, after incubation at 37°C for 90 min with periodic mixing, the cells were centrifuged, washed once, and resuspended in culture medium at 4×10^6/ml. For establishment of bulk cell lines, the cells were distributed either in 1-ml aliquots into 24-well cluster plates or in 5-ml aliquots into T25 flasks. To obtain cloned infected cell lines, cells were distributed at limiting dilutions ranging from 10^3 cells to 1 cell per well into 96-well plates containing a fibroblast feeder layer (BT6) derived from bovine fetal thymus tissue, as described previously (1). Cell line establishment was monitored microscopically, and wells with cell growth at a clonal frequency were expanded first into 24-well cluster plates and then into T25 flasks. Cell lines were maintained by subculture and addition of fresh culture medium every 2 to 3 days.

In some experiments, sorted cells infected with sporozoites were inoculated back into the donor animals after 48 h of culture in medium containing 2.5% T-cell growth factor derived from concanavalin A-stimulated PBMC as described previously (26).

The phenotypes of infected cell lines were determined by flow-cytometric analysis, on a FACStar Plus cell sorter, after staining by indirect immunofluorescence with a panel of MAbs specific for bovine leukocyte differentiation antigens as described elsewhere (18).

Infection of cattle. Two types of experiment were carried out in cattle. In the first, a group of 11 cattle was inoculated each with 10^5 (in one animal 5×10^4) autologous parasitized cells from cloned cell lines of different phenotypes. (For the origins and phenotypes of the cell lines, see Table 1.) They consisted of six $CD4^+$ or $CD8^+$ cell lines derived by cloning from cell lines established by infection of PBMC in vitro, one cell line obtained by infection and limiting dilution cloning of purified γ/δ T cells ($WC1^+$), and four cell lines obtained by infection and limiting dilution cloning of purified B cells. Three of the four $CD4^+$ cell lines coexpressed CD8 on a variable proportion of cells. The cloned cell lines had been in culture for periods ranging from 10 to 16 weeks at the time that they were used to inoculate cattle.

The second experimental approach involved inoculation of cattle with autologous PBMC or purified populations of T or B lymphocytes, which had been incubated in vitro with sporozoites for 48 h as described above. Because of the time that was required to obtain purified populations of lymphocytes by cell sorting, it was not possible to use large numbers of animals in a single experiment. Therefore, a series of four experiments, each involving three or four animals, was carried out (see Table 2).

Cells destined for inoculation into cattle were centrifuged, washed once in culture medium, and resuspended in 1 ml of phosphate-buffered saline, pH 7.2. The inocula were administered subcutaneously on the right side of the neck, and animals were monitored daily for rectal temperature and the appearance of parasitized cells, from day 5 until the experiments were terminated. Smears prepared from puncture biopsy samples of the right and left prescapular lymph nodes were fixed in cold acetone for 2 min and, after drying, stained with fluorescein isothiocyanate-labelled bovine antiserum to *T. parva* and examined microscopically for the presence of parasites. The percent parasitosis was determined by counting the number of schizonts in 1,000 cells.

All of the animals in the first experiment (see Table 1) and those that did not succumb to infection in the second set of experiments (see Table 2) were challenged, along with susceptible control animals, with a lethal dose of cryopreserved *T. parva* (Muguga) sporozoites (stabilate 836) between 4 and 12 weeks after initial infection. The parasites were injected subcutaneously on the right side of the neck and the animals were monitored clinically and parasitologically on a daily basis as described above for primary infections.

Phenotypes of parasitized cells in infected animals. In two of the experiments in which cattle were inoculated with purified populations of lymphocytes that had been incubated with sporozoites (experiments 3 and 4 [see Table 2]), the phenotypes of infected cells in the lymph nodes were examined on day 11 after infection. Lymph node puncture biopsy samples were collected into a 5-ml syringe containing Alsever's solution, and viable mononuclear cells were isolated by centrifugation over Ficoll Isopaque/Pharmacia at $900 \times g$ for 20 min. The cells were washed once in Alsever's solution and stained by indirect immunofluorescence with MAb specific for T-cell populations. The positive and negative cells in each stained population were sorted to >98% purity on a FACStar Plus cell sorter, and cytospin smears were prepared and stained by immunofluorescence for detection of parasitized cells in the same way as for lymph node puncture biopsy samples.

RESULTS

Reactions of animals inoculated with cloned autologous cell lines. Previous studies had shown that inoculation of cattle with 10^5 or more autologous parasitized cells from recently established cell lines resulted in severe, often fatal infections (5). In an attempt to investigate the influence of the phenotype of the infected cells on the severity of infection, 11 cattle were each inoculated with 10^5 (in one animal 5×10^4) autologous parasitized cells from established cloned cell lines. The phenotypes of the cell lines and the clinical reactions of the animals following inoculation are presented in Table 1.

The severity of the infections produced by the different cell lines varied markedly. Three animals that received B cells and one that received $CD8^+$ T cells showed no clinical evidence of infection. In a further five animals a few parasites were detected in the regional lymph node for 1 to 4 days and there was transient fever. Only in two animals (B944 and C165), both of which received $CD4^+$ T cells, was there a moderately severe reaction, with prolonged fever and high levels of parasitized cells (>10%) in the regional lymph nodes; however, these two animals cleared the infection on days 13 and 18 (Table 1).

There was therefore no consistent correlation between the phenotype of the infected cells and the severity of the infections that they produced.

All of the animals were challenged in two groups, along with susceptible controls, with a lethal dose of *T. parva* (Muguga) sporozoites at 4 and 12 weeks after the initial infection. All except two of the animals developed mild or inapparent reac-

TABLE 2. Reactions of cattle inoculated with different cell populations 48 h after infection in vitro with *T. parva* sporozoites

Expt	Animal	Cell population[a]	No. of cells	Fever		Parasitosis					
						Local node			Opposite node		
				Onset (day)	Duration (days)	Onset (day)	Level[b]	Duration (days)	Onset (day)	Level[b]	Duration (days)
1	E107	PBMC	2×10^5	7	>9	7	+++	>9	9	+++	>7
	D825	$CD2^+$	2×10^5	7	>9	6	+++	>9	9	+++	>7
	D822	sIg^+	2×10^5			9	+	3			
	D836	Supt									
2	D811	PBMC	3×10^5	6	>10	6	+++	>10	9	+++	>7
	E110	$CD2^+$	3×10^5	6	>10	6	+++	>10	8	+++	>8
	D826	sIg^+	3×10^5	6	4	6	++	5			
	D812	Supt									
3	F1	PBMC	3×10^4	9	>7	6	+++	>10	9	+++	>7
	E313	$CD4^+$	3×10^4	7	>9	6	+++	>10	9	+++	>7
	E332	$CD8^+$	3×10^4	6	>10	6	+++	>10	9	+++	>7
4	F22	PBMC	2×10^5	9	>7	6	+++	>10	9	+++	>7
	F10	$CD4^+$	2×10^5	7	>9	5	+++	>11	8	+++	>8
	F16	$CD8^+$	2×10^5	8	>8	6	+++	>10	9	+++	>7
	E312	sIg^+	2×10^6	9	4	7	++	8	10	+	1

[a] Supt, supernatant from infected PBMC after 48 h of culture.
[b] +++, ++, and +, levels of parasitosis of >10%, 1 to 10%, and <1%, respectively. All animals that underwent severe reactions were killed 16 days after infection.

tions, in some instances with a few parasites being detected for up to 4 days in the regional lymph node (data not shown), indicating that they had developed immunity. Two of the animals that initially showed no reaction following inoculation with infected B-cell lines (C193 and C672) developed severe infections with prolonged fever and high levels of parasitosis in regional and contralateral lymph nodes. One of these animals (C193) died on day 21, whereas the other cleared the parasites on day 22 and recovered.

Reactions of animals inoculated with purified cell populations infected in vitro with *T. parva*. The relatively mild infections produced by cloned parasitized cell lines were considered to reflect selection of infected cells and/or the parasite, during the period of culture required to clone, expand, and characterize the parasitized cell lines. To overcome this problem, four experiments were carried out in which various cell populations purified from individual animals by cell sorting were infected with sporozoites and inoculated into the donor animal after culture for 48 h. Doses ranging from 3×10^4 to 3×10^5 cells were administered in the different experiments. The resultant infections were compared with that produced by a similar dose of whole PBMC subjected to the same staining and sorting procedures and cultured under the same conditions. In these experiments, all animals that continued to exhibit fever and high levels of parasites in regional and other lymph nodes were killed on day 16.

The cell types that the animals received and the reactions of the individual animals following infection are presented in Table 2. In two experiments supernatants from infected whole PBMC were collected after 48 h of culture and inoculated into a susceptible animal. Both animals (D812 and D836) remained negative for parasites, showed no clinical signs of infection, and were fully susceptible to subsequent challenge with a lethal dose of sporozoites. On the basis of these findings and similar previous results (22), it was concluded that there were no surviving extracellular sporozoites in the 48-h cultures.

In all four experiments, animals that received infected PBMC developed severe reactions, with fever commencing between days 6 and 9 and parasites initially being detected in the regional lymph node on day 6 or 7 and attaining persistently high levels in both the regional and contralateral lymph nodes. In the first two experiments, individual animals were inoculated with purified autologous T cells ($CD2^+$) or B cells infected 48 h previously with sporozoites. In each instance, the animal that received T cells exhibited severe infection comparable to that observed in animals inoculated with infected PBMC. By contrast, the two animals that received infected B cells (D822 and D826) developed mild self-limiting infections, with parasites detected only in the regional lymph nodes. Parasitized cells were detectable for 3 and 5 days, reaching peak levels of 0.2 and 5%, respectively; only one of these animals exhibited fever.

In the third and fourth experiments (Table 2), infections produced after inoculation of animals with purified $CD4^+$ and after inoculation with $CD8^+$ T cells were compared. In each experiment, both T-cell subsets produced severe infections similar to that induced by whole PBMC. In the fourth experiment, an additional animal (E312) was included, and it received 2×10^6 infected purified B cells, i.e., 10 times the number of cells given to animals infected with PBMC, $CD4^+$ cells, or $CD8^+$ cells. Despite the higher dose of cells, this animal was able to control the infection. It developed fever and had detectable parasitized cells for 9 and 8 days, respectively, and had cleared the parasites by day 15. However, the level of parasitosis in the regional lymph node did not exceed 1% and parasites were detectable for only 1 day in the contralateral lymph node.

Given the previous findings that B cells and T cells can be infected at similar frequencies with *T. parva* (1), the results of these experiments indicate that infected B cells and T cells, or the parasites therein, differ in their capacity to replicate and cause disease in vivo.

All three animals that recovered from infection following inoculation with infected B cells were resistant to infection when challenged with a lethal dose of *T. parva* (Muguga) sporozoites 6 weeks later. Two of the animals exhibited fever for 1 day, and a few parasites were detected in the regional lymph node of only one animal (B312) for 3 days (data not

TABLE 3. Phenotypes of parasitized cells in lymph nodes of cattle inoculated with purified cell populations infected with *T. parva*

Animal	No. and type of infecting cells	Lymph node tested[a]	Sorted population	% Parasitosis
F1	3×10^4 PBMC	Regional	$CD4^+$	78.0
			$CD4^-$	4.0
E313	3×10^4 $CD4^+$	Regional	$CD4^+$	70.0
			$CD4^-$	<0.1
E332	3×10^4 $CD8^+$	Regional	$CD8^+$	69.0
			$CD8^-$	<0.1
F10	2×10^5 $CD4^+$	Contralateral	$CD4^+$	12.5
			$CD4^-$	<0.1
F16	2×10^5 $CD8^+$	Contralateral	$CD8^+$	1.0
			$CD8^-$	<0.1
E312	2×10^6 sIg^+	Regional	$CD2^+$	5.0
			$CD2^-$	<0.1

[a] All lymph nodes were tested on day 11 of infection.

shown). Challenge control animals developed sustained fever and high levels of parasitosis.

Phenotypes of parasitized cells in animals infected with defined cell populations. In six of the animals used in experiments 3 and 4 (Table 2), cells aspirated by a needle biopsy technique from lymph nodes on day 11 of infection were stained with a single T-cell marker and fractionated into positive and negative populations for detection of parasites. Cells from one animal that received PBMC (F1) and two animals that received $CD4^+$ T cells (E313 and F10) were sorted into $CD4^+$ and $CD4^-$ populations, while cells from two animals that received $CD8^+$ cells (E332 and F16) were sorted into $CD8^+$ and $CD8^-$ populations. Cells obtained from the animal inoculated with 2×10^6 purified B cells (E312) were sorted into $CD2^+$ and $CD2^-$ populations. The results of these analyses are shown in Table 3.

In the animal infected with whole PBMC, parasitized cells were found within both the $CD4^+$ and $CD4^-$ populations, although a majority were $CD4^+$. By contrast, in animals that received infected $CD4^+$ or $CD8^+$ cells, all of the detectable parasitized cells were within the respective $CD4^+$ or $CD8^+$ populations. Thus, as far as could be detected, the parasite was still confined to the T-cell type originally infected. However, in the cells from the animal that received 2×10^6 infected B cells, parasitized cells were detected in the $CD2^+$ but not in the $CD2^-$ population, indicating that most, if not all, of the patent infection on day 11 was attributable to T cells.

Infected B cells are prominent in early cultures of infected PBMC. Despite the previous finding that purified B cells and T cells become infected and establish cell lines at similar frequencies in vitro, the majority of cell lines established from mixed populations of cells have a T-cell phenotype (1, 21), suggesting that development of the parasite in B cells or growth of parasitized B cells might be impaired in the presence of other cell types. However, in none of the previous studies were infected cell lines derived from PBMC analyzed during the early stages of establishment to determine whether infected B cells were present. Whole PBMC were therefore infected in vitro with saturating levels of sporozoites and the level of infection and surface expression of Ig and the T-cell markers CD2, CD4, and CD8 were examined after different periods of culture. The results of the surface phenotype analyses for four cell lines are shown in Table 4. In this experiment, transformation of the cultures was apparent after 4 to 5 days, and by day 7 more than 85% of the cells were parasitized lymphoblasts. On day 7, large numbers of surface Ig^+ (sIg^+) cells were found in all four cultures (ranging from 22 to 68%), and in three of the four cultures sIg^+ cells outnumbered the cells expressing T-cell markers. However, by day 14, the numbers of sIg^+ cells had markedly declined (<8%), and by days 44 and 50 the cells in all four cultures were essentially all positive for T-cell markers. At this time, one of the cultures was predominantly $CD4^-$ $CD8^+$ whereas cells in the other three cultures were all $CD4^+$, with a variable proportion (16 to 79%) also expressing CD8. These results indicate that in mixed cell populations there is a substantial early growth of parasitized B cells, which are eventually overgrown by T cells. There is also an apparent selection of one T-cell subset or another in individual cultures.

DISCUSSION

Primary infections of cattle with *T. parva* are characterized by rapid multiplication of parasitized lymphoblasts which become disseminated throughout the lymphoid system and are responsible for much of the pathology associated with infection (16, 23). Thus, the disease is considered an inevitable consequence of the capacity of the parasite to induce transformation of host lymphocytes such that parasite replication outpaces protective cellular immune responses. The findings of the present study modify this view of the pathogenesis and demonstrate that the outcome of infection is influenced by the host cell type infected by the parasite.

Studies of the cell tropism of *T. parva* in vitro have demonstrated that B cells and $CD4^+$ or $CD8^+$ α/β T cells and γ/δ T cells are all susceptible to infection and transformation by the parasite at more or less similar frequencies (1). While most established lines of parasitized B cells lose expression of sur-

TABLE 4. Phenotypes of *T. parva*-infected cell lines at different times after establishment by infection of PBMC in vitro with sporozoites

Donor animal	Cell surface marker[a]	% of cells positive for marker on day after infection[b]			
		7	14	44	50
E98	IgM	52	5	1	2
	CD2	9	84	96	96
	CD4	5	54	91	91
	CD8	3	60	17	16
E182	IgM	22	2	NT	0
	CD2	39	82	NT	98
	CD4	29	54	NT	92
	CD8	6	54	NT	79
E223	IgM	51	2	0	0
	CD2	23	88	97	98
	CD4	16	67	98	98
	CD8	6	39	73	78
E293	IgM	68	8	1	1
	CD2	8	68	95	94
	CD4	5	49	2	7
	CD8	2	43	94	92

[a] The CD8-specific MAb used in this experiment (IL-A51) detects both the homodimeric (α/α) and heterodimeric (α/β) forms of CD8.
[b] NT, not tested.

face Ig, they can be distinguished from infected T cells by the absence of T cell markers. By contrast, phenotypic studies of cells from lymphoid tissues of animals infected with *T. parva* have demonstrated that the vast majority of parasitized cells are of the CD4 or CD8 α/β T cell lineage (11). This suggested either that relatively few B cells became infected in vivo or that B cells and T cells infected with *T. parva* differ in their capacities to undergo unregulated growth in vivo.

The initial approach taken to address this issue was to compare the infections established following inoculation of cattle with autologous, cloned cell lines of defined phenotypes. A dose of 10^5 cells was chosen on the basis of a previous study in which cattle inoculated with this dose of uncloned cells developed severe lethal infections (5). However, all except two of the animals which received 10^5 cells from cloned lines underwent mild or inapparent clinical reactions, with parasitized cells being detectable only for a few days. The remaining two animals exhibited prolonged parasitosis but eventually recovered; although both animals were infected with $CD4^+$ cells, other animals that received infected cells with the same phenotype exhibited mild clinical reactions. This experiment was therefore uninformative in terms of evaluating the pathogenic potential of the different cell types. The difference between the outcome of these infections and the outcome of those in our previous study (5) probably relates to the use of cloned cell lines which had been heavily selected for growth in vitro and had been maintained in culture for a longer period (10 to 16 weeks) to facilitate cloning and phenotyping. Recent studies have demonstrated that parasites transmitted to ticks from cattle undergoing mild infections initiated with autologous cloned cell lines are capable of establishing lethal infections upon subsequent transmission to cattle (24). Given the evidence that the parasites in these cell lines were clones, these data indicate that selection of the parasite at the stage of cloning cannot account for their relative avirulence. Alternatively, prolonged passage of the cloned cell lines may result in a large proportion of the parasites being selected for loss of virulence or, perhaps more likely, prolonged culture may cause changes in the host cell that undermine its ability to replicate in vivo.

Two of the four cattle that received parasitized B cell lines showed no clinical or parasitological reaction and were susceptible to subsequent challenge with sporozoites. The most likely explanation of this finding is that the cells failed to establish an infection. However, the possibility that these lines carried a parasite component of the Muguga stock that does not confer protection against challenge with the parent stock cannot be discounted, although there is no previous evidence of immunological heterogeneity within this stock.

As an alternative approach to investigate the pathogenicity of different cell types, animals were inoculated with purified populations of cells that had been incubated in vitro with sporozoites for 48 h, at the end of which time the culture supernatants were devoid of infectivity. Doses of 3×10^4 to 3×10^5 PBMC incubated with sporozoites gave typical acute infections which, had the animals not been killed, would undoubtedly have resulted in a fatal outcome. In these experiments it was not possible to quantify the numbers of parasitized cells in the inocula, because of the difficulty in detecting parasites after only 48 h of development. Previous studies in which sporozoite binding to bovine leukocytes was examined by immunofluorescence showed that about 30% of PBMC are capable of binding sporozoites (17). This observation, coupled with the findings of the present study, indicates that infection of less than 10^4 cells by sporozoites is sufficient to initiate a lethal infection. T lymphocytes of either the $CD4^+$ or $CD8^+$ lineage gave rise to infections of similar severity to that produced by whole PBMC, whereas three animals that received infected B cells all underwent mild self-limiting infections. The latter included one animal inoculated with a 10-fold-higher dose of B cells than comparable animals given T cells. While the frequency of cells infected with *T. parva* within the inoculated cell populations could not be determined, previous studies have shown that B and T cells isolated by procedures similar to those used herein contained similar frequencies of infected cells (1). Moreover, the prepatent period to detection of parasitized cells in the regional lymph node, which has been shown to be an indicator of the parasite dose received (7, 25), was not markedly different between animals inoculated with T cells and those inoculated with B cells. These observations indicate that the difference in outcome of infection in animals inoculated with infected B cells or T cells is related to differences in growth or regulation of the infected cells rather than merely reflecting the numbers of cells in which the parasite had established infection. The sequential phenotypic analyses of PBMC following infection with *T. parva* in vitro, which showed that there is a high level of representation of infected B cells in early cultures, also supports the notion that the frequency of infection of B cells is similar to, if not higher than, that of T cells.

The validity of the experimental approach used in this study to examine the pathogenicity of different cell types is dependent on the belief that the schizonts of *T. parva*, unlike those of *Theileria annulata*, have a limited capacity to transfer from one cell to another. This belief is based principally on the observation that in cattle inoculated with allogeneic parasitized cells, doses of 10^8 or more cells are required to achieve consistent establishment of infection in the cells of recipient animals and successful immunization (3). However, it has been suggested that transfer of infection might occur more readily between autologous or syngeneic cells (9). The finding, in animals inoculated with infected CD4 or CD8 cells, that the parasitized cells within the lymph nodes on day 11 were confined to the $CD4^+$ and $CD8^+$ populations, respectively, argues strongly against extensive transfer of the parasite between autologous cells. While the coexpression of CD8 on some $CD4^+$ infected cells (11) potentially complicates these results, infected $CD8^+$ $CD4^-$ parasitized cells should have been evident in the animals infected with $CD4^+$ cells if significant transfer of infection had occurred. Because of the small numbers of parasitized cells present in the lymph nodes, phenotypic analyses of infected cells in animals inoculated with B cells was possible only with the animal that received 2×10^6 cells, on day 11 of infection. Somewhat surprisingly, all of the infected cells were positive for the T-cell marker CD2. While this might be taken as evidence of transfer of infection from B cells to T cells, we believe that it is more likely to reflect the outgrowth of contaminating T cells in the original purified B-cell population, which, assuming 1% contamination, could have represented up to 10^4 cells in the inoculum. A more careful analysis of the phenotype of infected cells when they are first detected in the regional lymph node is required to resolve this issue.

The findings of this study raise important questions concerning the regulation of growth of *T. parva*-infected cells and its relevance to the pathogenesis of East Coast fever. There appear to be two possible explanations for the difference between infected B cells and T cells in the kinetics of growth in vivo. First, infected B cells may be inherently limited in their capacity to sustain a high rate of replication in vivo. Thus, they may undergo an initial phase of rapid replication resulting in a prepatent period similar to that seen with T cells, but subsequently either their overall replication rate may slow down or

some of the progeny cells may undergo only a limited number of cell divisions. Such phenomena could also be related to a greater propensity of the parasites within B cells to undergo differentiation to merozoites. Secondly, infected B cells and T cells may differ either in the types of immune responses they induce or in their susceptibility to host immune responses. Such differences could involve either specific or nonspecific cellular immune responses. The latter could account for changes in growth of parasitized B cells if such cells are susceptible to suppressive effects of cytokines or other growth factors generated during early host responses to infection. Further studies are required to investigate these issues.

The finding that infected B cells predominate in early cultures of PBMC infected with *T. parva* sporozoites is in marked contrast to the observation that established cell lines derived from PBMC, with very few exceptions, are composed entirely of T cells (1). This could be interpreted as mirroring the situation in vivo, where there appears to be rapid outgrowth of infected T cells during the second week of infection (11). However, further studies are required to investigate the replication rates of the different cell types in these cultures in order to determine whether outgrowth of T cells is merely a consequence of a superior replication rate or is associated with inhibition of B-cell growth by the T cells.

In conclusion, these experiments have provided evidence that despite the capacity of *T. parva* to infect and transform both B cells and T cells in vitro with similar efficiencies, the cell type infected in vivo has a marked effect on the pathogenicity of the parasite. An understanding of the factors that determine this difference could provide important clues to the pathogenesis of East Coast fever and how host immune responses to the parasite might be manipulated.

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REFERENCES

1. **Baldwin, C. L., S. J. Black, W. C. Brown, P. A. Conrad, B. M. Goddeeris, S. W. Kinuthia, P. A. Lalor, N. D. MacHugh, W. I. Morrison, S. P. Morzaria, J. Naessens, and J. Newson.** 1988. Bovine T cells, B cells, and null cells are transformed by the protozoan parasite *Theileria parva*. Infect. Immun. **56:** 462–467.
2. **Baldwin, C. L., A. J. Teale, J. G. Naessens, B. M. Goddeeris, N. D. MacHugh, and W. I. Morrison.** 1986. Characterisation of a subset of bovine T lymphocytes that express BoT4 by monoclonal antibodies and function: similarity to lymphocytes defined by human T4 and murine L3T4. J. Immunol. **136:**4385–4391.
3. **Brown, C. G. D.** 1981. Application of *in vitro* techniques to vaccination against theileriosis, p. 104–119. *In* A. D. Irvin, M. P. Cunningham, and A. S. Young (ed.), Advances in the control of theileriosis. Martinus Nijhoff, The Hague.
4. **Brown, C. G. D., M. P. Cunningham, M. J. Burridge, A. J. Musoke, R. E. Purnell, D. E. Radley, and C. Semphebwa.** 1973. Infection and transformation of bovine lymphoid cells *in vitro* by infective particles of *Theileria parva*. Nature (London) **245:**101–103.
5. **Buscher, G., W. I. Morrison, and R. T. Nelson.** 1984. Titration in cattle of infectivity and immunogenicity of autologous cell lines infected with *Theileria parva*. Vet. Parasitol. **15:**29–38.
6. **Clevers, H., N. D. MacHugh, A. Bensaid, S. Dunlop, C. L. Baldwin, A. Kaushal, K. Iams, C. J. Howard, and W. I. Morrison.** 1990. Identification of a bovine surface antigen uniquely expressed on CD4/CD8 negative TCR-gamma/delta positive T lymphocytes. Eur. J. Immunol. **20:**809–817.
7. **Cunningham, M. P., C. G. D. Brown, M. J. Burridge, A. J. Musoke, R. E. Purnell, D. E. Radley, and C. Semphebwa.** 1974. East Coast fever: titration in cattle of suspensions of *Theileria parva* derived from ticks. Br. Vet. J. **130:** 336–345.
8. **Davis, W. C., J. A. Ellis, N. D. MacHugh, and C. L. Baldwin.** 1988. Bovine pan T-cell monoclonal antibodies reactive with a molecule similar to CD2. Immunology **62:**1–3.
9. **Dolan, T. T., A. J. Teale, D. A. Stagg, S. J. Kemp, K. M. Cowan, A. S. Young, C. M. Groocock, B. L. Leitch, R. L. Spooner, and C. G. D. Brown.** 1984. A histocompatibility barrier to immunisation against East Coast fever using *Theileria parva*-infected lymphoblastoid cells. Parasite Immunol. **6:**243–250.
10. **Ellis, J. A., C. L. Baldwin, N. D. MacHugh, A. Bensaid, A. J. Teale, B. M. Goddeeris, and W. I. Morrison.** 1986. Characterisation by a monoclonal antibody and functional analysis of a subset of bovine T lymphocytes that express BoT8, a molecule analogous to human CD8. Immunology **58:**351–358.
11. **Emery, D. L., N. D. MacHugh, and W. I. Morrison.** 1988. *Theileria parva* (Muguga) infects bovine T lymphocytes *in vivo* and induces co-expression of BoT4 and BoT8. Parasite Immunol. **10:**379–391.
12. **Fawcett, D., A. Musoke, and W. Voigt.** 1984. Interaction of sporozoites of *Theileria parva* with bovine lymphocytes *in vitro*. 1. Early events after invasion. Tissue Cell **16:**873–884.
13. **Goddeeris, B. M., J. M. Katende, A. D. Irvin, and R. S. C. Chuma.** 1982. Indirect fluorescent antibody test for experimental and epizootiological studies of East Coast fever (*Theileria parva* infection in cattle). Evaluation of the cell culture antigen fixed and stored in suspension. Res. Vet. Sci. **33:**360–365.
14. **Hulliger, L., J. K. H. Wilde, C. G. D. Brown, and L. Turner.** 1964. Mode of multiplication of *Theileria* in cultures of bovine lymphocytic cells. Nature (London) **203:**728–730.
15. **Irvin, A. D., and W. I. Morrison.** 1987. Immunopathology, immunology and immunoprophylaxis of *Theileria* infections, p. 223–274. *In* E. J. L. Soulsby (ed.), Immune responses in parasitic infections: immunology, immunopathology and immunoprophylaxis, vol. III. CRC Press, Boca Raton, Fla.
16. **Jarrett, W. F. H., G. W. Crighton, and H. M. Pirie.** 1969. *Theileria parva*: kinetics of replication. Exp. Parasitol. **24:**9–25.
17. **Kinuthia, S. W.** 1987. Expression of parasite-specific receptors on bovine lymphocyte target cells for *Theileria annulata*. Ph.D. thesis. Universite Libre de Bruxelles, Brussells, Belgium.
18. **Lalor, P. A., W. I. Morrison, B. M. Goddeeris, R. M. Jack, and S. J. Black.** 1986. Monoclonal antibodies identify phenotypically and functionally distinct cell types in the bovine lymphoid system. Vet. Immunol. Immunopathol. **13:** 121–140.
19. **MacHugh, N. D., and P. Sopp.** 1991. Bovine CD8 (BoCD8). Vet. Immunol. Immunopathol. **27:**65–69.
20. **Malmquist, W. A., M. B. A. Nyindo, and C. G. D. Brown.** 1970. East Coast fever: cultivation *in vitro* of bovine spleen cell lines infected and transformed by *Theileria parva*. Trop. Anim. Health Prod. **2:**139–145.
21. **Morrison, W. I.** Unpublished data.
22. **Morrison, W. I., and G. Buscher.** 1983. The early events of infection with *Theileria parva* in cattle: infectivity for cattle of leukocytes incubated *in vitro* with sporozoites. Vet. Parasitol. **12:**145–153.
23. **Morrison, W. I., G. Buscher, M. Murray, D. L. Emery, R. A. Masake, R. H. Cook, and P. W. Wells.** 1981. *Theileria parva*: kinetics of infection in the lymphoid system of cattle. Exp. Parasitol. **52:**248–260.
24. **Morzaria, S. P., T. T. Dolan, R. A. I. Norval, R. P. Bishop, and P. R. Spooner.** 1995. Generation and characterisation of cloned *Theileria parva* parasites. Parasitology **111:**39–49.
25. **Radley, D. E., C. G. D. Brown, M. J. Burridge, M. P. Cunningham, M. A. Pierce, and R. E. Purnell.** 1974. East Coast fever: quantitative studies of *Theileria parva* in cattle. Exp. Parasitol. **36:**278–287.
26. **Teale, A. J., W. I. Morrison, B. M. Goddeeris, C. M. Groocock, D. A. Stagg, and R. L. Spooner.** 1985. Bovine alloreactive cytotoxic cells generated *in vitro*: target specificity in relation to BoLA phenotype. Immunology **55:**355–362.
27. **Williams, D. J. L., J. Newson, and J. Naessens.** 1990. Quantitation of bovine immunoglobulin isotypes and allotypes using monoclonal antibodies. Vet. Immunol. Immunopathol. **24:**267–284.

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