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A non-protective T helper 1 response against the intra-macrophage protozoan *Theileria annulata*

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SUMMARY

Theileria annulata is a protozoan parasite which infects and transforms bovine macrophages. Infected macrophages possess augmented antigen presentation capabilities, as they are able to activate the majority of T cells from unexposed animals. *In vivo*, T cells in the draining lymph node (principal site of parasite development) are activated 'non-specifically' by the parasite. This event is followed by failure of the immune response to control the infection. Protective immune responses against intra-macrophage protozoa are usually mediated by T helper 1 (Th1) T cell responses. Here we examine the cytokine responses made by *T. annulata*-activated T cells. We show that the outcome of *in vitro* activation of T cells by parasitized macrophages is a skewing of their cytokine responses towards preferential expression of interferon-gamma (IFN-γ) mRNA. The *in vitro* response is mirrored during *in vivo* infection, as greatly elevated amounts of IFN-γ protein are found in lymph efferent from infected lymph nodes, while expression of IL-4 mRNA within the node stops. IFN-γ production does not correlate with protection against the parasite, as infected cells flourish during peak IFN-γ production, and only very small amounts of IFN-γ are produced during the effective immune response of an immunized animal. Overproduction of IFN-γ and loss of IL-4 expression are also likely to account for the failure of B cells to reach the light zone of germinal centres, a developmental step which is tightly regulated by cytokines.

Keywords *Theileria annulata* cytokines T cells interferon-gamma

INTRODUCTION

The protozoan parasite of cattle *Theileria annulata* resides in macrophages during the pathogenic macroschizont stage of its life cycle [1–3]. This tick-borne parasite is of particular interest, as European cattle are extremely susceptible to disease caused by the parasite—Tropical Theileriosis—and this proves a serious barrier to improvement of cattle in endemic areas such as North Africa and India. The macroschizont stage of *T. annulata* does not reside in the endocytic system of the macrophage [4], and differs from other well characterized intra-macrophage protozoans such as *Leishmania* and *Toxoplasma* by 'transforming' the parasitized macrophage into continuous cell cycle. The parasite induces an aggressive infection in susceptible cattle, characterized by expansion of parasite-infected cells in the lymph node draining the tick bite site, accompanied by fever, anaemia, cachexia and anoxia [5,6]. The host immune response is apparently unable to contain the rapidly growing parasite, naive susceptible animals do not develop effective immunity, and death from experimental infection often occurs within 2–3 weeks, depending on the dose of parasite [7,8].

Intracellular protozoan parasites which reside in myeloid lineage cells are now recognized to be controlled largely by T helper-1 (Th1) cytokine responses. In particular, resolution of infection with both *Leishmania* spp., *To. gondii* and *Trypanosoma cruzi* depends on the action of IFN-γ, which may either have direct anti-parasite effects or induce activation of the parasitized macrophage to reject the parasite [9–13]. Both anti-*T. annulata* cytotoxic T lymphocytes (CTL) [14,15] and IFN-γ-mediated induction of nitric oxide (NO) production by macrophages [16] can be demonstrated from animals either rendered immune by treatment with the napthoquinone drug Butalex, or immunized with attenuated macroschizont infected cells (CTL responses only). However, *T. annulata*-susceptible animals are unable to produce similar effective responses during primary infections.

The principal candidate mechanism for immune response failure is the augmented T cell-activating capability displayed by *T. annulata.* Macroschizont-infected macrophages are able to activate most peripheral T cells from unexposed animals in a

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contact-dependent mechanism, with IL-1 production by the infected cells implicated in driving T cell activation [17–19]. Although the antigenic element responsible for T cell activation has yet to be identified, the stimulatory element is unlikely to be a mitogen, as the kinetics of T cell responses to infected cells is similar to antigen-stimulated cells [18].

We have recently shown that this altered T cell-activating ability associated with infected cells disrupts normal T cell activation during *in vivo* infection—the vast majority of draining lymph node T cells are activated 'non-specifically' by infected macrophages. This is followed by failure of T cells to enter normal immune response pathways [18]. The clearest evidence to date that this disrupts normal immune mechanisms is the loss of germinal centre morphology in the infected lymph nodes [18].

An understanding of the effects of this early T cell activation by the parasitized macrophages is an essential step towards an understanding of disease pathology, and ultimately the design of new therapies. Here we show that infection of susceptible animals with *T. annulata* sporozoites induces a rapid production of IFN-γ, and this response is not tightly controlled and damped down as found in immune animals. We further show that parasite-activated T cells preferentially produce IFN-γ mRNA. As well as having no inhibitory effect on the growth of infected macrophages, the unregulated IFN-γ response is associated with local failure of B cell responses, with B cells failing to reach the light zone of germinal centres in infected lymph nodes.

MATERIALS AND METHODS

Animals

Peripheral blood for *in vitro* studies was obtained from normal, healthy Friesian or Friesian cross female or castrated male cattle aged 6 months or older. Calves used for lymph node studies were aged 2 months or older (see individual sections below).

Theileria annulata-*infected cell lines*

Theileria annulata macroschizont-infected cell lines (TaCL) (Hissar or Ankara strain [20,21]) from the animals tested were prepared as previously described [17]. Cell lines were used at low passage number (2–20).

Cell preparation

Peripheral blood mononuclear cells (PBMC) were separated using Ficoll–Hypaque (Lymphoprep; Nycomed, Oslo, Norway) as previously described [22]. Complete tissue culture medium was used throughout the experiments [23].

Culture of PBMC

PBMC were cultured with TaCL essentially as described previously [18,19]. Briefly, PBMC were cultured with autologous irradiated (75 Gy) TaCL in 6×10 ml well plates (Life Technologies, Paisley, UK). PBMC (8×10^6/ml):TaCL ratios were either 10:1 or 20:1 (optimum stimulation ratios were determined previously (results not shown)). A total of seven different TaCL from four unrelated cattle were examined. PBMC ($2{\cdot}5 \times 10^6$/ml) were stimulated with 5 μg/ml concanavalin A (Con A; Sigma, Poole, UK) to provide a 'control' population of activated T cells. Stimulated cells were harvested at various times (1–7 days) for reverse transcriptase-polymerase chain reaction (RT-PCR) analysis.

Theileria annulata-*infected lymph node material*

Archival frozen or paraffin-embedded lymph nodes from *T. annulata*-infected animals were used to examine cytokine profiles and for more detailed examination of the previously reported germinal centre breakdown [18]. Animals had been infected with *T. annulata* sporozoites in the shoulder, and nodes excised during the course of infection [18]. Normal prescapular lymph nodes were examined alongside draining (i.e. site of parasite development) and non-draining lymph nodes removed from calves 2, 4, 8 and 10 days post-sporozoite infection. Thin (2·5–3 μm) sections were cut from paraffin blocks and used for immunohistology. Lymph nodes stored at −70°C in sterile OCT medium (BDH-Merck, Lutterworth, UK) were used for RT-PCR analysis (see below). In addition, cells isolated using a tissue homogenizer (Jencons Scientific, Leighton Buzzard, UK) from lymph nodes at the time of excision were assessed for cytokine mRNA.

Immunohistochemistry

Immunohistochemistry using the ABC system was carried out on lymph node sections using MoAb VPM30 (germinal centre B cells [18]), MIB 1 (Ki-67 proliferation antigen [24]) and anti-human CD3 (Dako A452; Dako, Glostrup, Denmark), as previously described [25], with the following modifications: sections to be stained with MoAb MIB 1 were microwave-treated as previously described [24]; sections for use with anti-human CD3 were treated with pronase as previously described [25]. Double staining was carried out by first staining sections with VPM30 or CD3 followed by microwave treatment and staining with MIB 1 (microwave treatment effectively 'kills' residual avidin-biotin or antibody activity from the first round of staining). Staining was visualized using diaminobenzidine (DAB) as a substrate for horseradish peroxidase (HRP) and vector red (Vector Labs, Peterborough, UK) as a substrate for alkaline phosphatase.

Efferent lymph samples

Cryopreserved cell-free bovine efferent lymph samples [27] generated during a previous study (A. K. Nichani *et al.*, manuscript in preparation) were examined for IFN-γ. Briefly, two of the animals were untreated and efferent lymph was collected for 5–6 days to monitor baseline IFN-γ levels. Two animals were infected with 0·1 TE (tick equivalent) of Gharb strain [28] *T. annulata* sporozoites and developed severe clinical theileriosis. The animals were treated with Butalex (2·5 mg/kg body weight; Pitman-Moore, Uxbridge, UK) as soon as feed intake declined, making a full recovery. Efferent lymph was collected for 10 or 11 days post-infection before drug treatment was necessary. One animal which had been rendered immune by infection with sporozoites and treatment with Butalex was re-infected with 2 TE Gharb strain sporozoites and monitored over the same period.

IFN-γ assay

IFN-γ was assayed in efferent lymph using a commercially available ELISA assay (bovine γ-interferon kit; CSL Diagnostics, Victoria, Australia) according to the manufacturer's instructions. Highly positive lymph samples were diluted up to eight times in PBS in order to obtain results within the range of the detection system. Quantification of IFN-γ was performed by titrating known quantities of recombinant bovine IFN-γ diluted in PBS in the ELISA plate alongside lymph samples. (Recombinant IFN-γ, activity 4×10^6 U/mg, kind gift of Dr R. A. Collins, IAH, Compton, UK.)

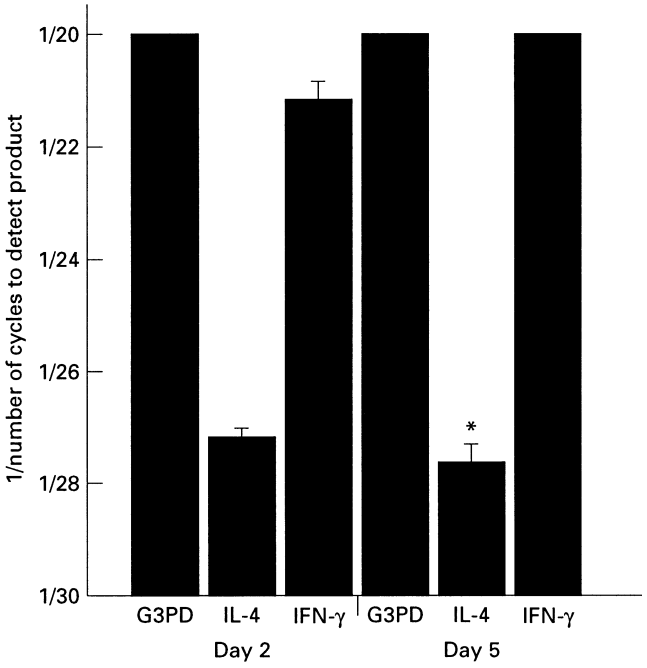

Fig. 1. Reciprocal of mean numbers of polymerase chain reaction (PCR) cycles required to detect glyceraldehyde-3-phosphodehydrogenase (G3PD), IL-4, and IFN-γ cDNA from *Theileria annulata* macroschizont-infected cell line (TaCL)-stimulated peripheral blood mononuclear cells (PBMC) after 2 days ($n = 6$) and 5 days ($n = 7$). Error bars = s.e.m. Where no error bars are present no variance was found between samples. *$n = 6$, one cell line did not induce any detectable IL-4 product in PBMC.

RT-PCR analysis

Although a bovine IFN-γ ELISA was used in this study, this kit is designed for use with serum products, and results with tissue culture samples were found to be unreliable. Also, a bioassay was unavailable for IL-4. As a result, cytokine production was assayed using RT-PCR analysis. *In vitro* stimulated PBMC or lymph node tissue were examined for the levels of expression of mRNA for IL-2, CD25, IL-4 and IFN-γ using a previously described semiquantitative RT-PCR technique [19,26]. Total RNA was isolated using the RNeasy system (Quiagen, Dorking, UK) according to the manufacturer's instructions. *In vitro* stimulated cells ($\geq 10^7$ cells) were washed twice in PBS before RNA isolation, frozen cells were allowed to thaw at 37°C and washed twice in PBS. Frozen lymph nodes were cut from the OCT medium using sterile scalpels and approximately 100 mg of tissue used for RNA isolation.

Total RNA (5 μg) was reverse transcribed using the Superscript system (Life Technologies). cDNA (2 μl) was subjected to different numbers of amplification cycles (20–30) and the products were examined on 2% agarose gels. Comparison of the cycle at which the PCR products became visible was used to assess the relative expression of the cytokine mRNA species [19]. Although this method does not allow direct comparison of mRNA abundance across cytokine primer pairs, it does afford a good estimate of the same mRNA expression after different treatment of cells. The expression of β-actin or glyceraldehyde-3-phosphodehydrogenase (G3PD) was used as an internal control for these reactions. Primer sequences for β-actin have been previously described [26], primer sequences for G3PD were the kind gift of Dr B. Mertens (ILRI, Kenya).

RESULTS

Incubation with T. annulata *macroschizont-infected cells induces a dominant IFN-γ response in peripheral blood T cells*

When T cells are activated by autologous TaCL, peak levels of surface activation marker expression (CD25 and MHC class II) are achieved within 48 h, with maximum proliferation at day 5 [18]. The cytokine profiles of cells responding to TaCL were examined at these timepoints in this study (Figs 1 and 2). All seven TaCL cell lines consistently induced higher levels of IFN-γ mRNA

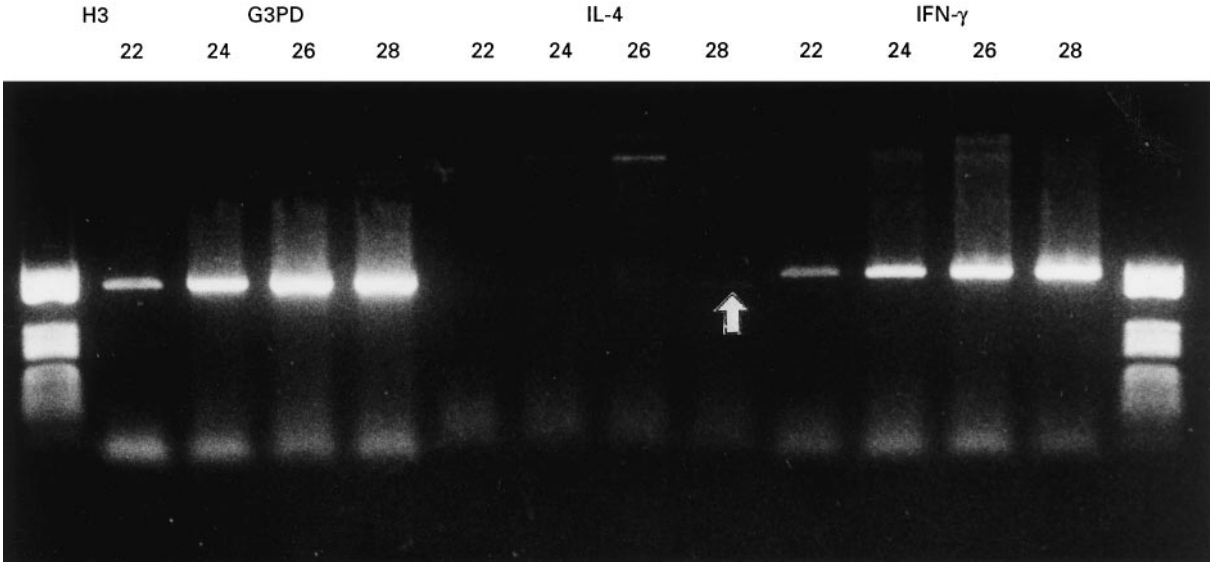

Fig. 2. Limited cycle analysis (cycles 22–28) from a representative animal after 5 days stimulation of peripheral blood mononuclear cells (PBMC) with an autologous *Theileria annulata* macroschizont-infected cell line (TaCL). While glyceraldehyde-3-phosphodehydrogenase (G3PD) and IFN-γ are easily detected after 22 cycles, IL-4 is only faintly detected at cycle 28 (arrowed). H3, *Hae*III markers.

Table 1.

Animal	Treatment	Cycles to detect IL-4	Cycles to detect IFN-γ
12 929	TaCL	NP	20
	Con A	22	20
13 050	TaCL	28	20
	Con A	22	20
10 814	TaCL	28	20
	Con A	20	20

NP, No product detected; TaCL, *Theileria annulata* macroschizont-infected cell line; Con A, concanavalin A.

production than IL-4 in responding PBMC, with the former product easily detectable after 20 cycles in all but one case (22 cycles, 48 h incubation). IL-4 products were never detected until at least 26 cycles of amplification. One cell line did not induce any detectable IL-4 message at day 5.

Working at maximum efficiency, PCR amplification is logarithmic, indicating that each molecule of IFN-γ cDNA, when detected at 20 cycles, required 2^{18} (262 144) amplifications to detect a product. When IL-4 was detected at all, 26–30 cycles were required—2^{24} (4194 304) to 2^{28} (268 435 456) amplifications. IFN-γ cDNA is therefore present in far greater amounts than IL-4 cDNA in TaCL-activated PBMC.

The consistent low abundance of IL-4 mRNA was not due to primer inefficiency, but specifically associated with the activation of PBMC by TaCL. When levels of IL-4 *versus* IFN-γ mRNA production by *T. annulata*-activated PBMC from three animals were compared with Con A-stimulated PBMC at their time of peak proliferation (5 and 3 days, respectively), Con A activation induced very similar levels of IL-4 mRNA and IFN-γ mRNA, whereas incubation with parasite-infected cells always induced far higher levels of IFN-γ mRNA production than IL-4 (Table 1). Simple 'activation' of PBMC therefore does not lead to noticeable differences in levels of IL-4 and IFN-γ mRNA amplified, suggesting that incubation with TaCL is specifically inducing higher IFN-γ production.

In vivo *infection induces an extremely strong Th1 response*

To determine whether high IFN-γ levels were also found during *in vivo* infection, lymph efferent from infected nodes was examined by ELISA. Lymph from uninfected resting lymph nodes showed no

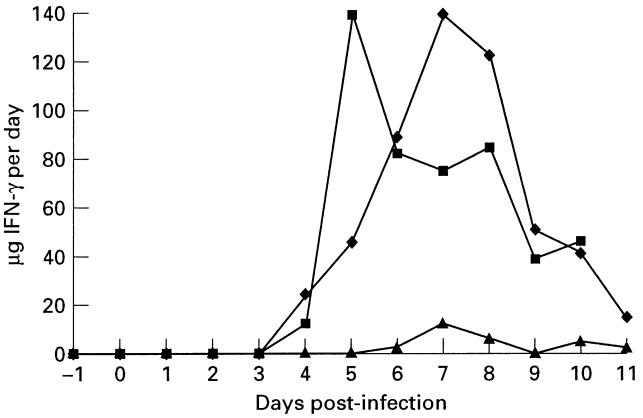

Fig. 3. Levels of IFN-γ protein detected in the efferent lymph of two naive *Theileria annulata*-infected animals and one previously immunized animal. ■, Naive 1; ♦, naive 2; ▲, immune.

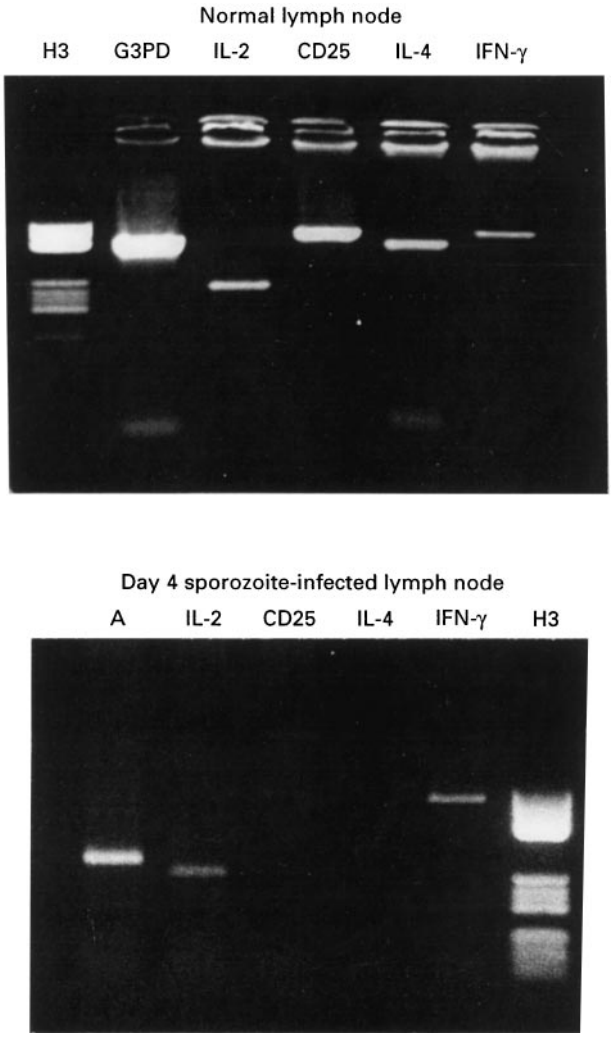

Fig. 4. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of bovine lymph nodes. In a normal node, IL-2, CD25, IL-4 and IFN-γ mRNA are present. Four days after infection with *Theileria annulata*, IL-4 and CD25 products can no longer be detected in the draining lymph node. G3PD, glyceraldehyde-3-phosphodehydrogenase. H3, *Hae*III markers.

Fig. 5. Germinal centre within a non-draining lymph node 8 days post-sporozoite infection. Both MIB-1^+ dark zone cells (D, stained brown) and VPM30^+ light zone B cells (L, stained red) are present. (×400, DAB and vector red.)

Fig. 6. Residual germinal centre within a day 8 sporozoite-infected draining lymph node (same animal as Fig. 5). Despite the continued presence of both a $CD3^+$ T cell zone (T, stained red) and MIB 1^+ dark zone (D, stained brown), no VPM30 staining was found in the germinal centre. Mantle zone marked with dotted line. (×650, DAB and vector red.)

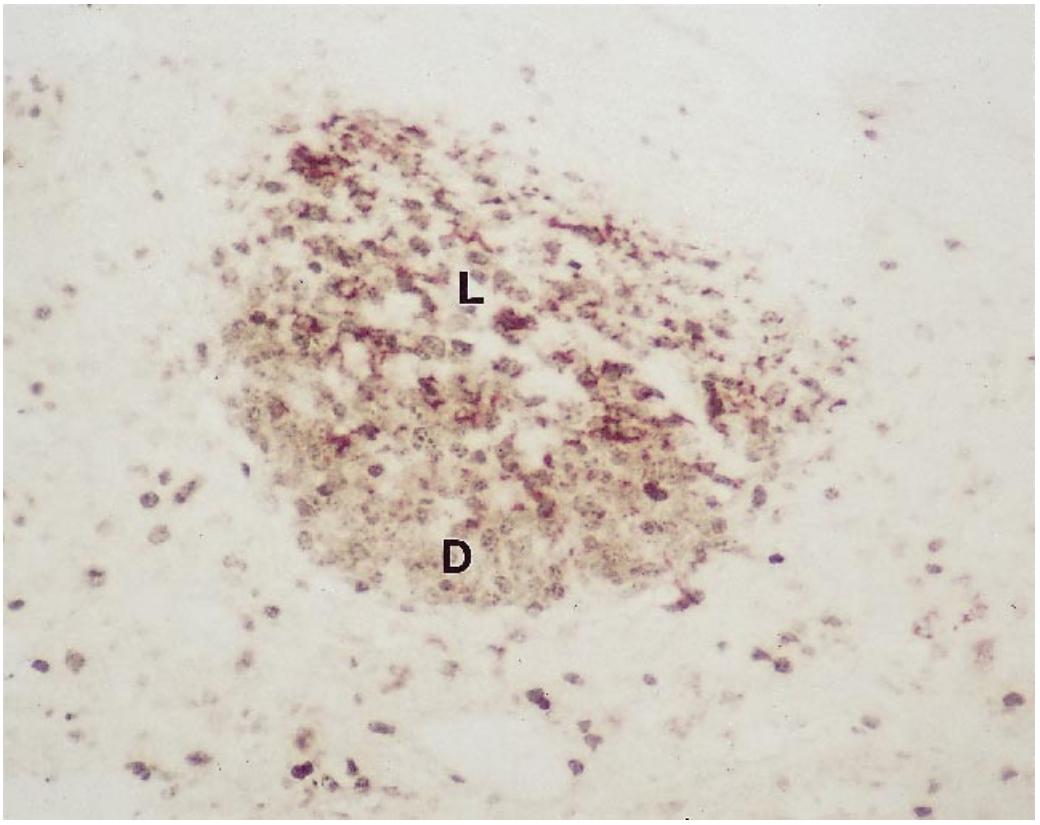

Figure 5

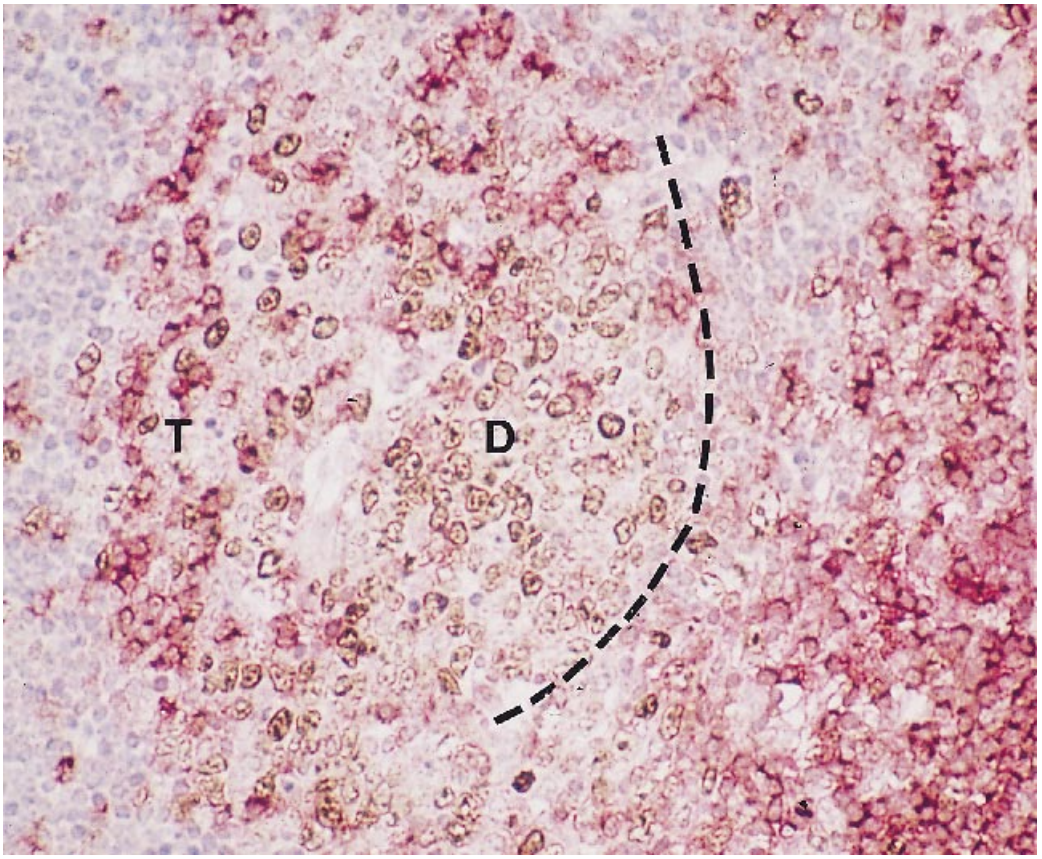

Figure 6

elevation of IFN-γ levels (results not shown). Lymph efferent from nodes draining the site of sporozoite infection in two naive susceptible animals showed very large increases in IFN-γ production (Fig. 3). Over a 6-day period (days 4–9 of infection) a total of $\geq 2 \times 10^6$ U of IFN-γ was detected in the efferent lymph of both naive infected animals. In contrast, lymph efferent from infected draining lymph nodes of an immune animal showed only transient increases in IFN-γ levels (Fig. 3), containing $1{\cdot}1 \times 10^5$ U of the cytokine over the same 6-day period. IFN-γ production in the immune animal appeared to 'shut down' quickly, as the small peak of cytokine production present 7 days post re-infection quickly dropped.

Draining lymph node cytokine mRNA production was assessed by RT-PCR during the peak period of IFN-γ production. Easily detectable in normal lymph nodes, IL-4 mRNA or CD25 mRNA was not detectable in draining lymph node cells within 4 days of sporozoite infection (Fig. 4). This loss of IL-4 mRNA production was found both in fresh preparations from isolated lymph node cells and archival frozen lymph node tissue blocks.

Germinal centre pathology is consistent with a dominant Th1 cytokine response

In vitro, IFN-γ has been demonstrated to interfere with B cell development by inhibiting expression of surface immunoglobulin after the initiation of proliferation [29–31]. We have previously shown that *T. annulata*-infected lymph nodes lose germinal centre (GC) light zone morphology from 8 days post-infection [18]. Here we examined GC morphology in more detail. T cells, proliferating dark zone cells, and VPM30$^+$ light zone B cells were found to be present within GC in normal and non-draining lymph nodes from infected animals (Fig. 5). However, although T cells and proliferating dark zones were found in infected lymph node GC (Fig. 6), VPM30 light zone B cell staining was completely lost from all infected GC by day 8 of infection. The previously reported loss of germinal centre morphology was therefore due to a failure of B cells to pass into the light zone rather than physical destruction by the growing parasite.

DISCUSSION

Theileria annulata-infected macrophages possess augmented antigen presentation capabilities, as they are capable of activating up to 70% of peripheral T cells from unexposed animals [18]. In *T. annulata* infection of susceptible animals, there is evidence of large alterations in immune responses. Infected lymph nodes are characterized by rapidly proliferating infected cells, T cells are primarily activated 'non-specifically' by the parasite, and there is a virtually complete loss of B cell GC. The parasite has therefore developed a survival strategy which not only allows infected cells to flourish, but which also disrupts normal immune responses. Highly successful survival strategies have been developed by intramacrophage protozoans such as *Leishmania* and *To. gondii* based on inducing Th2 cytokine responses in their hosts, which counteract the Th1 responses which can effectively clear infections [9–13]. In this study we have examined cytokine responses induced by *T. annulata*-mediated activation of T cells both *in vitro* and *in vivo*, and their implications for the induction of effective immune responses. During infection of naive animals leading to severe clinical theileriosis, IFN-γ levels in efferent lymph rose sharply, with approximately 20 times more cytokine produced by naive animals than by an immune animal. The high IFN-γ levels were accompanied by a loss of IL-4 mRNA production in naive animals. Unlike other intra-macrophage parasites such as *Leishmania* spp. and *To. gondii*, the skewing of the host response towards Th1 cytokine production does not confer immunity in *T. annulata* infection. Indeed, the peak time of IFN-γ production (4–9 days) coincides with the time when parasite-associated leuco-proliferation is greatest in infected lymph nodes, accompanied by the appearance of large numbers of macroschizont-infected cells [18]. *Theileria annulata* not only survives in the face of greatly increased IFN-γ production, but appears to actively encourage the induction of a Th1 response. IFN-γ is unlikely to be produced directly by *T. annulata*-infected cells, as mRNA for this cytokine is not usually found in parasitized macrophages [19]. *In vitro* activation of T cells by all *T. annulata*-infected cell lines induced the production of far higher levels of IFN-γ mRNA (at least $\times$64 more) than IL-4 mRNA.

Most infected draining lymph node T cell activation is 'non-specific' in response to the parasite [18]. As production of IFN-γ in the efferent lymph peaks after large numbers of *T. annulata*-activated T cells are established in the node [18], it seems likely that the parasite is inducing IFN-γ production via non-specific activation of T cells. Although remaining greatly elevated above levels seen from an immune animal, efferent lymph IFN-γ levels do drop partially after 8 or 9 days post-infection. This corresponds to the times when parasite-activated T cells are seen to leave the lymph node in the efferent lymph (A. K. Nichani *et al.*, manuscript in preparation) and further strengthens the link between T cells 'non-specifically' activated by parasitized cells and IFN-γ production.

The specific induction of an IFN-γ-dominated response from *T. annulata*-activated T cells goes some way towards clarifying the impact of the wide range of cytokine mRNAs produced by parasite-infected macrophages upon immune responses to the parasite. Parasitized cells express mRNA for IL-1α, IL-1β, IL-6, IL-10, tumour necrosis factor-alpha (TNF-α) [19] and the p35 and p40 subunits of IL-12 (J. D. M. Campbell and D. J. Brown, unpublished observations). Although a role for IL-1α and IL-6 has been proposed in the induction of T cell proliferation by the infected cells [19], the biological outcomes of the simultaneous production of antagonistic cytokines such as IL-10 and IL-12 [32–35] from the same cell were unknown. The results of this study clearly show that the net influence of *T. annulata*-infected cells on their environment is induction of a Th1 response, both *in vitro* and *in vivo.*

The induction of a Th1 response by *T.annulata*-infected cells via T cells is not likely to be purely driven by the antigen responsible for the T cell activation. When T cells were simply 'turned on' using Con A, the numbers of PCR cycles required to detect IL-4 or IFN-γ were similar, agreeing with other studies which showed that mitogen activation of T cells without the addition of cytokines to skew responses has the potential to induce either Th1 or Th2 responses [36]. This also holds true for 'genuine' antigens—identical *Leishmania* infections in the same inbred strain of mice can lead to either a Th1 or Th2 response, depending on the cytokines used to influence the response [37]. Also, staphylococcal superantigens induce a non-skewed cytokine response in reacting T cells in the absence of other stimuli [38]. It seems most likely that the induction of a dominant Th1 response in T cells responding to activation by *T. annulata* is due to the influence of infected cell-derived cytokines after the initial 'hit' of activation by the parasite. In an immune animal, IFN-γ production appeared to be tightly controlled, as only two small bursts of

cytokine were seen in the efferent lymph. As this control mechanism is evidently absent from naive infected animals, this would again suggest that the *T. annulata*-infected cells are producing factors which strongly promote IFN-γ production and are antagonistic to normal control mechanisms. One obvious candidate for the induction of IFN-γ from responding T cells in naive animals is IL-12 production by infected cells [39], and although mRNA for this cytokine is made by infected cells, further work will be required to determine its significance.

Protection afforded by IFN-γ against pathogens which parasitize macrophages is mediated through activation of the host cell to reject the parasite [9]. This is obviously not a protective mechanism in *T. annulata*, although the NO intermediates associated with killing of intra-macrophage organisms can be detected from infected cells [16]. As *T. annulata* does not parasitize the endocytic system of the macrophage, but exists in discrete membrane-bound vacuoles in the cytoplasm [4], it is likely that it can avoid lysosome-mediated damage. Indeed, it is perhaps unsurprising that *T. annulata* does not appear to be killed by responses designed to activate macrophages, as infection with the parasite induces an activated surface phenotype in the host macrophage [3].

IFN-γ has been demonstrated *in vitro* to interfere with B cell development, after B cells have been induced to proliferate [29]. In this study we demonstrate a similar phenomenon *in vivo*. Once high levels of IFN-γ were produced in infected lymph nodes accompanied by an apparent loss of IL-4 production, germinal centres were found to be 'arrested' at the proliferating dark zone stage, with no B cells entering the light zone. Entering the light zone is dependent upon the expression of antigen-specific immunoglobulin [40]. B cells are particularly sensitive to IFN-γ after initial proliferation, before immunoglobulin is expressed [29], and a lack of IL-4 also inhibits immunoglobulin production [30,31]. It is likely that the failure of B cells to progress to light zones in the infection studied here is due to the action of IFN-γ inhibiting immunoglobulin-dependent selection.

In this study we have shown that, contrary to recognized mechanisms for rejection or survival of similar organisms, the intra-macrophage protozoan *T. annulata* specifically induces the production of greatly elevated levels of IFN-γ. The critical step in the induction of IFN-γ is the 'non-specific' activation of T cells by *T. annulata*-infected macrophages. These findings provide the first evidence of a mechanism for *T. annulata* to avoid destruction by the immune system. Identification of both the antigenic element and infected cell cytokines which induce the non-protective Th1 response in parasite-activated T cells is now essential.

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REFERENCES

1 Spooner RL, Innes EA, Glass EJ, Brown CGD. *Theileria annulata* and *T. parva* infect and transform different mononuclear cells. Immunology 1989; **66**:284–8.

2 Glass EJ, Innes EA, Spooner RL, Brown CGD. Infection of bovine monocyte/macrophage populations with *Theileria annulata* and *Theileria parva.* Vet Immunol Immunopathol 1989; **22**:355–68.

3 Campbell JDM, Brown DJ, Glass EJ, Hall FR, Spooner RL. *Theileria annulata* sporozoite targets. Parasite Immunol 1994; **16**:501–5.

4 Jura WGZO, Brown CGD, Perry M. Comparative autoradiographic study of parasite-host-cell cyclical relationship in lymphoblastoid cell-lines infected with *Theileria annulata* and *T. parva in vitro.* Vet Parasitol 1985; **18**:339–48.

5 Neitz WO. Theileriosis, gonderioses and cytauxzoonoses: a review. *Ondersteport J Vet Res* 1957; **27**:275–431.

6 Barnett SF. *Theileria.* In Kreier JP, ed. Parasitic Protozoa, Vol. IV. New York: Academic Press, 1977:71–113.

7 Samantaray SN, Bhattacharyulu Y, Gill BS. Immunisation of calves against bovine tropical theileriosis (*Theileria annulata*) with graded doses of sporozoites and irradiated sporozoites. Int J Parasitol 1980; **10**:355–8.

8 Preston PM, Brown CGD, Bell-Sakyi L, Richardson W, Sanderson A. Tropical theileriosis in *Bos taurus* and *Bos taurus* cross *Bos indicus* calves: response to infection with graded doses of sporozoites of *Theileria annulata.* Res Vet Sci 1992; **53**:230–43.

9 Murray HW. Interferon-gamma, the activated macrophage, and host defence against microbial challenge. Ann Intern Med 1988; **108**:595–608.

10 Scott P, Natovitz P, Coffman RL, Pearce E, Sher A. Immunoregulation of cutaneous Leishmaniasis. J Exp Med 1988; **168**:1675–84.

11 Gaafar A, Kharazmi A, Ismail A *et al.* Dichotomy of the T cell response to *Leishmania* antigens in patients suffering from cutaneous leishmaniasis; absence or scarcity of Th1 activity is associated with severe infections. Clin Exp Immunol 1995; **100**:239–45.

12 Gazinelli RY, Hakim FT, Hieny S, Shearer GM, Sher A. Synergistic role of $CD4^+$ and $CD8^+$ T-lymphocytes in IFN-γ production and protective immunity induced by an attentuated *Toxoplasma gondii* vaccine. J Immunol 1991; **146**:286–92.

13 Gazinelli RY, Wysocka M, Hayashi S, Denkers EY, Hieny S, Caspar P, Trinchieri G, Sher A. Parasite induced IL-12 stimulates early IFN-γ synthesis and resistance during acute infection with *Toxoplasma gondii.* J Immunol 1994; **153**:2533–43.

14 Preston PM, Brown CGD, Spooner RL. Cell-mediated cytotoxicity in *Theileria annulata* infection of cattle with evidence for BoLA restriction. Clin Exp Immunol 1983; **53**:88–100.

15 Innes EA, Millar P, Brown CGD, Spooner RL. The development and specificity of cytotoxic cells in cattle immunized with autologous or allogeneic *Theileria annulata* infected lymphoblastoid cell lines. *Parasite Immunol* 1989; **11**:57–68.

16 Visser AE, Abraham A, Bell-Sakyi LJ, Brown CGD, Preston PM. Nitric oxide inhibits establishment of macroschizont-infected cells lines and is produced by macrophages of calves undergoing bovine tropical theileriosis or East Coast fever. Parasite Immunol 1995; **17**:91–102.

17 Glass EJ, Spooner RL. Parasite-accessory cell interactions in Theileriosis. Antigen presentation by *Theileria annulata*-infected macrophages and production of continuously growing antigen-presenting cell lines. Eur J Immunol 1990; **20**:2491–7.

18 Campbell JDM, Howie SEM, Odling KA, Glass EJ. *Theileria annulata* induces aberrant T cell activation *in vitro* and *in vivo.* Clin Exp Immunol 1995; **99**:203–10.

19 Brown DJ, Campbell JDM, Russell GC, Hopkins J, Glass EJ. T cell activation by *Theileria annulata* infected macrophages correlates with cytokine production. Clin Exp Immunol 1995; **102**:507–14.

20 Gill BS, Bansal GC, Bhattacharyulu Y, Kaur D, Singh A. Immunological relationship between strains of *Theileria annulata* Dschunkowsky and Luhs 1904. Res Vet Sci 1980; **29**:93–97.

21 Schein E. On the life cycle of *Theileria annulata* (Dschunkowsky and Luhs, 1904) in the midgut and haemolymph of *Hyalomma anatolicum excavatum* (Koch, 1844). Zeitschrift fur Parasitenkunde 1975; **47**:165–7.
22 Glass EJ, Spooner RL. Requirement for MHC class II positive accessory cells in an antigen specific bovine T cell response. Res Vet Sci 1989; **46**:196–201.
23 Glass EJ, Spooner RL. Generation and characterization of bovine antigen-specific T cell lines. J Immunol Methods 1990; **128**:267–75.
24 Key G, Becker MHG, Baron B, Duchrow M, Schulter C, Flad HD, Gerdes J. New Ki-67 equivalent murine monoclonal antibodies (MIB 1-3) generated against bacterially expressed parts of the Ki-67 cDNA containing three 62 base pair repetitive elements encoding for the Ki-67 epitope. *Lab Invest 1993;* **68**:629–36.
25 Ramos-Vara JA, Miller MA, Lopez E, Prats N, Brevik L. Reactivity of polyclonal human CD3 antiserum in lymphoid tissues of cattle, sheep, goats, rats and mice. Am J Vet Res 1994; **55**:63–6.
26 Van Lierop MJC, Nilsson PR, Wagenaar JPA, van Noort JM, Campbell JDM, Glass EJ, Joosten I, Hensen EJ. The influence of MHC polymorphism on the selection of T cell determinants of foot and mouth disease virus in cattle. Immunology 1995; **84**:79–85.
27 Glass EJ, Spooner RL. Cellular immunity in cattle. In: Weir D, Herzenberg L, Herzenberg L, eds. The handbook of experimental immunology, 5th edn. Cambridge, MA: Blackwell Scientific Publications, 1996.
28 Ouhelli H. Theileriose Bovine a *Theileria annulata* (Dschunkowsky and Luhs, 1904). Recherche sur la biologie des vecteurs (*Hyalomma* spp.) et sur les interactions hote-parasite. These de Doctorat de Sciences 1985; I.N.P. Toulouse.
29 Abed NS, Chace JH, Cowdery JS. T cell-independent and T cell-dependent B cell activation increases IFN-γR expression and renders B cells sensitive to IFN-γ-mediated inhibition. J Immunol 1994; **153**:3369–77.
30 Armitage RJ, Macduff BM, Spriggs MK, Fanslow WC. Human B cell proliferation and Ig secretion induced by recombinant CD40 ligand are modulated by cytokines. J Immunol 1993; **150**:3671–80.
31 Donckier V, Abramowicz D, Bruyns C *et al.* IFN-γ prevents Th2 cell-mediated pathology after neonatal injection of semiallogenic spleen cells in mice. J Immunol 1994; **153**:2361–8.
32 de Waal Malefyt R, Abrams J, Bennet B, Figdor CG, de Vries JE. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J Exp Med 1991; **174**:1209–20.
33 Fiorentino DF, Zlotnik A, Mosmann TR, Howard M, O'Garra A. IL-10 inhibits cytokine production by infected macrophages. J Immunol 1991; **147**:3815–22.
34 Hseih CS, Heimberger AB, Gold JS, O'Garra AO, Murphy KM. Differential regulation of T helper phenotype development by interleukins 4 and 10 in an αβ T-cell-receptor transgenic system. Proc Natl Acad Sci USA 1992; **89**:6065–9.
35 Hseih CS, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM. Development of TH1 $CD4^+$ T cells through IL-12 produced by *Listeria*-induced macrophages. Science 1993; **260**:547–9.
36 Rocken M, Saurat JH, Hauser C. A common precursor for $CD4^+$ T cells producing IL-2 or IL-4. J Immunol 1992; **148**:1031–6.
37 Wang ZE, Reiner SL, Zheng SC, Dalton DK, Locksley RM. $CD4^+$ effector cells default to the Th2 pathway in interferon-gamma deficient mice infected with *Leishmania major*. J Exp Med 1994; **179**:1367–71.
38 Lagoo A, Lagoodeenadavalin S, Byrne J, Lorenz HM, Hardy KJ. Cytokine gene expression in superantigen-activated naive and memory T cells from adult human peripheral blood. J Immunol 1993; **150**:A269.
39 Trinchieri G. Interleukin-12 and interferon-γ. Do they always go together? Am J Pathol 1995; **147**:1534–8.
40 MacLennan ICM. From the dark zone to the light. Current Biol 1994; **4**:70–72.