Cytotoxic T Cells in Immunity to *Theileria parva* in Cattle

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1 Introduction

Theileria are tick-borne protozoan parasites which in their mammalian hosts successively utilise leukocytes and erythrocytes for completion of their life cycle. The parasites are found predominantly in ruminants. In domestic cattle, there are two species, namely *T. annulata* and *T. parva*, which cause economically important diseases. *T. parva* occurs throughout a large part of East and Central Africa where it causes an acute, usually fatal disease known as East Coast fever (ECF) (W.I. Morrison et al. 1986; Irvin and Morrison 1987). Control of the disease relies largely on regular application of acaricides to prevent tick infestation. This practice is costly, time consuming and can lead to selection of acaricide-resistant ticks. Thus an effective method of imunising against the disease would have a major impact on cattle production in ECF-endemic areas. To this end, studies have been undertaken to define the host immune responses involved in immunity against *T. parva* with the aim of identifying protentially protective antigens. These studies have yielded evidence that major histocompatibility complex (MHC) restricted cytotoxic T cells are important in mediating immunity.

In this paper, we review current information on *T. parva*-specific cytotoxic T cell responses and, in particular, focus on the role of the MHC in determining the parasite strain specificity of the responses.

2 The Host-Parasite Relationship

2.1 The parasite life cycle

Infection of cattle with *T. parva* is initiated when sporozoites, deposited by the tick vector during feeding, gain entry into lymphocytes. This occurs by receptor-mediated endocytosis, and during the subsequent 12–24 h the host cell membrane

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surrounding the parasite is destroyed so that the parasite comes to lie free in the cytoplasm (FAWCETT et al. 1984). Within 24–48 h of invading a lymphocyte, the sporozoite starts to develop to a multinucleate body, termed a schizont. This is associated with activation of the host cell which begins to proliferate. At each cell division, the parasite also divides so that both daughter cells are infected (HULLIGER et al. 1964). Thus the infection is established and becomes disseminated by clonal expansion of the small population of lymphocytes initially infected by the parasite. Later in the infection, schizonts undergo merogony, and merozoites thus formed infect erythrocytes, giving rise to prioplasms which are infective for the tick.

Susceptible cattle usually die within 4 weeks of infection with the parasite. In such animals, large numbers of parasitised lymphoid cells are found throughout the lymphoid system and are associated with extensive lymphocytolysis (MORRISON et al. 1981; IRVIN and MORRISON 1987). Parasitised cells also invade non-lymphoid tissues, particularly the lungs and gastrointestinal tract and, in the lungs, commonly result in severe pulmonary oedema. Thus, it is the schizont stage of the parasite which is responsible for most of the pathology of the disease.

The relationship of the parasite with the host cells enables parasitised cells to grow continuously in culture; parasitised cell lines can be established either by culturing cells from infected cattle or by infecting normal lymphocytes in vitro with sporozoites (BROWN et al. 1973; BROWN 1983). The latter culture system has proved extremely useful for experiments to identify the host cell types infected with *T. parva* and for studies of T-cell responses to the parasite.

2.2 Host Cell Types Infected by the Parasite

The production in the past few years of monoclonal antibodies (MAb) specific for different populations of bovine lymphocytes and accessory cells has made it possible to define which cell types are infected with *T. parva*. The susceptibility of different populations of cells to infection and transformation by the parasite in vitro has been tested by incubating cells purified with a fluorescence-activated cell sorter, with suspensions of *T. parva* sporozoites, and subsequently monitoring the establishment of cell lines following distribution of the cells at limiting dilution in the wells of microtitre plates (BALDWIN et al. 1988). These experiments showed that B-lymphocytes, both major subpopulations of T-lymphocytes ($BoT4^+$ and $BoT8^+$) and an additional population of lymphocytes lacking B-cell and T-cell markers could readily be infected and establish cell lines. Purified monocytes and granulocytes did not give rise to cell lines. However, when unfractionated populations of peripheral blood mononuclear cells (PBM) were infected with the parasite, the cell lines which established invariably had the phenotype of T cells. Moreover, phenotypic analyses of infected cells in cattle undergoing lethal infections with *T. parva* have demonstrated that the majority of infected cells in vivo are T-lymphocytes (EMERY et al. 1988). These findings indicated that while all of the presently defined subpopulations of lymphocytes are susceptible to infection with *T. parva*, T-lymphocytes dominate the infections in vivo.

2.3 Properties of Parasitised Cells

The growth characteristics of *Theileria*-infected lymphocytes are similar to those of neoplastically transformed cells. This apparent transformed state is dependent on the continued presence of the parasite, as the removal of parasites by specific drug treatment causes cells to revert to a non-proliferative state (PINDER et al. 1981). Constitutive production, by infected cells, of a growth factor with functional properties and apparent molecular weight similar to those of interleukin 2 (IL-2) has been demonstrated (BROWN and LOGAN 1986) and suggested as an important contributory factor to maintenance of growth of infected cells. These experiments were conducted with a BoT8$^+$ T-cell clone which was infected in vitro with *T. parva* and prior to infection did not produce the growth factor. Experiments carried out by DOBBELAERE et al. (1988) have also demonstrated that recombinant human IL-2 supports and potentiates the growth of infected cells when they are cultured at low cell densities. While these observations indicate that an autocrine mechanism of production and consumption of growth factors may contribute to the growth of infected cells, they do not shed light on the primary events within the cell which lead to the transformed state. Nevertheless, the observation that infected cells produce and are responsive to growth factors has potentially important implications in the pathogenesis and immunology of the disease. Thus, on the one hand, functional changes induced in host lymphocytes by the parasite might influence the generation of parasite-specific immune responses and, on the other, soluble mediators elaborated during the induction of such immune responses may modulate the growth or behaviour of the parasitised cells.

3 Immunity Against *T. parva*

3.1 Immunisation of Cattle

Cattle can be immunised against *T. parva* by inoculating them with a potentially lethal dose of sporozoites followed by treating them for several days with the antibiotic oxytetracycline (NEITZ 1953; RADLEY et al. 1975a, d). This regimen results in a transient mild infection from which the animals recover and are subsequently immune to challenge with the same parasite. Attempts to immunise cattle with non-viable parasite antigens have generally been unsuccessful (reviewed in MORRISON et al. 1986).

3.2 Parasite Strain Heterogeneity

A shortcoming of the infection and treatment immunisation procedure which has limited its practical application is that immunisation with one stock of the parasite

does not provide protection against all other stocks (RADLEY et al. 1975b, c; IRVIN et al. 1983). However, parasite strain heterogeneity is probably limited as the use of mixtures of two or three stocks for immunisation has provided protection against experimental challenge with a number of stocks isolated in different geographical locations and against field challenge (IRVIN 1985). The precise immunological characterisation of parasite stocks is complicated by the fact that in cross-protection experiments, breakthrough infections usually do not occur reciprocally between parasite stocks. Thus, there are many examples of a parasite stock that protects against another stock but not vice versa. Moreover, when breakthrough infections occur, they often do so only in a proportion of animals. These findings may be due in part to the fact that the parasite stocks are uncloned and therefore may be heterogeneous mixtures. Support for this notion comes from recent studies with parasite-specific MAbs and DNA probes. DNA sequences present as multiple copies in the *Theileria* genome have been cloned and, when used as probes on Southern blots of DNA prepared from different parasite stocks, detect restriction fragment length polymorphism (CONRAD et al. 1987). These probes also detect differences in the DNA from parasites within cloned cell lines derived from the same parasite stock (P.A. CONRAD et al., in press). Similar differences between and within parasite stocks have been observed with MAbs specific for a parasite antigen which shows marked polymorphism among populations of *T. parva* (SHAPIRO et al. 1987; B.M. GODDEERIS and P.G. TOYE, unpublished data). It is likely that genetic recombination between parasite populations contributes to these phenotypic and genotypic differences. Such recombination is well documented in other protozoan parasites such as malaria (WALLIKER et al. 1987) and trypanosomes (JENNI et al. 1986), and in *Theileria* there is morphological evidence of zygote formation from gametes during development in the tick (MELHORN and SCHEIN 1976; SCHEIN et al. 1977). In order to elucidate this question and to provide defined parasites for further immunological studies, efforts are currently being devoted to obtaining cloned populations of *Theileria* parasites.

3.3 Mechanisms of Immunity

Although cattle repeatedly exposed to sporozoites produce antibodies which, in vitro, neutralise the infectivity of sporozoites (MUSOKE et al. 1982), only a minor anti-sporozoite response is detected after a single immunisation by infection and treatment. In animals immunised in this way the protective immune responses are believed to be directed against schizont-infected lymphocytes (reviewed in W.I. MORRISON et al. 1986). This is based on the observations that schizont-infected cells are often transiently detectable 7–9 days after challenge of immune cattle with sporozoites, and that cattle can be immunised with large numbers of viable schizont-infected cells, i.e. in the absence of exposure to sporozoites.

Given that the schizont stage of the parasite remains in an intracellular location during replication, it is difficult to envisage antibodies being effective against this stage of the parasite. Indeed, although immunisation results in readily detectable anti-schizont antibody responses, there is no evidence that such antibodies play a

role in protection. Nor is there any evidence that cattle produce antibodies to parasite-specific antigens on the surface of infected cells (CREEMERS 1982). By inference, therefore, it would seem that immunity is likely to involve cell-mediated immune responses. This is supported by the findings that transfer of thoracic duct lymphocytes from immune to naive chimaeric twin calves protected against concurrent challenge with *T. parva* (EMERY 1981) whereas attempts to transfer immunity with serum from immune cattle were unsuccessful (MUHAMMED et al. 1975).

4 *Theileria*-Specific T-Cell Responses

4.1 Cytotoxic T-Cell Responses

The first direct evidence that infections with *T. parva* induce cell-mediated immune responses was derived from experiments in which cattle were infected or immunised with the parasite and their PBM monitored for cytotoxic activity on parasitised cell lines. In cattle undergoing lethal infections, cytotoxic cells capable of killing allogeneic infected cells as well as murine YAC-1 cells were detected in the peripheral blood during the later stages of the infection (EMERY et al. 1981). Somewhat surprisingly, these effectors did not kill autologous infected cells. The absence of killing of autologous cells may have been due to the presence of infected cells in the effector population which might act as cold target inhibitors of any effectors specific for autologous cells. At any rate, the cytotoxic cells detected clearly do not recognise target molecules on autologous cells. The identity of these effector cells and their target antigen specificity have not been investigated further.

By contrast to findings in cattle undergoing lethal infections, immunisation or challenge of cattle with *T. parva* results in the induction of cytotoxic T cells specific for parasitised lymphoblasts. These cytotoxic cells are transiently detectable in the peripheral blood at the time of remission of infection (EUGUI and EMERY 1981; EMERY et al. 1981). They kill autologous parasitised cells but not uninfected lymphoblasts or cells from unrelated cattle infected with the same parasite. By selecting panels of target cells of defined MHC phenotypes and by testing the capacity of MAb specific for class I or class II MHC molecules to inhibit cytotoxicity, the cytotoxic cells were shown to be restricted by class I MHC determinants (MORRISON et al. 1987b). In the majority of animals, there was a marked bias in the restriction of the cytotoxic T-cell response to one or other of the MHC haplotypes (all animals were heterozygous). Moreover, with the class I specificities represented, responses restricted by some specificities (or haplotypes) consistently predominated over responses restricted by others (MORRISON et al. 1987b). The results indicated that there is a hierarchy in dominance among the class I MHC molecules that restrict the response. Subsequent studies of the effectors following stimulation in vitro demonstrated that they are T-lymphocytes belonging to the T8 subset (GODDEERIS et al. 1986a).

The temporal relationship of the cytotoxic T-cell response with disappearance of parasitised cells in cattle undergoing challenge with *T. parva* indicates that the response may be important in control of the infection. Moreover, such a mechanism of immunity is compatible with the finding that establishment of infection in the animal is required for immunity to develop because of the need for T cells to recognise antigenic changes on the cell surface in the context of self MHC molecules.

To date the parasite-induced antigens recognised by the cytotoxic T cells have not been identified. In one instance, an apparently parasite-specific cell surface antigen was detected with a MAb (NEWSON et al. 1986); however when the parasitised cells were cloned and used to infect an allogeneic animal, infected cells isolated from the recipient animal did not express the antigen. In retrospect, with the information which has recently emerged on how viral antigens are presented to class I restricted T cells as small processed peptides on the surface of infected cells (TOWNSEND et al. 1986), it is not surprising that a parasite-specific cell surface antigen could not be detected with antibodies.

4.2 Helper T-Cell Responses

The evaluation of non-cytotoxic T-cell responses against *Theileria*-infected cells has been difficult because of the fact that, in in vitro proliferative assays, parasitised cells induce proliferative responses of similar magnitude and kinetics in PBM from immune or naive cattle (PEARSON et al. 1979; GODDEERIS and MORRISON 1987). However, experiments in which lymphocyte cultures were restimulated at weekly intervals in vitro with autologous parasitised cells showed that proliferative cultures of T cells from immune cattle could be maintained for 1–3 months, whereas similar cultures from naive cattle often showed a gradual diminution of proliferative activity (GODDEERIS et al. 1986a). A large proportion of the proliferating cells from immune cattle were $BoT4^+$ $BoT8^-$ T-lymphocytes. Cloned populations of these cells were obtained and were shown to be specific for parasitised cells and restricted by class II MHC determinants (BALDWIN et al. 1987). These cloned population had properties characteristic of helper T cells in that they proliferated, upon antigenic stimulation, in the absence of exogenous growth factors and were shown to produce T-cell growth factor activity.

In studies of murine and human T-cell responses, the observation that class I-restricted cytotoxic T cells grown in vitro usually require exogenous growth factors for maintenance of proliferation has led to the prevailing belief that class II restricted helper T cells are required for the generation of a cytotoxic T-cell response. However recent experiments carried out in mice (HEEG et al. 1987a, b) have demonstrated the existence of a population of $Ly2^+$ ($T8^+$) T cells which produce IL-2 following antigenic stimulation. Moreover, several studies in vivo in mice have shown that generation of virus-specific cytotoxic T-cell responses (BULLER et al. 1987) and graft versus host and graft rejection responses (SPRENT et al. 1986) can be mediated by $Ly2^+$ T cells in the absence of $L3T4^+$ ($T4^+$) T cells. In view of these findings, the role of $BoT4^+$ T cells in providing help in the generation of class I restricted cytotoxic T cells specific for *Theileria*-infected cells must remain speculative.

4.3 Parasite Strain Specificity of the Cytotoxic T-Cell Responses

Since the immunity induced by one stock of *T. parva* often does not provide protection against challenge with all other stocks of the parasite, one would expect to observe a degree of strain specificity in the protective immune responses. To address this question, the parasite strain specificity of *Theileria*-specific cytotoxic T-cell responses in cattle immunised with *T. parva* (Muguga), has been analysed on target cell lines infected with the Muguga and Marikebuni stocks. These two stocks are immunologically different in that cattle immunised with Marikebuni are immune to challenge with Muguga, whereas a proportion of animals immunised with Muguga are susceptible to challenge with Marikebuni (IRVIN et al. 1983). In order to preclude the argument that differences in cytotoxicity might be due to the two parasites infecting different cell types, cloned T cells infected with the two parasites were prepared as target cells for most of the animals. In most instances, these cell lines were also cloned after infection to ensure that they did not contain mixed parasite populations.

The results from six cattle, three of which were reported on previously (MORRISON et al. 1987b), are shown in Table 1. Cytotoxic cells generated in two of the animals were completely specific for Muguga-infected target cells, whereas effector cells from the remaining four cattle showed killing of target cells infected with either Muguga or Marikebuni parasites. Although these particular animals have not been challenged with the Marikebuni stock to determine whether the specificity of the response correlates with protection, the results clearly demonstrate

Table 1. Parasite strain specificity of the *Theileria*-specific cytotoxic T-cell response in six immune cattle challenged with *T. parva* (Muguga)

Donor of effector cells	Target cell line	BoLA phenotype[a]	Percent cytotoxicity on targets infected with[b]	
			T. parva (Muguga)	*T. parva* (Marikebuni)
B641	B641	w10/KN18	41	2
	T11.49(B641)[c]	w10/KN18	36	0
	B171	w7/KN18	59	0
C447	T16.13(C447)[c]	w7/w10	63	4
	C167	w4/w10	42	1
C234	C234	w6/w10	42	23
	C165	w6/w7	31	13
	T3.5(C196)[c]	w6/w7	44	2
C196	T3.5(C196)[c]	w6/w7	52	15
	C165	w6/w7	35	25
	C234	w6/w10	82	61
D247	D247	w6.2/w7	56	59
	D232	w6.2/w2	34	31
	T19.4(C887)[c]	w6.2/w10	47	37
D409	D409	w7/w10	65	73
	D247	w6.2/w7	43	76

[a] Cattle and parasitised cell lines were typed serologically for class I antigens as described by TEALE et al. (1983)
[b] Results obtained at an effector to target ratio of 40:1 in a 4-h ^{51}Cr-release assay are presented.
[c] T-cell clone infected with *T. parva*.

that, as with protection, there is considerable heterogeneity in the specificity of the cytotoxic T-cell response among individual animals immunised with the same parasite stock.

5 Analyses with Cytotoxic T-Cell Clones

5.1 Generation of Cytotoxic T Cells In Vitro

The use of effector cells generated in vivo to analyse the antigenic relationship of different parasites is limited because of the likelihood that such populations contain cells with different antigenic specificites. Moreover, the levels of cytotoxicity detected in PBM of cattle undergoing immunisation or challenge with *T. parva* are often low and are detectable only for a few days. Therefore, for further studies of the antigenic specificity of the cytotoxic T cells, it was necessary to culture the effector cells in vitro and to obtain cloned populations. Details of the techniques used are described elsewhere (GODDEERIS and MORRISON 1988a).

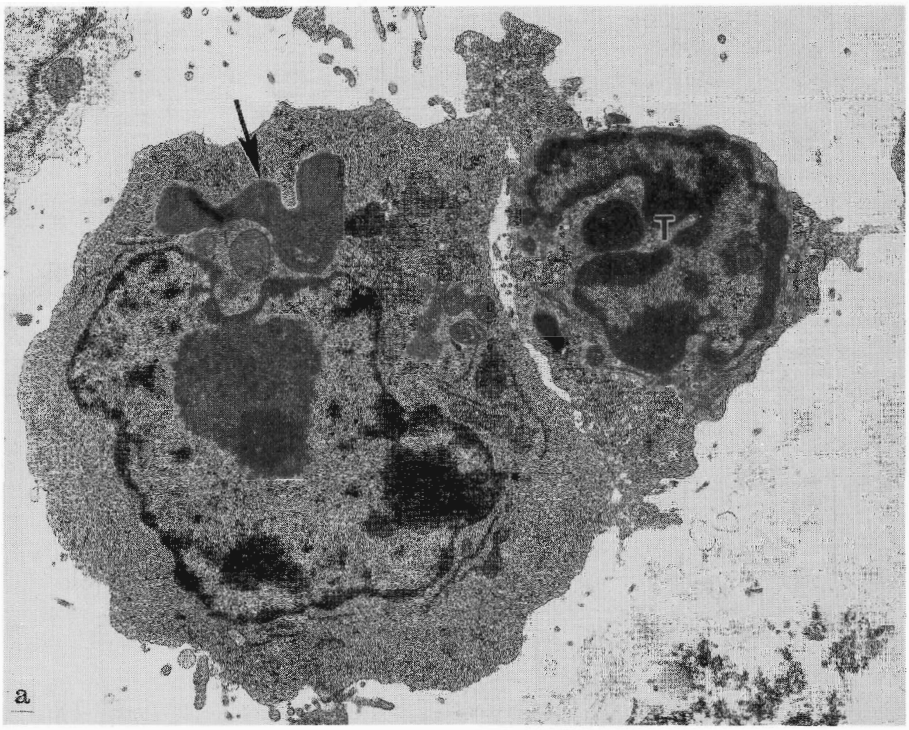
Cultures containing *T. parva*-specific cytotoxic T cells are readily generated in vitro from PBM of immunised animals by repeated stimulation with autologous infected cells on a weekly basis. As with the in vivo response, the effector cells have been shown to be MHC class I restricted and are within the $BoT8^+$ T-cell subset (GODDEERIS et al. 1986a). In general the MHC restriction and strain specificity of these cultures are similar to those observed in vivo. In only one of eight cattle in which both the responses in vivo and in vitro were analysed was the MHC restriction of the effectors generated in vitro different from that of the in vivo effectors. In this case, there was evidence that this was due to a decrease in the level of expression of one of the MHC class I antigens on the infected cell line used to stimulate the culture.

Cytotoxic T-cell clones were derived from these cultures following removal of the $BoT4^+$ lymphocytes, which usually constituted over 60% of the cells, by complement-mediated lysis with a BoT4-specific MAb (GODDEERIS et al. 1986b). These clones showed potent killing activity (Fig. 1), giving maximum cytotoxicity at effector to target ratios of between 1:1 and 4:1. Schizonts were apparently released intact; however, since this stage of the parasite has a very poor capacity to infect lymphocytes, killing of the infected cell essentially eliminates the parasite.

5.2 Influence of the Immunising Parasite on Strain Specificity of the Cytotoxic T Cells

Cytotoxic T-cell clones generated from cattle immunised with the Muguga stock of *T. parva* varied in their parasite strain specificity. In some instances they killed

Fig. 1a, b. Electron micrographs showing loose binding of a cytotoxic T-cell (*T*) to a parasitised lymphoblast observed 30 min after mixing the two populations (**a**) and lysis of a parasitised cell by a cytotoxic T-cell (*T*) observed 60 min after mixing the two populations (**b**). In each instance, the schizont is indicated with an *arrow*. Ultrastructural studies of the cells were carried out by Dr. S. Ito. (× 6000)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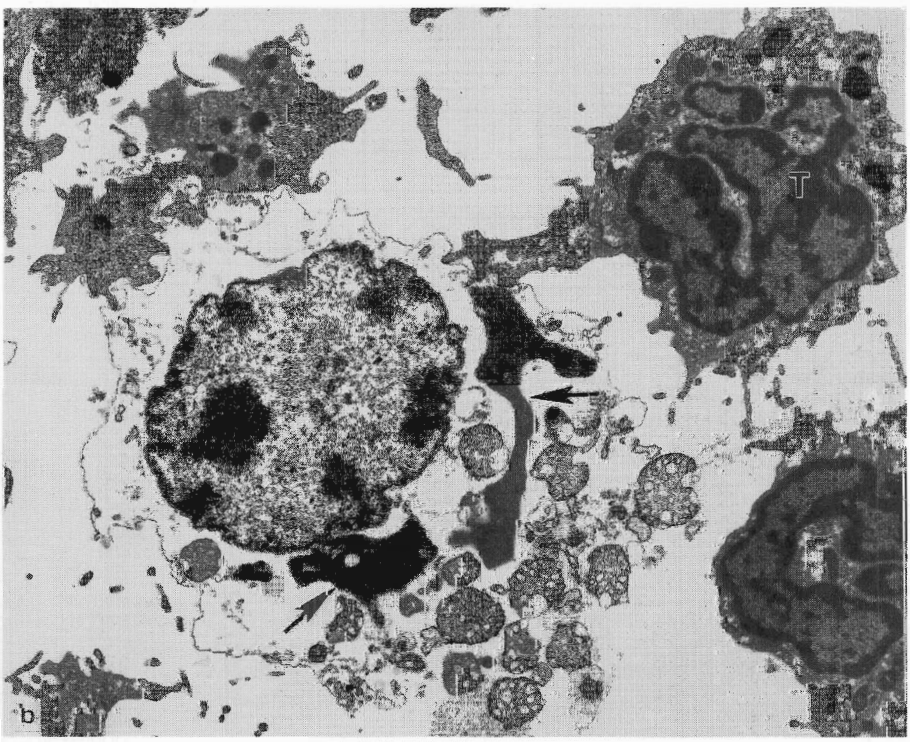
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cell lines infected with either the Muguga or Marikebuni stocks (B.M. GODDEERIS and W.I. MORRISON, unpublished data), whereas in other cases at least some of the Marikebuni-infected target cell lines were not killed (GODDEERIS et al. 1986b). Thus, as was observed in vivo, the parasite strain specificity of the cytotoxic T cells apparently differed between individual cattle.

Cytotoxic T-cell clones have been derived from only one animal immunised with the Marikebuni stock. These clones killed target cells infected with either the Muguga or Marikebuni parasites (MORRISON et al. 1987a). The level of killing varied from one target cell to another, but this was shown to be due to cyclical changes in the target cells rather than heterogeneity within the individual cell lines. Clones restricted by class I molecules encoded by both MHC haplotypes were identified. Clones restricted by the same specificities (w6.2 and KN104) had also been derived from animals immunised with Muguga. Thus, it was possible to compare the parasite strain specificities of these clones using the same target cells. The results with two pairs of clones are shown in Table 2. The w6.2-restricted clones from animals immunised with Muguga or Marikebuni exhibited similar strain specificities in that they killed target cells infected with either parasite (B.M. GODDEERIS and W.I. MORRISON, unpublished data). By contrast, one KN104-restricted T-cell clone from the Marikebuni-immunised animal killed targets infected with either parasite whereas similarly restricted clones derived from a Muguga-immune animal were specific for targets infected with Muguga (GODDEERIS and MORRISON 1988b). The latter finding indicates that the immunising parasite can influence the antigenic specificity of the cytotoxic T-cell response. This is consistent with results of cross-protection in that the specificity of the immunity induced by the Muguga and Marikebuni parasites also differs.

However, the situation is complicated by the fact that, at least in the case of the Marikebuni stock, the parasite stock is antigenically and genetically heterogeneous.

Table 2. Influence of the immunising parasite stock on the parasite strain specificity of cytotoxic T-cell clones derived from immue cattle

Donor animal (MHC phenotype)	Immunising parasite stock	T-cell clone	Restricting MHC specificity[a]	Percent cytotoxicity on infected target cells of animal[b]			
				D409 (KN104/w7)		D247 (w6.2/w7)	
				Muguga	Marikebuni	Muguga	Marikebuni
C887 (KN104/w6.2)	Marikebuni	T21.7	KN104	32	35	0	2
		T19.7	w6.2	1	2	40	32
B641 (KN104/ KN18)	Muguga	T20.40	KN104	72	0	—	—
D232 (w2/w6.2)	Muguga	T23.110	w6.2	—	—	62	41

[a] The cytotoxicity of clones T21.7 and T20.40 was specifically inhibited by MAb IL-A4, which reacts with the KN104 specificity, and that of clones T19.7 and T23.110 was inhibited by MAb B4/18 which reacts with all $w6^+$ animals

[b] Results of cytotoxicity obtained at an effector to target ratio of 2:1 in a 4-h ^{51}Cr-release assay are presented

Thus, using MAbs specific for a *Theileria* antigen which differs in molecular weight between parasite populations, it has been possible to demonstrate four different forms of the antigen in cloned cell lines derived from the Marikebuni stock (B.M. GODDEERIS and P.G. TOYE, unpublished data). In some instances, cytotoxic T-cell clones generated from Muguga-immunised cattle could also distinguish between these cell lines infected with different Marikebuni parasites.

5.3 Influence of MHC Phenotype on Strain Specificity of the Cytotoxic T cells

There is considerable heterogeneity in the strain-specificity of the cytotoxic T-cell response and the immunity induced by the *T. parva* (Muguga) parasite. One factor which is likely to contribute to this is the MHC phenotype of the animal. In order to address this issue, we have analysed the strain specificity of cytotoxic T-cell clones which differed in MHC restriction specificity but originated from the same animal (B.M. GODDEERIS et al., submitted for publication). The restriction specificities were determined in the same way as for effector cells generated in vivo, i.e. by testing the effectors on panels of target cells of defined phenotypes and by inhibition with MAb specific for polymorphic class I determinants. Parasite strain specificity was evaluated on target cell lines infected with different Marikebuni parasites on the same animal cell background.

The first point to make is that sets of clones which exhibited similar MHC restriction specificities also had the same parasite strain specificity. By contrast, clones from the same animal restricted by different MHC determinants showed different strain specificities. The results with two representative clones from an animal immunised with *T. parva* (Muguga) are shown in Table 3. In each instance, the clones were assayed on one Muguga-infected target cell and on two different Marikebuni-infected target cells on the same animal cell background. These findings provide convincing evidence that the restricting MHC molecules, by selecting particular antigenic epitopes, can influence the parasite strain specificity of the T-cell response. Similar observations have been made in the cytotoxic T-cell responses of mice to influenza A viruses (VITIELLO and SHERMAN 1983). The influence of the MHC

Table 3. Influence of the restricting MHC molecule on parasite strain specificity of cytotoxic T-cell clones derived from an animal immunised with *T. parva* (Muguga)

Donor animal (MHC phenotype)	T-cell clone	Restricting MHC specificity	Percent cytotoxicity on autologous cells infected with:[a]		
			Muguga	Marikebuni 5	Marikebuni 16
C196 (w6/w7)	T26.20	w7	37	4	23
	T26.44	Undefined molecule on w6 haplotype	63	31	0

[a]Results of cytotoxicity obtained at an effector to target ratio of 2:1 in a 4-h ^{51}Cr-release assay are presented. Differences in the parasites within the Marikebuni 5 and Marikebuni 16 cell lines were detected with parasite-specific MAb

on selection of antigenic epitopes may be due either to differences within the T-cell repertoires in the frequencies of T cells specific for certain antigen-MHC combinations or to differences in the capacity of processed antigenic peptides to associate with different MHC class I molecules.

6 Concluding Remarks

Information on the kinetics and specificity of the cytotoxic T-cell response of cattle to *T. parva* indicates that the response is important in mediating immunity. Our studies have shown that the immunising parasite population and the MHC phenotype of the animal can have an influence on the parasite strain specificity of the cytotoxic T-cell response. The immunity engendered in cattle by infection and treatment with one stock of *T. parva* also displays a degree of strain specificity. However, since the parasite stocks currently under study are not antigenically homogeneous, definitive evidence for a correlation between specificity of the cytotoxic T-cell response and protection must await experiments with cloned populations of parasites.

Identification and characterisation of the antigens recognised by the cytotoxic T cells is essential in order to define the molecular basis of the antigenic differences between parasite populations and to explore the immunogenicity of such antigens. In view of the available evidence that the T cells are exquisitely specific for parasitised cells and exhibit parasite strain specificity, it seems likely that the target antigens are encoded by the parasite. The current thinking that antigens within the cytosol of cells tend to be processed and presented in the context of MHC class I molecules (Moore et al. 1988) is also consistent with the concept that the T cells recognise a parasite-derived antigen, since the *Theileria* parasite lies free within the cytoplasm of the host cell and therefore can presumably secrete or shed proteins directly into the cytosol.

The *Theileria* parasite is a relatively complex organism, having a genome of almost 10^7 base pairs (Morzaria et al. 1988). Over 200 proteins have been detected in two-dimensional gels prepared from lysates of radiolabelled purified schizonts (Sugimoto et al., in press). Strategies must, therefore, be devised for identification of, T-cell target antigens within this complex mixture. As already alluded to, antibodies are unlikely to detect antigens on the cell surface in a form in which they are recognised by T cells. Therefore, the parasite-specific T cells themselves must be used as screening reagents. In the case of class II restricted T cells, this can readily be done by examining the response of T cells to soluble antigenic fractions added to antigen-presenting cells. Indeed, $BoT4^+$ T-cell cultures specific for *T. parva*-infected cells, generated in vitro from immune cattle, have been shown to proliferate specifically in response to crude antigenic extracts from parasitised cells (Brown et al., in press). By contrast, class I restricted T cells generally recognise only antigens that are actively being synthesised within the presenting cells (L.A. Morrison et al. 1986) or are artificially introduced into the cytosol (Moore et al. 1988).

Consequently, the strategy to identify these target antigens is to isolate candidate parasite proteins or genes and determine whether the proteins or gene products are recognised by T cells following introduction, by appropriate means, into mammalian cells expressing the relevant bovine MHC molecules.

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