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Strain specificity of bovine *Theileria parva*-specific cytotoxic T cells is determined by the phenotype of the restricting class I MHC

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SUMMARY

To determine whether the major histocompatibility complex (MHC) phenotype of cattle could affect the parasite strain specificity of immunity to *Theileria parva* by influencing the antigenic specificity of *Theileria*-specific cytotoxic T lymphocytes (CTL), we investigated the parasite strain specificity of *Theileria*-specific CTL clones derived from cattle of different class I MHC phenotypes. Thirty-one class I-restricted CTL clones were generated from four cattle immunized with the Muguga stock of *T. parva*. The MHC restriction and parasite strain specificities were determined for each clone utilizing as targets, parasitized cell lines of different MHC phenotypes and cloned cell lines containing different parasite strains. CTL clones restricted by the same MHC determinant had similar parasite strain specificities. On the other hand, clones restricted by different MHC determinants exhibited different parasite strain specificities. This was true whether the clones were generated from the same animal or from different cattle and tested on a target cell line expressing both MHC determinants. These results provide strong evidence that differences in the strain specificities of CTL derived from animals immunized with the same parasite stock, are determined by the class I MHC phenotype of the immunized animal.

INTRODUCTION

In recent years, evidence has emerged that class I major histocompatibility complex (MHC)-restricted T-cell responses play an important role in immunity to diseases caused by intracellular protozoan parasites, most notably bovine theileriosis (Morrison *et al.*, 1989) and human malaria (Good, Berzofsky & Miller, 1988). The most important species of *Theileria* in African cattle, *Theileria parva*, infects lymphocytes and causes an acute, usually fatal, disease known as East Coast fever (reviewed by Morrison *et al.*, 1986). Cattle can be immunized against the disease by infection with sporozoites and simultaneous treatment with long-acting tetracyclines (Radley *et al.*, 1975). It is currently believed that this immunity is mediated by cytotoxic T lymphocytes (CTL), which recognize as yet unidentified parasite antigens on the surface of infected lymphocytes (Goddeeris *et al.*, 1986b; Morrison *et al.*, 1987b). In previous studies, we have analysed *Theileria*-specific CTL lines and clones from animals immunized with either *T. parva* (Muguga) (Goddeeris, Morrison & Teale, 1986a; Goddeeris *et al.*, 1986b) or *T. parva* (Marikebuni) (Morrison, Goddeeris & Teale, 1987a). Cytotoxic T-cell lines or clones generated against the Marikebuni stock recognized targets infected with either parasite stock, whereas of those generated against the Muguga stock some killed only Muguga-infected cells while others killed Muguga- and Marikebuni-infected cells (Morrison *et al.*, 1989). Moreover, recently we observed that certain CTL clones of Muguga-immunized animals recognized some but not other Marikebuni-infected cell lines (Goddeeris & Morrison, 1988a).

These results on the strain specificity of CTL appeared to be consistent with cross-protection, in that cattle immunized with the Marikebuni stock are protected against challenge with Muguga whereas some Muguga-immunized animals are susceptible to challenge with the Marikebuni stock (Irvin *et al.*, 1983). However, the situation is further complicated by the recent demonstration that there is considerable parasite heterogeneity within the Marikebuni stock. Polymorphism in the restriction fragment lengths of parasite DNA (Conrad *et al.*, 1989), differences in the protein spot patterns of two-dimensional gel electrophoresis of schizonts (Sugimoto *et al.*, 1989), and differences in the relative molecular weight (MW) of a parasite-specific polymorphic molecule (Goddeeris, Toye & Morrison, 1989; P. G. Toye and B. M. Goddeeris, in preparation) have been detected between different populations of the Marikebuni stock.

The aim of this study was to determine whether the parasite strain specificity of *Theileria*-specific CTL is influenced by the MHC phenotype of the immunized animal. This question was addressed by analysing the strain specificity of CTL clones generated from cattle of different MHC phenotypes, immunized with *T. parva* (Muguga).

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MATERIALS AND METHODS

Animals

Four cattle with different MHC phenotypes were selected. Animals C196 and E98 were Boran (*Bos indicus*), animal D232 was a Guernsey (*B. taurus*) and D409 was a crossbred between Boran and Guernsey. The four animals were immunized with the Muguga stock of *T. parva* by simultaneous inoculation with a lethal dose of sporozoites from cryopreserved stabilate (836) and long-acting oxytetracycline (Pfizer, Sandwich, Kent, U.K.) (Radley *et al.*, 1975). Animals D409 and E98 received a homologous challenge 11 months and 2 months after immunization, respectively, and animal C196 received two homologous challenges 3 months and 12 months after immunization.

Cattle that were matched and mismatched for their MHC phenotypes with the four immunized animals were selected as cell donors for the preparation of *T. parva*-infected cell lines. At the outset of the experiments, sera of all animals were screened for the presence of anti-schizont antibodies, as described previously (Goddeeris *et al.*, 1982) and all were negative. During the experiments, all animals were maintained indoors and were sprayed weekly with acaricide (Delnav, Wellcome Kenya Ltd, Nairobi).

Cell lines

Cell lines infected with *T. parva* were established from peripheral blood mononuclear (PBM) cells, and in one instance from a T-cell clone, by infection *in vitro* with sporozoites obtained from dissected tick salivary glands. The cell lines were maintained in culture as described previously (Goddeeris & Morrison, 1988b). The sporozoites were from stabilate 3087 of the Muguga stock and from two different stabilates of the Marikebuni stock of *T. parva*: sporozoite stabilate 2245 was used to prepare the *T. parva* (Marikebuni)-infected cell line D409MaI while stabilate 3014, which was derived by one tick/cattle passage from stabilate 2245, was used to prepare all other *T. parva* (Marikebuni)-infected cell lines. The infected cell lines T3.5Ma5 and T3.5Ma16 were clones from an infected cell line T3.5Ma, which had been obtained by infecting a cloned alloreactive BoT4$^+$ T-cell line (T3.5) of animal C196 with *T. parva* (Marikebuni).

Uninfected lymphoblasts were used as target cells to define parasite specificity. These were derived by stimulation of PBM with concanavalin A and subsequently maintaining the cells as a cell line in medium containing T-cell growth factor (TCGF), as described previously (Goddeeris & Morrison, 1988b). On the day before use of the uninfected cells as targets, they were passaged with TCGF containing 0·1 M alpha-methyl-D-mannoside to bind any residual concanavalin A in the TCGF.

Typing for bovine MHC antigens

Peripheral blood mononuclear cells and cell lines were typed for bovine lymphocyte antigens (BoLA) using alloantisera and monoclonal antibodies in a microlymphocytotoxicity assay (Teale *et al.*, 1983). These reagents define class I MHC specificities, the majority of which are encoded by a single locus, the BoLA-A locus. Animals C196, D232, D409 and E98 had the BoLA phenotypes w6/w7, w2/w6.2, w10,KN104/w7 and w10,KN104/w10,KN104, respectively. Animal E98 was produced by interbreeding half-siblings and was shown to be homozygous at the MHC. Recent experiments with this animal have demonstrated the expression of two distinct class I MHC products bearing the w10 and KN104 specificities, respectively (P. G. Toye, N. D. MacHugh, A. M. Bensaid, S. Alberti, A. J. Teale and W. I. Morrison, manuscript submitted. Animal D247, which is one of the animals used for the generation of infected cell lines, was a half-sibling of animal D232 and was haploidentical with the latter for the w6.2-bearing haplotype.

Immunoblot analysis of the infected cell lines

All *T. parva*-infected cell lines were analysed in Western blots with mAb BT5/2.2.5 (produced at ILRA), which identifies a parasite-specific molecule expressed on the surface of the intracellular schizont (Pinder & Hewett, 1980; Shapiro *et al.*, 1987). This molecule differs in MW between stocks of *T. parva* (Shapiro *et al.*, 1987) and between subpopulations of the Marikebuni stock (Goddeeris *et al.*, 1989) and will be referred to in this paper as the polymorphic molecule.

To prepare cell lysate, infected cells were incubated for 2 hr at 4° in lysis buffer at a concentration of 2×10^7 cells per ml. The lysate was centrifuged at 10,000 ***g*** for 10 min to remove debris, the supernatant collected and stored at −20°. The lysis buffer consisted of 10 mM Tris-HCl, pH 7·5, 5 mM EDTA, 0·15 M NaCl, 5 mM iodoacetamide, 40 μg/ml leupeptin, 1 mM TLCK, 1 mM PMSF, 5 μg/ml aprotinin and 0·5% Nonidet P-40 (Bensaid *et al.*, 1989). Lysates were mixed with an equal volume of sample buffer (40 mM Tris-HCl, pH 6·8, 20% glycerol, 2% SDS, 0·42 M 2-ME, 0·02% bromophenol blue) and heated for 2 min in a boiling waterbath. Aliquots of 50 μl, equivalent to 5×10^5 cells per lane, were then resolved by electrophoresis (100 mA/gel) in a 7·5% SDS–PAGE slab gel at 10°. The molecules were transferred to nitrocellulose electrophoretically (500 mA for 1 hr at 10°). Thereafter the nitrocellulose blot was saturated for 30 min in 10% skimmed milk in phosphate-buffered saline (PBS) and reacted with mAb BT5/2.2.5 (ascitic fluid at 1/400 in 1% skimmed milk in PBS) for 2 hr. After three washes of 10 min each in PBS containing 0·1% skimmed milk and 0·05% Tween 20, the blot was incubated for 1 hr with sheep anti-mouse Ig conjugated to horseradish peroxidase (Amersham International plc, Amersham, Bucks, U.K.; dilution of 1/400 in 1% skimmed milk in PBS). After three washes of 10 min each, the blot was developed using hydrogen peroxide as substrate and 3,3-diaminobenzidine (1 mg/ml) as chromogen in PBS with 1% skimmed milk.

Restriction fragment length polymorphism

Preparation of DNA from *Theileria*-infected cells followed procedures described previously (Conrad *et al.*, 1987). Restriction endonuclease digestion of approximately 20 μg of *Theileria*-infected cell DNA was carried out for 6 hr using 4 U of EcoR1 per μg of DNA. Fragments of DNA were separated in horizontal 0·8% (w/v) agarose gels at 40 volts for 16 hr and the restriction fragments transferred onto nitrocellulose filters (Hybond C; Amersham International).

The filter was probed with a 623 base pair (bp) Sau96 1 fragment that had been cloned from a repetitive region of the *T. parva* (Muguga) genome by ligation of a gel fragment into the Sma1 site of pUC8 (Allsopp & Allsopp, 1988). Labelling of the gel-purified 623 bp repetitive DNA fragment to a specific activity of 2×10^8 c.p.m. per μg was by random priming (Feinberg & Vogelstein, 1983) using a commercial kit (Boehringer, Mannheim, FRG). The filter was prehybridized at 65° for 6 hr in a solution containing 4 × SSC (1 × SSC = 150 mM NaCl, 15 mM Na citrate), 10 × Denhardt's solution, 0·1% SDS

and 0·1% Na pyrophosphate. Labelled probe was then added and hybridization carried out overnight. The filters were washed four times for 30 min to a stringency of 4 × SSC at 65°.

Cytotoxic T-cell clones

T. parva-specific CTL clones were established from cultures of T cells established by stimulation of PBM *in vitro* several times, as described previously (Goddeeris *et al.*, 1986b; Goddeeris & Morrison, 1988b). Ten days after the last challenge, PBM from animals D409, E98 and C196 were stimulated twice *in vitro* with autologous *T. parva* (Muguga)-infected cells, first at a responder to stimulator ratio of 20:1, subsequently, 1 week later, at a responder to stimulator ratio of 2:1. On both occasions the cultures were established in culture medium containing 5% TCGF. In the case of animal D232, PBM were collected 3 months after immunization and stimulated three times *in vitro* with autologous-infected cells but without TCGF. All CTL clones were derived from the restimulated populations after elimination of the BoT4$^+$ cells with mAb IL-A11 and complement to enrich for the BoT8$^+$, class I-restricted T cells (Goddeeris & Morrison, 1988b). All CTL clones used in this study were analysed with mAb CH128A, IL-A11 and IL-A17, which define the bovine T-cell differentiation antigens BoT2, BoT4 and BoT8, respectively (Davis *et al.*, 1988; Baldwin *et al.*, 1986; Ellis *et al.* 1986).

Cytotoxicity assay

Cytotoxic activity of the CTL clones was tested in a 4-hr ^{51}Cr-release assay conducted in 96-well flat-bottomed microculture plates as described previously (Goddeeris & Morrison, 1988b). Maximum release was evaluated by subjecting the target cells to two cycles of rapid freezing and slow thawing. The cytotoxicity was calculated as:

$$\%\ \text{cytotoxicity} = 100 \times \frac{\text{test release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}}.$$

To define the MHC restriction, panels of parasitized target cells were chosen to provide targets that were matched or mismatched for BoLA specificities with the effector T-cell clone. For the same purpose, several mAb specific for polymorphic or monomorphic class I MHC determinants were tested for their capacity to inhibit cytotoxicity. The inhibition assay was performed by preincubating the target cells with the appropriate mAb for 30 min at room temperature in the assay plates before adding the effector cells. The cells were then incubated and harvested as for a normal ^{51}Cr-release assay. The mAb were added to the test at a final concentration that gave 100 times saturating levels of binding, as assessed by indirect immunofluorescence.

The following mAb were used in the inhibition assay. Monoclonal antibody IL-A4 recognizes a polymorphic determinant on the KN104 BoLA molecule (Bensaid *et al.*, 1988), mAb IL-A9 recognizes a determinant specific for the w4 and w7 class I specificities (A. J. Teale, W. I. Morrison and S. J. Kemp, unpublished data), mAb B4/18 recognizes a class I MHC determinant associated with the w6 specificity (Spooner & Pinder, 1983) and mAb IL-A19 recognizes a non-polymorphic determinant on bovine class I MHC molecules (Bensaid *et al.*, 1989).

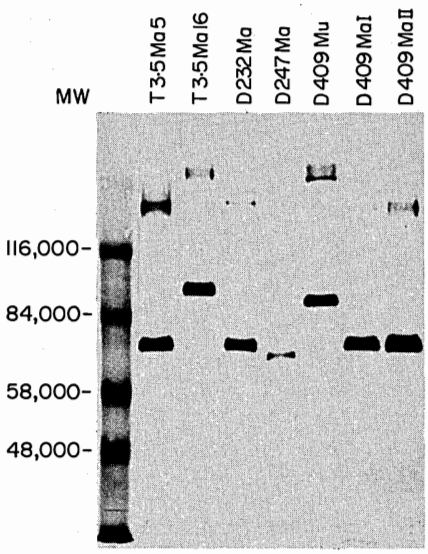

Figure 1. Western blot analysis with mAb BT5/2.2.5 of lysates of cell lines infected with the Muguga (Mu) or Marikebuni (Ma) stocks of *T. parva*. The infected cell lines were T3.5Ma5 and T3.5Ma16, both subcloned after infecting a T-cell clone of animal C196, D232Ma, D247Ma, D409Mu, D409MaI and D409MaII.

RESULTS

Parasite characterization of the infected target cell lines

The parasite-specific polymorphic molecule recognized by BT5/2.2.5 had the same MW, namely 93,000, in all cell lines infected with the Muguga stock of *T. parva* (Fig. 1; D409Mu). By contrast, three different MW were found for the parasite-specific polymorphic molecule of cell lines infected with the Marikebuni stock of *T. parva*. The mAb BT5/2.2.5 recognized a molecule with a MW of 98,000 for cell line T3.5Ma16, 74,000 for cell lines T3.5Ma5, D232Ma, D409MaI and D409MaII and 71,000 for cell line D247Ma (Fig. 1).

Five of the cell lines infected with the Marikebuni stock of *T. parva*, namely T3.5Ma5, T3.5Ma16, D232Ma, D247Ma and D409MaI, exhibited no obvious differences when their DNA was probed with the 623 bp repetitive fragment from *T. parva* (Muguga) (Fig. 2). By contrast in the sixth cell line, D409MaII, most of the bands seen by the repetitive probe differed in size and intensity from those seen in the other five cell lines (Fig. 2), suggesting that the repetitive genomic region was organized differently in this cell line.

Analysis of the MHC restriction

A total of 31 parasite-specific CTL clones were analysed. All clones were BoT2$^+$, BoT4$^-$, BoT8$^+$ and lysed *T. parva* (Muguga)-infected target cells but not uninfected target cells. Their origin and MHC restrictions are summarized in Table 1.

Eight of the CTL clones were generated from animal E98, which is class I MHC-homozygous and expresses the w10 and KN104 class I specificities. Based on cytotoxicity obtained on a panel of *T. parva* (Muguga)-infected target cells and on inhibition of cytotoxicity with mAb IL-A4, all clones were shown to be restricted by the KN104 molecule (Fig. 3a, one clone presented, hatched bars). Five clones were generated from

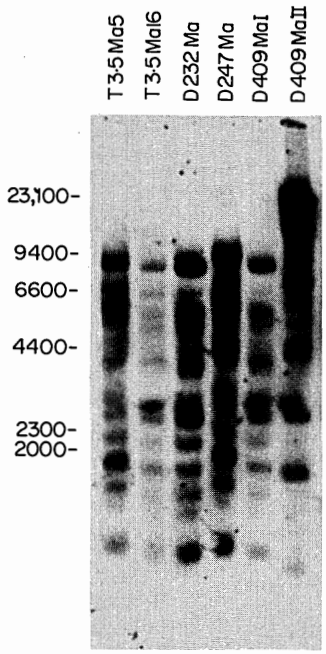

Figure 2. EcoR1-digested DNA of T3.5Ma5, T3.5Ma16, D232Ma, D427Ma, D409MaI and D409MaII were transferred onto nitrocellulose and probed with a radiolabelled 623 bp repetitive DNA fragment from *T. parva* (Muguga). The size markers are Lambda DNA digested with Hind III and sizes are given in bases.

Table 1. Origins and MHC restrictions of the *T. parva*-specific CTL clones

		MHC restriction		
Animal	BoLA phenotype	Number	Haplotype	Specificity
E98	w10,KN104/w10,KN104	8	w10,KN104	KN104
D409	w10,KN104/w7	5	w7	w7
C196	w6/w7	9	w6	Undefined
		1	w7	w7
D232	w6.2/w2	4	w6.2	w6.2
		4	w6.2	Undefined

animal D409 with the w7/w10, KN104 class I phenotype. These clones were all shown to be restricted by a product of the w7-bearing haplotype. Their cytotoxicity was specifically inhibited by preincubation of target cells with mAb IL-A9, which is specific for the w4 and w7 class I specificities, indicating that they are restricted by the w7 molecule (Fig. 3a, one clone presented, solid bars). However, the clones killed only w7-matched target cells which originated from a *B. taurus* animal and not those from a *B. indicus* animal.

Ten clones were generated from animal C196, which has the w6/w7 BoLA phenotype. When tested on a panel of target cells matched for one or other BoLA haplotype, nine clones were shown to be restricted by the w6-bearing haplotype (Fig. 4a, one clone presented, solid bars) and one by the w7-bearing haplotype (Fig. 4a, hatched bars). The cytotoxic activity of the latter was blocked by incubating the targets with mAb IL-A9, indicating that it is restricted by the w7 specificity. In contrast

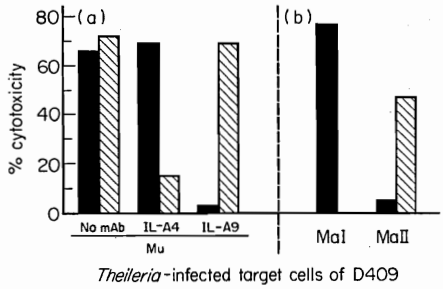

Figure 3. Inhibition of cytotoxicity with anti-class I mAb (a) and analysis of the strain specificity (b) of two *Theileria*-specific CTL clones, one from animal D409 (solid bars) and another from animal E98 (hatched bars). The clones were tested for cytotoxicity at an effector to target ratio of 2:1. The target cell lines were from animal D409 (w10,KN104/w7) and were infected with the Muguga stock (Mu) and with two different isolates of the Marikebuni stock, respectively (MaI and MaII).

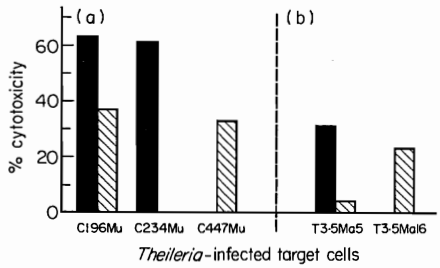

Figure 4. Analysis of MHC restriction (a) and parasite strain specificity (b) of two *Theileria*-specific CTL clones of animal C196 (w6/w7). The clones were tested for cytotoxicity at an effector to target ratio of 2:1. Target cell lines comprised an autologous cell line, a w6-matched cell line of animal C234 (w6/w10) and a w7-matched cell line of animal C447 (w7/w10), all infected with the Muguga stock (Mu) of *T. parva*, and two Marikebuni-infected autologous cell lines, T3.5Ma5 and T3.5Ma16.

with the w7-restricted clones from animal D409, this clone killed w7-matched target cells of *B. indicus* origin and not of *B. taurus* origin. Thus, although the w7 antigens of *B. taurus* and *B. indicus* animals are serologically indistinguishable, they are recognized as different by *Theileria*-specific T cells.

Finally, eight clones were generated from animal D232. All clones were shown to be restricted by products of the w6.2-bearing haplotype, as defined by screening on a panel of *T. parva* (Muguga)-infected targets from MHC-matched animals. All eight clones were restricted by class I MHC determinants, as their cytotoxic activities were inhibited by mAb IL-A19, which recognizes a non-polymorphic determinant on bovine class I MHC molecules (Fig. 5a, two clones presented). However, the cytotoxicity of only four of the clones could be inhibited by the mAb B4/18, which recognizes a public class I specificity associated with w6 (Fig. 5a, one clone presented, hatched bars). These findings indicate that the clones are restricted by different MHC determinants, possibly on different class I products, on the same haplotype.

Analysis of the parasite strain specificity

The parasite strain specificities of all CTL clones were analysed on *T. parva* (Marikebuni)-infected cell lines. The clones of

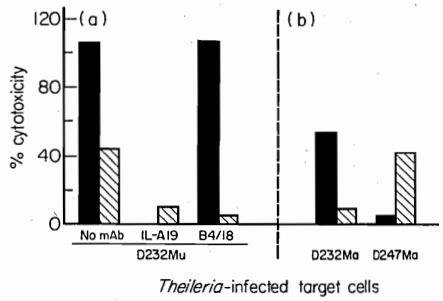

Figure 5. Inhibition of cytotoxicity with anti-class I mAb (a) and analysis of the strain specificity (b) of two *T. parva*-specific cytotoxic T cell clones from animal D232 (w2/w6.2). The clones were tested for cytotoxic activity at an effector to target ratio of 4/1 (solid bars) and 10/1 (hatched bars), respectively. Target cells consisted of autologous cell lines infected with the Muguga (D232Mu) or Marikebuni (D232Ma) stock of *T. parva* and a w6.2 MHC-haploidentical target cell line (D247Ma) infected with the Marikebuni stock.

animals E98 and D409 which were KN104- and w7-restricted, respectively, were tested on the same target cell lines D409MaI and D409MaII with the BoLA phenotype w7/w10,KN104. Although these two cell lines had polymorphic molecules with similar MW, namely 74,000 (Fig. 1), they were infected with different Marikebuni parasites, as demonstrated by analysis of their DNA in Southern blots with a parasite-specific repetitive DNA probe (Fig. 2). The two sets of clones differentiated between these *T. parva* (Marikebuni)-infected cell lines. Results with representative clones of each set are shown in Fig. 3b. The w7-restricted clones of animal D409 exhibited high cytotoxicity on the D409MaI cells and only low cytotoxicity on the D409MaII cells, while the KN104-restricted clones of animal E98 killed only the D409MaII cells.

Clones of animal C196 were tested on two autologous cell lines, namely T3.5Ma5 and T3.5Ma16, which had been cloned from a *T. parva* (Marikebuni)-infected T-cell clone. Although these two infected clones originated from the same infected cell line, their polymorphic molecule, as defined with mAb BT5/2.2.5, differed in MW, namely 74,000 for T3.5Ma5 and 98,000 for T3.5Ma16 (Fig. 1). The two different sets of CTL clones of C196 differentiated between these two infected cell lines. Results with representative clones of each set are shown in Fig. 4b. The clones restricted by the w6 haplotype killed only the T3.5Ma5 clone while the cytotoxic T-cell clone restricted by the w7 haplotype exhibited high cytotoxicity on the T3.5Ma16 clone but low cytotoxicity on the T3.5Ma5 clone.

Cytotoxic T-cell clones of animal D232 were tested on an autologous (D232Ma) and a w6.2 haploidentical (D247Ma) target cell line infected with *T. parva* (Marikebuni). It is important to note that all clones killed equally well the autologous (D232) and the w6.2 haploidentical (D247) targets infected with the Muguga stock. The two *T. parva* (Marikebuni)-infected targets had been infected with the same stabilate, but on analysis in Western blot their polymorphic immunodominant molecules had different MW, namely 74,000 for D232Ma and 71,000 for D247Ma (Fig. 1). The Marikebuni-infected cell lines were also recognized as different by the *T. parva*-specific CTL clones. Results with representative clones of each set are shown in Fig. 5b. The four clones restricted by the w6.2 specificity manifested high cytotoxicity on the D247Ma cell line and low cytotoxicity on the D232Ma cell line, while the four clones restricted by another MHC specificity on the w6.2 haplotype killed only the D232Ma cell line. It is of note that the D247Ma cell line was also recognized by the w7-restricted clones of animal D409 (Table 2).

Table 2. Analysis of the strain specificity of three sets of CTL clones (two clones in each set) restricted by different class I MHC molecules on target cell lines of animal D247 with the BoLA phenotype w6.2/w7

Clones		Cell lines of D247 infected with	
Donor	Restricting specificity	Muguga	Marikebuni
D232	Undefined molecule	89*	3
	on w6.2 haplotype	81	5
D232	w6.2 molecule	46	38
		62	41
D409	w7 molecule	52	26
		71	62

* Percentage cytotoxicity at an effector to target ratio of 2:1.

DISCUSSION

This study has yielded important information on the influence of the MHC on the strain specificity of *Theileria*-specific cytotoxic T cells. Among the 31 clones analysed, those restricted by the same class I MHC molecule had similar parasite strain specificities. By contrast, those restricted by different MHC molecules often had different strain specificities, even when they originated from the same animal. These results provide strong evidence that differences in the strain specificities of CTL derived from animals immunized with the same parasite stock are determined by the class I MHC phenotype of the immunized animal. A similar influence of the MHC on the strain specificity of CTL has been demonstrated in studies with influenza A virus, where mice with different MHC phenotypes were found to react to different viral antigenic epitopes, resulting in differences in the virus strain specificity of the CTL (Vitiello & Sherman, 1983).

The MHC restriction and parasite strain specificities of short-term T-cell cultures from the four animals in this study, in each instance, were similar to those of the CTL clones (data not shown), indicating that the clones were representative of the CTL response in the animal. A striking feature of the CTL responses in all four cattle was that a large component of the response was restricted by one or two class I MHC specificities. Indeed, in two cattle all of the clones were restricted by one MHC molecule, in another animal nine out of ten clones were restricted by one molecule and in the fourth animal the clones were restricted by two specificities encoded by the same MHC haplotype. A bias in MHC restriction has been observed previously for *Theileria*-specific CTL responses *in vivo* (Morrison *et al.*, 1987b). Moreover, similar observations are documented for T-cell responses to viral infections in man and mice (Townsend & McMichael, 1985). This bias in the MHC restriction of the response to one or two MHC molecules emphasizes the influence that MHC can exert on the strain specificity of the T-cell response, if a particular MHC molecule also selects particular antigen epitopes.

Two phenomena, which are not mutually exclusive, may explain the marked differences between individual animals in parasite strain specificity of the *Theileria*-specific CTL response. First, it is possible that major differences exist in the T-cell receptor repertoire of naive cattle so that T cells specific for particular antigen–MHC combinations are absent or are less frequent than others and therefore are poorly represented in the response. This would account not only for the finding that the response is directed towards particular antigenic epitopes of the parasite but also for the observed bias in the response towards certain MHC specificities. The antigen receptor on the surface of T lymphocytes recognizes antigen in the form of processed peptides associated with MHC molecules on the surface of antigen-presenting cells (Dembic *et al.*, 1986). There is evidence that the cleft between the alpha helices of the first and second domains of the heavy chain of class I molecules provides a binding site for antigenic peptides (Bjorkman *et al.*, 1987). The generation of the T-cell receptor repertoire occurs in the thymus in a process which involves expression of rearranged T-cell receptor genes and selection of T cells whereby those with high avidity for self-MHC molecules (with or without self peptides) are deleted (Blackman, Kappler & Marrack, 1988). Thus, the generation of a T-cell repertoire for a given restricting MHC molecule will be influenced by other MHC molecules and self-polymorphic molecules which can associate in the form of peptides with the restricting MHC molecule. Hence, it might be expected that, if the T-cell repertoire is important in determining antigenic specificity of the CTL response to *Theileria*, T cells which are restricted by the same MHC molecule but originate from different cattle would show different antigenic specificities. Further studies will be required to answer this question.

The second factor which is likely to influence the specificity of the CTL response is that certain antigen–MHC combinations are more immunodominant than others possibly due to the strength of binding between the MHC molecule and the antigenic peptide which could result in a higher concentration on the surface of the infected cell. Thus, different peptides would have different avidities for different MHC molecules and compete with each other for the same MHC molecule, or indeed, certain peptides might not be able to associate with a particular MHC molecule. This has been confirmed in studies with class II molecules, in which differences in binding between immunogenic peptides and Ia have been observed, and T-cell responses were directed predominantly to those Ia–peptide combinations which showed strongest binding (Buus *et al.*, 1987). In the case of *Theileria*, we have shown previously that at least two different parasite epitopes on Muguga-infected cells are recognized in association with the same class I MHC molecule (KN104) by CTL generated from animals immunized with different parasite stocks (Goddeeris & Morrison, 1988c). However, in animals immunized with *T. parva* (Muguga) only one of these epitopes was able to induce a detectable CTL response. A higher concentration of this epitope (in association with the KN104 MHC molecule) on the cell surface and/or a higher frequency of its specific T cells and/or a stronger binding with its specific T-cell receptors could explain why the CTL response was directed towards this epitope.

Regardless of how the selection of particular antigen/MHC combinations occurs in CTL responses to *T. parva*, its major significance is that, depending on whether or not the epitopes are conserved between strains, differences in the strain specificity of the CTL response may determine whether or not animals immunized with one stock of the parasite are protected against other stocks. In the case of *T. parva* (Muguga), it is likely that the results obtained in the present study account for the variation between individual animals in the degree of immunity to challenge with the Marikebuni stock.

For *T. parva*, the parasite molecules which contain the T-cell epitopes have not yet been identified. Although in three instances differences in T-cell recognition correlated with size differences in the polymorphic parasite antigen, there is no evidence that the T cells recognize epitopes on this molecule. Moreover, in the case where T cells detected differences in cell lines which expressed polymorphic parasite molecules of similar MW, analysis of the parasite DNA showed other obvious differences between the Marikebuni parasites. Thus, the Western and Southern blots together with the CTL clones identified four different Marikebuni parasites. In view of this parasite heterogeneity and the finding that individual animals immunized with the Muguga stock recognize different components of the Marikebuni stock, interpretation of the CTL specificity in relation with cross-protection between the two stocks is extremely complicated. Consequently, in future studies it will be necessary to use cloned populations of parasites to address this question.

These findings have considerable implications in the search for vaccines against intracellular protozoal diseases in which CTL are considered to play a major protective role. An important question to address is whether differences in responses of individual animals to different antigenic epitopes are due to inherent differences in the capacity of particular antigen–MHC combinations to induce a response, or are associated with a hierarchy in dominance among antigenic epitopes due to competition for association with MHC molecules, as has been observed for murine class II molecules (Buus *et al.*, 1987). In the former case, it would be necessary for vaccines to incorporate multiple antigenic epitopes to ensure that all individuals will mount a strong T-cell response. In the latter case, if protective epitopes exist which are common to different parasite strains and are able to associate with a broad spectrum of MHC molecules, it would be advantageous to present them to the host in isolation from strain-specific epitopes which bind more strongly with the MHC. Elucidation of these problems is of major importance in efforts to develop a vaccine against East Coast fever, and is of relevance to human malaria, in which CTL responses are now considered to play a role in immunity (Good *et al.*, 1988).

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