Parasite strain specificity of bovine cytotoxic T cell responses to Theileria parva is determined primarily by immunodominance

Parasite Strain Specificity of Bovine Cytotoxic T Cell Responses to *Theileria parva* Is Determined Primarily by Immunodominance

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The parasite strain specificity of CTL responses to *Theileria parva* varies among cattle immunized with the same parasite stock. We have investigated the influence of class I MHC on the strain specificity of CTL responses to *T. parva* in 19 cattle of defined class I phenotype immunized with either of two *T. parva* populations, in which protection to subsequent reciprocal challenge correlated with CTL strain specificity. In the majority of animals the response was restricted by the products of one MHC haplotype and there was a consistent bias to some haplotypes in preference to others. In 10 of 13 cattle expressing the molecularly defined MHC specificities A10 and KN104 on one haplotype, the CTL response was restricted entirely by this haplotype, thus allowing a precise analysis of the MHC restriction specificities. The MHC restriction specificity and the parasite population used for immunization both influenced the strain specificity of the response. By examining responses in identical twins immunized with different parasites or in animals before and after challenge with heterologous parasites, animals that mounted a strain-specific response to primary infection were shown to be capable of responding to Ags shared by the two parasite populations. These findings indicate that the strain specificity of CTL responses to *T. parva* is not determined primarily by immune response genes that define the inherent capacity to respond, but rather is a consequence of the response in individual animals being biased toward a limited number of immunodominant peptide-MHC determinants. ***The Journal of Immunology,*** **1995, 155: 4854–4860.**

A number of apicomplexan protozoan parasites have been shown to induce class I MHC-restricted T cell responses specific for the parasite stages that replicate within nucleated cells (1–3). Such responses have been studied most extensively in infections with *Plasmodium* species in rodents and humans, and during infections with *Theileria parva* in cattle (4, 5). Studies in mice involving depletion and adoptive transfer of T cell subsets indicate that $CD8^+$ T cells are involved in immunity to malaria parasites (6–9). Direct evidence that $CD8^+$ T cell responses can mediate protection against *T. parva* has also been provided by recent experiments utilizing identical twin calves in which adoptive transfer of lymphocytes, highly enriched for $CD8^+$ T cells, conferred the ability to control a primary infection (10). These observations highlight the potential for employing parasite Ags recognized by $CD8^+$ T cells for immunization.

The intralymphocytic stage of *T. parva*, against which $CD8^+$ T cell responses are directed, is responsible for most of the pathology associated with the infection. Development of the schizont following invasion of lymphocytes by sporozoites stimulates proliferation of the host cell. By dividing at the same time as the host cell the parasite maintains infection in the daughter cells and thus is able to undergo rapid multiplication without being exposed to the extracellular environment. In susceptible animals, this generally results in overwhelming parasitosis and death within 2 to 4 wk of infection. Infection and treatment immunization procedures have been developed to immunize cattle, and such animals are immune to subsequent challenge with the same parasite population. Parasite-specific CTL are detected transiently in the blood of infected animals at the time of remission of primary or challenge infections (11, 12). Memory CTL precursors (CTLp)[2] can also be detected in the blood, for many months after immunization, by restimulation of T cells in vitro with parasitized lymphoblasts (1, 13).

A striking feature of the CTL response to *T. parva* is the variation in parasite strain specificity of the response among animals immunized with the same parasite stock. In studies utilizing two immunologically distinct stocks of *T. parva*, Muguga and Marikebuni, some animals immunized with Muguga were found to generate CTL that recognized both parasites, whereas others produced a strain-specific CTL response (12, 14). Recently, the production of cloned parasite populations has enabled us to examine the relationship between the strain specificity of CTL responses and cross-protection between the Muguga and Marikebuni parasite populations. The application of a limiting-dilution (LD) analysis assay to detect CTLp facilitated a quantitative evaluation of strain specificity (13). In a group of cattle immunized with either of the two parasite populations and subsequently challenged with the reciprocal parasite, those animals that developed a cross-reactive CTL response were found to be immune to challenge, whereas those that generated strain-specific CTL developed moderate to severe clinical reactions following challenge (15). These results

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[2] Abbreviations used in this paper: CTLp, CTL precursors; LD, limiting dilution.

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corroborated the evidence that CTL responses are involved in mediating immunity to *T. parva* and emphasized the need to understand the basis of the inter-animal variation in parasite strain specificity.

Materials and Methods

Animals

Nineteen female or castrated male Boran (*Bos indicus*) cattle, aged between 12 and 36 months, were used. These included two pairs of monozygotic twins born of dams implanted with split embryos. All cattle were reared indoors from birth under parasite-free conditions and at the outset of the study were clinically normal and negative for Ab to *T. parva*.

Class I MHC typing

The animals were selected on the basis of their class I MHC phenotype. PBMC and parasitized cell lines derived from the animals were typed for class I using specific alloantisera in a microlymphocytotoxicity assay essentially as described previously (16). The majority of the alloantisera used in the assay define class I MHC specificities encoded by one locus; these comprise specificities defined in international comparison tests utilizing *Bos taurus* animals (17) as well as locally defined specificities (prefix KN), some of which are confined to *B. indicus* animals. One specificity (KN104) has been shown to be encoded by a second class I locus (18, 19); this specificity, which is defined by an alloantiserum and a mAb, is frequently expressed along with the A locus molecule A10 in *B. indicus* cattle. Seventeen of the animals were the progeny of three sires: seven from sire 1422 (class I phenotype A7/A10,KN104), six from sire 103 (A10,KN104/A30), and four from sire 50 (A6/KN8). All animals expressed at least one of the three A locus specificities, A10, A6, and KN8, and in all cases KN104 was expressed along with A10. In *B. taurus*, A6 is a supertypic class I specificity within which the subtypes w17, A18, and A19 can be defined (17). These subtypes are not frequently identified in *B. indicus* animals. However, we have been able to subdivide $A6^+$ haplotypes in our population of *B. indicus* cattle on the basis of their reactivity with a class I-specific mAb, FJ101. Moreover, a set of *T. parva*-specific CTL clones restricted by products of an $A6^+$ haplotype were found to kill $A6^+FJ101^+$ but not $A6^+FJ101^-$ target cells (S. J. Kemp and W. I. Morrison, unpublished data). Of the eight $A6^+$ animals in the present study, six were $FJ101^+$.

Parasites and parasitized cell lines

Two immunologically distinct populations of *T. parva*, namely the Muguga stock (stabilate 3087) and a cloned subpopulation of the Marikebuni stock (stabilate 3219), were used for immunization and challenge of cattle and for establishment of parasitized cell lines. The Muguga stock appears to be antigenically homogeneous; Muguga-infected cell lines generally exhibit a constant profile with parasite-specific mAb (20, 21) and are consistently recognized by class I MHC-restricted Muguga-specific CTL clones (14). Furthermore, animals infected by inoculation with autologous cloned Muguga-infected cell lines are immune to challenge with the parent stock (22). By contrast, the parent Marikebuni stock has been shown, with parasite-specific mAb and DNA probes, to be genetically and antigenically heterogeneous (20, 23). Previous studies had demonstrated that a proportion of animals immunized with the Muguga stock were susceptible to challenge with the Marikebuni stock, whereas animals immunized with the Marikebuni stock were immune to challenge with either the Marikebuni or Muguga stock (24). The cloned population of Marikebuni was obtained by tick pickup of parasites from animals infected with cloned parasitized cell lines as described previously (15). In contrast to animals immunized with the parent stock, some animals immunized with Marikebuni 3219 were susceptible to challenge with Muguga (15).

Parasitized cell lines were established by infection of PBMC, isolated from peripheral blood by density gradient centrifugation, with *T. parva* sporozoites obtained from ground-up salivary glands dissected from infected adult *Rhipicephalus appendiculatus* ticks, as described previously (25). The cell lines were maintained in RPMI 1640 medium (Sigma Chemical Co., Poole Dorset, U.K.) supplemented with 10% (v/v) FCS (Life Technologies Ltd., Paisley, Scotland), 20 mM HEPES buffer (pH 7.2), 5×10^{-5} M 2-ME, 2 mM L-glutamine, and 50 μg of gentamicin per milliliter. All cell lines were tested by indirect immunofluorescence with a panel of parasite-specific mAbs that detect polymorphic determinants on parasite Ags (21), and by Western blotting with a mAb that detects size polymorphism in a schizont Ag (26). All of the cell lines derived with each parasite gave identical profiles, but the two sets of cell lines showed differences with both assays.

Immunization with Theileria parva

Thirteen (nine female and four male) of the cattle were immunized with *T. parva* (Muguga) and six (three female and three male) were immunized with the cloned population of Marikebuni 3219. The animals were allocated to the two groups primarily on the basis of MHC phenotype to facilitate comparison between groups of animals with identical or similar phenotype. The range of ages of animals within the two groups was similar. Immunization was achieved by infection with an LD_{100} of cryopreserved sporozoite stabilate administered s.c. and simultaneous treatment with long-acting oxytetracycline given by i.m. injection (27). Establishment of the immunizing infection was confirmed by the detection of an anti-schizont Ab response. Six months after immunization, eighteen of the animals were challenged, along with susceptible controls, with the heterologous parasite, again as an LD_{100} of sporozoites administered s.c. The animals were monitored clinically and parasitologically as described previously (15). Briefly, parasitosis (percent parasitized lymphocytes) was assessed in the regional and contralateral lymph nodes by microscopic examination of smears of aspirated lymph node cells stained with FITC-conjugated antiserum to *T. parva*. A rectal temperature of 39.5°C or higher, associated with schizont parasitosis, was considered a pyrexic response. Animals were considered to be immune when detectable parasitosis was confined to the regional lymph node.

Limiting-dilution assay for CTL

All cultures were performed in RPMI 1640 medium without HEPES (Sigma Chemical) supplemented with 10% (v/v) of a selected batch of heat-inactivated FCS, 5×10^{-5} M 2-ME, 2 mM L-glutamine, and 50 μg of gentamicin per milliliter. LD microcultures were established as described previously (13). Briefly, doubling dilutions of PBMC were distributed in multiple replicate wells of microtiter plates along with a fixed number of irradiated autologous parasitized lymphoblasts in culture medium containing bovine T cell growth factors. After culturing at 37°C for 7 days in an atmosphere of 5% CO_2 in air, the well contents were assayed for cytotoxic activity.

Cytotoxic T cell clones

T. parva-specific CTL clones were generated from cultures of PBMC stimulated three to five times in vitro in autologous *Theileria* MLC essentially as described previously (1, 25). Four CTL clones were isolated from PBMC of two animals, D580 and E81 (two from each), 3 mo after immunization with *T. parva* (Muguga). All four clones were $CD2^+$, $CD4^-$, $CD8^+$.

Cytotoxicity assays

Cytotoxicity assays were conducted in RPMI 1640 medium supplemented with 10% (v/v) of a batch of FCS screened to give low levels of spontaneous isotope release from labeled target cells. Target cells comprised lymphocytes infected with either parasite stock maintained as continuously growing cell lines. Cells used as targets were in the logarithmic phase of growth and were screened for class I MHC type before use.

Cytotoxicity generated in the LD microcultures was measured using an [^{111}In]indium oxine-release assay performed in V-bottom 96-well assay plates (Greiner, Nuertingen, Germany) as described previously (28). In some assays, individual cultures were split into two aliquots and assayed on different target cells (split-well assays). Cultures were scored as positive if they had isotope-release values above three times the SD of the mean of the background release values. The natural logarithm of the fraction of negative wells was plotted against the number of responder cells per well and the best linear fit to the data was obtained by computerized maximum likelihood analysis (29). The frequency of CTLp was then calculated directly from the gradient of the plot, and this conformed to the Poisson model of single-hit kinetics (30).

Killing activity of the CTL clones was tested in a standard 4-h [^{51}Cr]chromium-release assay conducted in half-area 96-well flat bottom culture plates (Costar, Cambridge, MA). The percentage of specific lysis was calculated by the equation: (experimental release − spontaneous release/maximum release − spontaneous release) × 100. Maximum release was evaluated by subjecting the target cells to two cycles of rapid freezing and slow thawing. Spontaneous release was obtained by incubating target cells in assay medium alone.

The capacity of mAbs specific for polymorphic or monomorphic determinants on bovine class I to inhibit cytotoxic activity of the CTL clones was tested, as part of a series of experiments to define their MHC restriction specificities. The mAbs were added to labeled target cells and incubated at room temperature for 30 min before adding the effector cells. The final concentration of mAbs in the assay represented 10- to 20-fold excess

Table I. *MHC restriction of the CTL response in cattle immunized with* Theileria parva *(Muguga) or* T. parva *(Marikebuni 3219)*

Animal	Sire	Class I MHC Phenotype[b]	CTLp Frequencies[a]								Immunity to Challenge[c]
			Self MHC target	MHC-mismatch target	MHC-matched targets						
					A10,KN104	KN8	A6	A7	KN12	KN18	KN103	
Muguga-immunized animals												
D580	1422	A10.KN104/KN8	3,090	–	2,550	–						+
E81	1422	A10.KN104/KN8	5,331	–	4,973	–						–
E118	1422	A10.KN104/KN8	14,735	–	15,318	–						–
F25	103	A10.KN104/KN8	9,765	–	10,000	–						–
E260	103	A10.KN104/A6^2	5,061	–	4,973		–					–
E292	1422	A10.KN104/A6^1	4,921	–	–		5,321					+
E45	1422	A10.KN104/KN103	3,090	–	2,550						–	–
E49	1422	A10.KN104/A7	2,199	–	2,731			2,394				+
E249	50	A6^1/KN8	8,300	–		–	7,083					–
E216	50	KN8/KN12	10,980	–		12,230			–			–
F304	102	KN8/KN18	9,570	–		–				10,124		–
D761	50	A6^1/A7	4,050	–			4,601	4,326				+
D717	nk	A6^1/A7	2,569	–			3,091	–				–
Marikebuni 3219-immunized animals												
F104[d]	103	A10.KN104	4,929	–								ND
E59	103	A10.KN104/KN8	4,100	–	5,051	–						–
E259	103	A10.KN104/A6^2	2,495	–	2,222		–					+
E82	1422	A10.KN104/KN8	4,100	–	5,051	–						–
D540	1422	A10.KN104/A6^1	3,785	–	–		4,011					+
E212	50	A6^1/KN8	3,290	–		–	3,850					+

[a] Results are expressed as the reciprocal of the CTL presursor frequencies calculated from LD analyses. Two LD microcultures were established from each animal, utilizing stimulator cells infected with the parasite used for immunization; the cultures were split and one assayed for cytotoxicity on autologous and MHC-mismatched targets, while the other was assayed on target cells matched for each MHC haplotype. Negative values (–) indicate that CTLp were not detectable at the maximal responder input of 2×10^4 cells/well.

[b] The MHC haplotype inherited from the sire is underlined where known. The A6 haplotypes are designated A6^1 or A6^2 based on positive or negative reactivity respectively with the mAb FJ101.

[c] Animals immunized with Muguga were challenged with Marikebuni 3219 and *vice versa*. Animals were considered to be immune (+) when only low levels of parasitized cells (<1%) were detected in the regional lymph node for less than 3 days.

[d] F104 is MHC-homozygous and was not assayed on MHC-matched targets.

* *nk, Not known.*

in relation to the end-point titers obtained with stained cells analyzed by flow cytometry. The following mAbs were used: IL-A19 (IgG_{2a}) recognizes a conserved determinant on bovine class I MHC molecules (31); IL-A4 (IgM) defines the KN104 class I specificity (32); IL-A10 (IgM) reacts only with determinants on the A10 molecule, in the population of *B. indicus* cattle studied, (A. J. Teale and S. J. Kemp, unpublished data); IL-A21 (IgG_{2a}) recognizes bovine class II MHC molecules (33).

Results

Protection against heterologous parasite challenge

Details of the parasite strain specificity of the CTL responses following immunization and the susceptibility of the animals to challenge with the heterologous parasite populations are reported elsewhere (15). The outcome of challenge is summarized in Table I. Four of the thirteen Muguga-immunized animals and three of the five Marikebuni 3219-immunized animals mounted cross-reactive CTL responses and were found to be immune to challenge. The remaining animals all had strain-specific CTL responses and developed moderate to severe clinical reactions following challenge.

There was no obvious correlation between the magnitude or strain specificity of the CTL responses and the age or sex of the animals.

MHC restriction of the CTL response is biased to one haplotype

The precursor frequencies of CTL capable of lysing autologous target cells or targets mismatched or matched with the effector cells, with respect to either class I MHC haplotype, were measured for each animal in two split-well LD microcultures. As shown in Table I, CTLp frequencies ranging from 1:14,735 to 1:2,199 were detected with autologous target cells. CTLp were not detectable with any of the targets that did not share class I specificities with the effectors. CTLp frequencies similar to those detected on autologous targets were obtained with MHC-matched targets, but in all except two animals (E49 and D761) the response was restricted entirely to one haplotype. Among the three most frequently represented MHC haplotypes expressing A6, KN8, or A10,KN104 specificities, there was a bias in the response to some in preference to others. Thus, in five of the six animals that were A6$^+$ FJ101$^+$ (A6^1), the response was completely restricted to this haplotype and in the sixth animal (D761), it was restricted by both haplotypes. In twelve animals that expressed A10 and KN104 on one haplotype, the response was completely directed to this haplotype in nine animals and was partially restricted by the same haplotype in another animal (E49). The two animals (E292 and D540) in which there was no response directed to the A10,KN104 haplotype both expressed A6 and the FJ101 determinant on the other haplotype. By contrast, only one (E216) of 10 animals expressing KN8 had CTLp restricted by the KN8-bearing haplotype.

Strain specificity and MHC restriction in A10$^+$,KN104$^+$ animals

Recent studies in which the genes that encode the A10 and KN104 specificities have been cloned and sequenced have demonstrated that the molecules bearing these specificities represent the products of distinct class I loci (18, 19). The observation that the CTL response in most of the A10$^+$,KN104$^+$ animals was biased toward

Table II. *Parasite strain and MHC restriction specificities of the CTL response after immunization with* T. parva *in animals expressing the A10 and KN104 class I specificities*

Animal	MHC Class I Phenotype	Strain Specificity of CTLp[a]		MHC Restriction of CTLp[a]	
		Muguga	Marikebuni	A10	KN104
Muguga-immunized					
E260[b]	A10,KN104/A6	6,302	–	–	7,128
E81[c]	A10,KN104/KN8	5,136	–	–	4,831
F25	A10,KN104/KN8	8,570	–	–	9,437
D580	A10,KN104/KN8	2,159	3,355	–	2,844
E45	A10,KN104/KN103	5,133	–	ND	ND
E118	A10,KN104/KN12	14,200	–	ND	ND
Marikebuni 3219-immunized					
E259[b]	A10,KN104/A6	2,867	2,071	–	3,344
E82[c]	A10,KN104/KN8	–	2,231	2,563	–
E59	A10,KN104/KN8	–	4,231	4,915	–
F104	A10,KN104/A10,KN104	3,271	4,110	3,711	8,191

[a] The results are expressed as the reciprocal of the frequency of CTLp calculated from LD analyses. Negative values (–) represent no detectable CTLp at a maximum responder input 2×10^4 cells/well. LD cultures were established with stimulator cells infected with the parasite used for immunization.
[b,c] Sets of identical twins.

this haplotype provided the opportunity to examine the relationship between the MHC restriction and parasite strain specificities of the response in these animals.

Two split-well LD microcultures were established from each animal and assayed for cytotoxicity on targets infected with Muguga or Marikebuni 3219 and targets expressing A10 or KN104, infected with the parasite strain used for immunization. The results obtained in 10 animals, six immunized with Muguga and four immunized with Marikebuni 3219, in which the CTL response was restricted entirely by the A10,KN104 haplotype, are presented in Table II. In five of the six Muguga-immunized animals, the CTL response was specific for Muguga-infected targets while the remaining animal (D580) exhibited a cross-reactive response. MHC restriction was analyzed in four of these animals, including animal D580, which exhibited a cross-reactive CTL response; in all four animals the response was restricted entirely by KN104.

These findings indicate that in $A10^+$,$KN104^+$ animals immunized with Muguga, the KN104 molecule is a dominant restricting element and in most animals presents strain-specific antigenic determinants; however, animal D580 was exceptional in that it mounted a KN104-restricted, cross-reactive response. To confirm that the response measured by the LD microassay reflected the specificities of the T cells at the clonal level, CTL clones were generated from two animals (E81 and D580), one of which gave a strain-specific and the other a cross-reactive CTL response. The parasite strain and MHC restriction specificities of two CTL clones from each animal were analyzed; representative results are presented in Figure 1. Based on cytotoxicity on target cells of different class I MHC phenotype and susceptibility to blocking of cytotoxicity by mAbs specific for A10 or KN104, all four clones were shown to be restricted by KN104. The two clones derived from D580 killed target cells infected with either parasite population whereas those derived from E81 were specific for Muguga-infected targets. Thus, these two animals responded to different antigenic epitopes, one specific to Muguga and the other conserved between the two parasites, presented by the same class I molecule.

In two of the four animals immunized with Marikebuni 3219, the CTL were specific for the immunizing parasite, while in the other two (E259 and F104), they killed targets infected with either

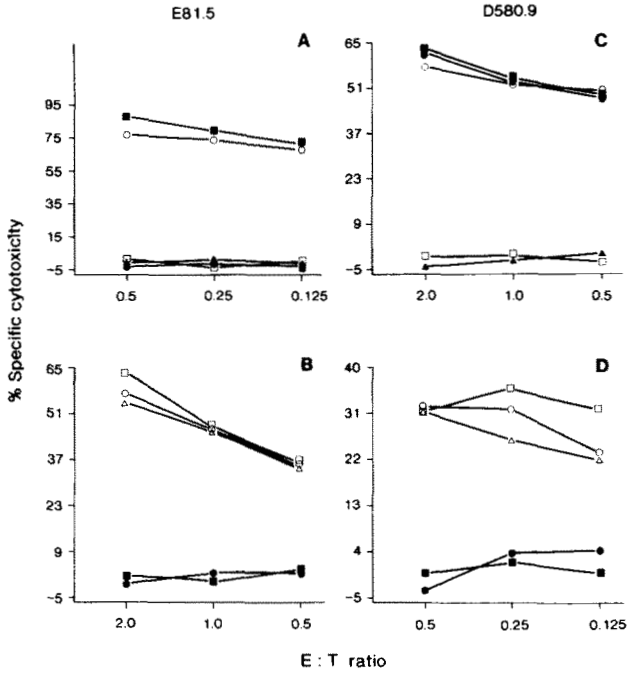

FIGURE 1. Parasite strain and MHC restriction specificities of two CTL clones, E81.5 and D580.9, derived from animals E81 (A10,KN104/KN8) and D580 (A10,KN104/KN8), respectively, are shown in *A* and *C*. Cytotoxicity was analyzed on autologous cells infected with the Muguga (○) or Marikebuni 3219 (●) populations of *T. parva* to determine strain specificity of the clones. Cytotoxicity was analyzed on Muguga-infected target cells expressing either the A10 (□) or KN104 (■) class I molecules or unrelated class I molecules (▲) to determine the class I MHC restriction specificity of the clones. The results of experiments to confirm the MHC restriction specificities of the clones by blocking of target cell lysis with class I-specific mAbs are shown in *B* and *D*. Cytotoxicity was analyzed on autologous target cells infected with Muguga. The following mAbs were used: ○, no mAb; ●, IL-A19 (recognizes a class I monomorphic determinant); ■, IL-A4 (specific for a KN104-associated epitope); □, IL-A10 (recognizes an A10-associated epitope); △, IL-A21 (specific for a class II monomorphic determinant).

Table III. *Comparison of the parasite strain-specificity of the CTL response in* T. parva *(Muguga)-immunized animals pre- and post-challenge with* T. parva *(Marikebuni 3219)*

	Strain Specificity of CTLp[a]			
	Pre-challenge		Post-challenge	
Animal	Muguga	Marikebuni	Muguga	Marikebuni
E81	4,903	–	3,700	3,498
F25	8,337	–	4,730	4,500

[a] Results are expressed as the reciprocal of the frequency of CTLp calculated from LD analyses. LD microcultures established pre- and post-challenge both utilized Muguga-infected stimulator cells.

Table IV. *Comparison of the MHC restriction specificities of the CTL response in* T. parva *(Muguga)-immunized animals pre- and post challenge with* T. parva *(Marikebuni 3219)*

	Pre-Challenge[a] CTLp		Post-Challenge[a] CTLp			
	Muguga-infected stimulator and target cells		Muguga-infected stimulator and target cells		Marikebuni-infected stimulator and target cells	
Animal	A10	KN104	A10	KN104	A10	KN104
E81	–	4,831	–	3,572	1,783	3,289
F25	–	9,437	–	5,513	4,030	5,731

[a] Results are expressed as the reciprocal of the frequency of CTLp calculated from LD analyses.

parasite. In contrast to the strain-specific responses to Muguga, which were KN104-restricted, the Marikebuni 3219-specific responses in both animals (E59 and E82) were restricted entirely by A10. The cross-reactive response was restricted by KN104 in one animal (E259) and in the other (F104) by both KN104 and A10.

Comparison of the responses to different parasites in identical twins

The experiment for which results are presented in Table II included two sets of identical twins; the co-twins of each set had been immunized with the different parasite populations. In one set of twins, E81 and E82, the CTL response was specific for the parasite used for immunization in both animals and in the Muguga-immunized twin the response was KN104 restricted while in the Marikebuni 3219-immunized twin the response was A10 restricted. This result confirms that the difference in MHC restriction is a function of the parasite used for immunization rather than being due to individual animal variation. In the second set of twins, E259 and E260, the CTL response was strain specific in the Muguga-immunized animal and cross-reactive in the Marikebuni 3219-immunized animal; however, the response in both animals was restricted entirely by KN104. Given that these animals are genetically identical, this finding indicates that animal E260 mounted a strain-specific response despite having the genetic capability of responding to epitopes common to the two parasites.

Specificity of CTL responses following challenge with the heterologous parasite

Many of the animals that developed strain-specific CTL responses to Muguga after challenge with Marikebuni 3219 underwent severe clinical reactions but recovered; thus, they provided the opportunity to compare the responses before and after heterologous challenge. The results of split-well LD analyses to examine the strain specificity of the responses in two animals are presented in Table III. Muguga-infected cells were used as stimulator cells before and after challenge. In both animals, CTL capable of killing both parasite populations were elicited by stimulation with Muguga-infected cells following challenge with Marikebuni 3219. Comparable split-well assays established to examine the role of A10 and KN104 class I molecules in restricting the response (Table IV) demonstrated that, as with the strain-specific CTL detected before challenge, the cross-reactive CTL detected following challenge were KN104 restricted. When Marikebuni 3219-infected stimulator cells were used in the LD microcultures established following challenge, CTLp restricted by both KN104 and A10 were detected, indicating the presence of an A10-restricted Marikebuni 3219-specific population of CTL. These findings further emphasize the role of the parasite in determining the MHC restriction of the response and demonstrate that animals that mounted a strain-specific CTL response to the Muguga parasite are inherently capable of responding to antigenic determinants shared by the two parasite populations.

Discussion

The principal aim of this study was to define the basis of the observed variation between animals in the parasite strain specificity of CTL responses to *T. parva*. The study utilized a group of cattle in which the strain specificity of the response had been shown to correlate with immune status following challenge with heterologous parasites. In view of the well-established influence of the MHC on selection of antigenic epitopes in laboratory animal models, efforts were focused on defining the role of class I MHC phenotype in determining strain specificity.

The initial experiments demonstrated that, in most of the animals, the CTL response was restricted entirely by the products of one MHC haplotype and, where tested, to one MHC molecule. Among the most frequently represented haplotypes, there was a hierarchy in dominance, A6 ($FJ101^+$) being dominant over A10,KN104 and both of these being dominant over KN8. These findings are consistent with results of previous, less detailed analyses of MHC restriction of the response (12).

A more precise definition of the MHC restriction was conducted in animals expressing A10 and KN104, ten of which exhibited a CTL response that was restricted entirely by this haplotype. The genes encoding these specificities have been cloned and shown to represent distinct loci (18, 19). Five of six $A10^+$,$KN104^+$ animals immunized with the Muguga parasite exhibited a strain-specific response and in four animals in which MHC restriction was examined, the response was restricted entirely by KN104. These results, together with previous findings that KN104-restricted CTL clones from Muguga-immunized animals were all parasite strain specific (14, 34, 35), indicate a strong association of KN104 with strain-specific responses to the Muguga parasite. However, this association is clearly not absolute since one animal (D580) had a strong KN104-restricted cross-reactive CTL response. The veracity of this result is reinforced by the finding that this animal also was solidly immune to challenge with Marikebuni 3219. Since animal D580 inherited the A10,KN104 haplotype from the same sire as several of the other animals that displayed strain-specific responses, and its parasitized cells were recognized by strain-specific KN104-restricted CTL, the difference in specificity is unlikely to be due to polymorphism in the KN104 molecule. The detection of the Muguga-specific epitope on the D580 cell line also argues against a difference in Ag processing, such as has been described in alloreactive responses in rats as a consequence of polymorphism in their peptide transporter molecules (36). A more likely explanation is that the TCR repertoire differed in this animal, such that

the affinity or frequency of CTLp specific for the Muguga-specific epitope presented by KN104 was lower than in the other animals. It is well established that MHC molecules other than the restricting element, as well as minor histocompatibility Ags, can influence the composition of the TCR repertoire and thus the fine specificity of T cell responses (37–39).

In contrast to the KN104 restriction of strain-specific CTL elicited by the Muguga parasite, strain-specific CTL responses in two animals immunized with Marikebuni 3219 were restricted by A10. The fact that this difference was found in identical twin calves immunized with different parasites indicates that the parasite population used for immunization has a strong influence on the MHC restriction and hence the precise antigenic specificity of the response.

In view of the antigenic complexity of *T. parva* and its site of replication within the cytoplasm of host cells it might be expected that a variety of antigenic peptides would be available for association with class I molecules and recognition by $CD8^+$ T cells. However, the findings that the CTL response was frequently restricted by a single class I molecule and, in many animals, was parasite strain specific, imply that the response is focused on a limited number of antigenic determinants in each animal. This view is supported by analyses of CTL clones, which have shown that panels of clones derived from individual animals usually fall into one or two predominant groups based on their MHC and parasite strain specificities and patterns of killing (5, 14). Focusing of CTL on individual class I restriction elements and on a limited number of antigenic epitopes has also been observed in responses to viral infections in mice and humans (40–42) and, in some instances, has been shown to influence virus strain specificity (43).

Perhaps the most significant finding in the present study was that animals that mounted a parasite strain-specific CTL response were shown to be capable of responding to antigenic determinants shared between the two parasite populations. This conclusion is based on two observations. First, animals in which immunization with the Muguga parasite resulted in strain-specific CTL, developed cross-reactive CTL restricted by the same MHC molecule following recovery from challenge with Marikebuni 3219. Second, the CTL response in identical twin calves immunized with different parasites was KN104 restricted in both animals but was parasite strain specific in the Muguga-immunized twin and cross-reactive in the Marikebuni 3219-immunized twin. These results indicate that the variation in strain specificity of the response is not due to conventional immune response genes that determine the inherent capability of animals to respond to particular antigenic determinants. Rather, they imply that there is a hierarchy in dominance of different MHC-peptide combinations and that the variation in specificity in different animals is a consequence of the response being focused on a few of the most dominant determinants in each animal. This model is consistent with the notion that both the MHC of the animal and the antigenic composition of the parasite used for immunization will influence the specificity of the response. The concept of immunodominance within a hierarchy of antigenic determinants is not new. In early studies of immune response gene control of CTL responses to viral infections in mice, Zinkernagel et al. (40) proposed "immunodominance" as an explanation for the lack of a response to a particular MHC-viral Ag combination in F1 mice, despite the detection of a response in the parent strain and the demonstration that T cells capable of recognizing the determinant were present in the nonresponder F1 mice. Further evidence to support the concept of immunodominance has come from studies of CTL responses to minor histocompatibility Ags (44) and flaviviruses (45) in mice. The data from the present study provide the first evidence that immunodominance exerts an influence on the specificity of CTL responses to protozoan parasites.

The question of why certain "less dominant" antigenic determinants fail to induce a detectable CTL response is a key issue. The efficacy of induction of $CD8^+$ T cell responses to a particular antigenic determinant depends on the precursor frequency and avidity of T cells capable of responding to the Ag and the concentration of the antigenic peptide bound by the presenting MHC molecule. Given the evidence that the animals immunized with *T. parva* were capable of mounting a vigorous response to a common parasite epitope presented by KN104, it seems more likely that the relative dominance of epitopes is determined by some form of Ag competition at the level of the APC. Assuming the presence of similar frequencies of T cell precursors specific for different antigenic determinants, the relative quantities of peptide bound by different class I molecules would be critical in determining the specificity of the response. Competition could occur in the association of different peptides with the same MHC molecule or between T cells of different specificities at the level of recognition of parasitized cells. Clearly, antigenic peptides generated in abundance during the early stages of infection would be favored. Differences between parasite strains in the expression of antigenic proteins could also influence which determinants are immunodominant. Killing of parasitized cells by the dominant component of the CTL response, by rapidly reducing the pool of APCs, may be a significant factor in the failure to achieve activation of T cells that recognize the less dominant epitopes. Experiments to resolve these issues will only be possible once the target parasite Ags have been identified.

The results of this study have important implications for vaccine development strategies. There is now strong experimental evidence that class I MHC-restricted CTL responses play an important role in immunity to *T. parva*. In the past, the strain specificity of these responses has been seen as a significant obstacle to future attempts to exploit CTL target Ags for vaccination. However, our findings indicate that many, if not all, animals may be capable of responding to a wider range of epitopes than occurs during a single infection with the parasite. Consequently, it may be possible, in designing a vaccine, to induce responses preferentially against conserved epitopes or to present the Ags in such a way that responses are induced against a wider variety of epitopes than occurs during infection, thus achieving protection against the majority of parasite strains.

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