

The non-structural proteins of bluetongue virus are a dominant source of cytotoxic T cell peptide determinants

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Virus-specific, CD8⁺ cytotoxic T lymphocytes (CTLs) were generated in two strains of mice (BALB/c, CBA/Ca) against bluetongue virus serotype 10 (BTV-10). Recombinant vaccinia viruses (VV) expressing the individual structural and non-structural proteins of BTV were used to infect syngeneic target cells. We found that in both BALB/c (*H-2^d*) and CBA/Ca (*H-2^k*) mice, polyclonal CTL populations recognized target cells expressing the non-structural proteins better than those expressing the structural proteins. CTLs generated against other BTV serotypes also predominantly recognized the non-structural proteins. However, the extent

of cross-reactivity was dependent on the *H-2* background of the animals immunized. No CTLs cross-reactive to the BTV-10 heterotype were demonstrated with the panel of molecularly cloned recombinants in the *H-2^d* haplotype. The outer capsid proteins VP2 and VP5 which vary considerably between serotypes were not recognized by heterotypic CTLs. Using this murine model we have determined which BTV proteins are the major targets of the CTL response. The implications for the design and development of subunit vaccines are discussed.

Introduction

Bluetongue (BT) is an arthropod-borne viral disease of sheep and other domestic and wild ruminants. BTV (genus *Orbivirus*, family *Reoviridae*) was initially isolated in South Africa and has subsequently been isolated in both tropical and subtropical regions of the world including the Far and Middle East, North and South America and Australia. The virus is transmitted by biting gnats of the genus *Culicoides*. To date, 24 serotypes have been identified by neutralization tests (Obdeyn, 1987). The BTV genome consists of 10 segments of double-stranded RNA, which is enclosed by a complex capsid structure consisting of a nucleocapsid containing five proteins (major, VP3 and VP7; minor, VP1, VP4 and VP6) which is surrounded by an outer capsid structure containing two major proteins (VP2 and VP5). In addition to the seven structural proteins, three non-

structural (NS) proteins, NS1, NS2 and NS3 are synthesized in infected cells (Roy, 1989). Amongst BTV serotypes the genes coding for the non-structural proteins and the internal proteins are highly conserved, whereas the outer capsid proteins, VP2 and VP5, vary considerably (Ritter & Roy, 1988).

Over 70% of the world sheep population is in BTV endemic areas and although sporadic epizootics do not normally give rise to high mortality rates they can account for high morbidity rates (Parsonson, 1992). Modified live-attenuated vaccine strains of BTV have been developed for use in the Republic of South Africa and in the United States of America. In South Africa sheep are vaccinated two to three times with pentavalent live-attenuated vaccine at 3 week intervals and in the USA, although five serotypes have been identified (BTV-2, -10, -11, -13 and -17), a modified live virus vaccine is available only for BTV-10. Conventional live-attenuated vaccines have certain inherent disadvantages as they can cause fetal infection with teratogenic consequences. Moreover, interference can occur between BTV serotypes, resulting in the development of incomplete immunity. In view of the importance of the disease to animal health and the export industries of the world involving international movement of animals or germplasm, safe and effective non-infectious vaccines are required.

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Studies to delineate the repertoire of immune responses associated with protective immunity and virus clearance in BTV infection have been impeded by the inconsistency in experimentally reproducing clinical disease in sheep. Therefore most investigators currently use development, titre and duration of post-challenge viraemia as a primary correlation of protective immunity (Stott *et al.*, 1985a). During natural infections, protection against reinfection is provided by neutralizing antibodies, either directly or by the rapid response of memory B and T helper cells. There is some evidence to support the hypothesis that BTV-specific cytotoxic T lymphocytes (CTLs) may play a role in protection against BTV infection. Inactivated vaccine preparations can confer protection in the absence of detectable levels of neutralizing antibodies (Stott *et al.*, 1979) and adoptive transfer of BTV-specific CTLs to monozygotic sheep has been shown to confer partial protection (Jeggo *et al.*, 1984, 1985). Furthermore, in a laboratory-based mouse model, BTV-specific cross-reactive CTLs have been demonstrated (Jeggo & Wardley, 1982). These data are also supported by *in vitro* studies. BTV antigen-specific ovine T cell lines have been shown to induce cross-reactive stimulation by a number of different BTV serotypes (Ghalib *et al.*, 1985a, b; Stott *et al.*, 1979). Takamatsu & Jeggo (1989) showed that some CTL lines could reduce BTV replication in autologous skin fibroblast cells.

We describe in this paper the generation of serotype cross-reactive, BTV-specific CTLs in mice of the *H-2^k* major histocompatibility complex (MHC) class I haplotype. Using vaccinia virus (VV) recombinants that individually express each of the ten BTV gene products, we show that both the structural and non-structural proteins are targets for lysis by homotypic CTLs. Statistical analysis of the data indicates that non-structural protein peptides are the predominant source of homotypic and heterotypic CTL recognition.

Methods

Cell lines and viruses. BHK-21 cells were grown as monolayers in Eagle's minimal essential medium containing 10% fetal bovine serum (FBS) at 37 °C in 5% CO₂. Target cells, P815 (*H-2^d*) and L929 (*H-2^k*) were obtained from the Sir William Dunn School of Pathology, Oxford, UK. Both cell lines were propagated in RPMI 1640 medium containing 10% FBS (RPMI-10) and maintained as above.

United States prototype isolates of BTV-2, -10 and -17, and Australian BTV-15, were originally obtained from the Animal Disease Research Laboratory, Denver, Colorado, USA, or the Veterinary Research Institute, Onderstepoort, South Africa, respectively. Each of the viruses was plaque cloned, using monolayers of BHK-21 cells and the serotypes were verified by monotypic serum neutralization tests.

Recombinant VV expressing the full-length VP3 gene of BTV-17 and the other nine full-length genes of BTV-10 have been generated in our laboratory. Recombinant VV were generated using either the transfer plasmid pSC11-BgIII (kindly provided by J. C. S. Clegg, CAMR, Salisbury, UK) or pSC11-SmaI (kindly provided by B. Moss,

Laboratory of Viral Diseases, Bethesda, USA). Both of these transfer plasmids contain a moderate-strength compound early/late promoter derived from a gene encoding a 7.5 kDa protein which is expressed both early and late in VV infection. VP1 was inserted into the *BgIII* site and the remaining BTV genes into the *SmaI* site. The DNA was digested with either *BgIII* or *SmaI* and cloned into the pSCII vectors using standard methods (Sambrook *et al.*, 1989). Recombinant plasmids in the correct transcriptional orientation were selected by restriction analysis and verified by sequencing across both the *BgIII* or *SmaI* junctions. Transfection and selection of recombinant VV were undertaken as previously described (Shiu *et al.*, 1992). Wild-type VV (strain WR) was used to generate all the recombinant viruses. Recombinant viruses were plaque purified three times in selective growth medium prior to production of high titre stocks. Expression of the BTV proteins was shown by indirect immunofluorescence antibody test (IFAT) and Western blot analysis. The levels of expression of each BTV protein by recombinant VV were relatively low in comparison to the proteins synthesized in BTV-infected cells. However, synthesis of all ten proteins was comparable (manuscript in preparation).

CTL generation and assays. Female BALB/c (*H-2^d*) and CBA/Ca (*H-2^k*) mice, age 8–12 weeks old (obtained from Harlan Olac UK, Bicester, UK), were inoculated intraperitoneally with 7.0 log₁₀ p.f.u. BTV serotypes -2, -10, -15, or -17. Mice were sacrificed 7 days post-inoculation and spleens were removed. Splenocytes were prepared using standard methods (Offit *et al.*, 1991) and resuspended in RPMI-10 containing 10 mM-HEPES, 100 U/ml penicillin, 100 µg streptomycin, 0.03% glutamine and 30 µM-2-mercaptoethanol (RPMI CM). Splenocytes (putative effector cells) from immunized mice were co-cultured for 7 days with syngeneic BTV-infected γ -irradiated (20 Gy) spleen cells from non-immunized mice. Antigen presenting cells (APC) were infected with the immunizing virus at an m.o.i. of 10 p.f.u./cell and co-cultivated with effector cells at a ratio of 1:2. Effector cells (pooled from 6 mice) and APC were resuspended in RPMI CM and incubated at 37 °C in 5% CO₂. Target cells (P815, L929) were either mock-infected or infected with recombinant VV or homologous BTV serotypes at an m.o.i. of 10 p.f.u./cell, or 50 p.f.u./cell, for 2 h or 24 h, respectively, at 37 °C. Cells were washed twice in RPMI-10 and resuspended to a final concentration of 5 × 10⁴ cells/ml. Infection of the cells was confirmed by IFAT analysis.

Putative effector cells from each bulk culture were tested in a non-radioactive cytotoxicity assay (Cytotox 96; Promega). This assay is a colorimetric alternative to ⁵¹Cr release cytotoxicity assays and quantitatively measures lactate dehydrogenase (LDH), a stable cytosolic enzyme that is released upon cell lysis. Released LDH in culture supernatants is measured with a 30 min coupled enzyme assay which results in the conversion of tetrazolium salt to a red formazan product.

Target cells (100 µl) were added to triplicate wells in a 96-well plate for testing at effector:target (E:T) cell ratios of 40:1, 20:1 and 10:1. Target cell spontaneous release was measured by adding the same concentration of target cells to triplicate sets of wells containing 100 µl of culture medium. To measure the maximum target cell LDH release, 20 µl of lysis buffer (Cytotox 96; Promega) was added to triplicate wells containing target cells, 45 min prior to harvesting the supernatants. Spontaneous release in the absence of CTLs was < 15% of maximal release by lysis buffer in all experiments. Determination of spontaneous release of LDH from effector cells was assessed by adding 100 µl of each cell concentration to triplicate wells containing 100 µl of culture medium. Triplicate sets of wells containing 200 µl of culture medium were also included as a control to correct for contributions caused by phenol red and LDH activity present in culture medium containing serum. A further control to correct for the volume increase when measuring maximum LDH from target cells was included: 20 µl of lysis buffer was added to triplicate sets of wells containing 200 µl of culture medium 45 min prior to harvesting the supernatant and incubation.

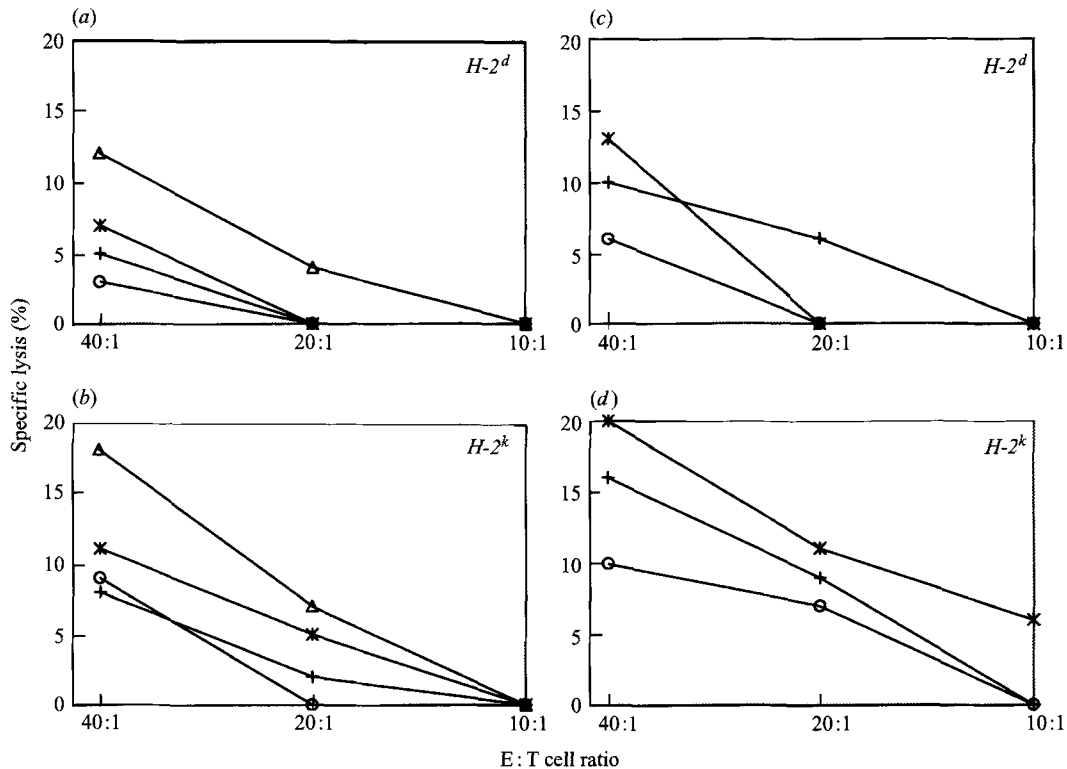


Fig. 1. Lymphocyte effector cells derived from mice inoculated with either BTV serotype -2 (○), -10 (△), -15 (+) or -17 (*) were prepared as in Methods. Target cells infected for 24 h with 50 p.f.u./cell of either BTV10 (a and c) or homologous virus (b and d) were incubated with effector cells for 4 h in a non-radioactive cytotoxicity assay.

Cytotoxicity assay plates were centrifuged at 275 *g* for 4 min prior to incubation at 37 °C in 5% CO₂ for 4 h. Plates were then centrifuged at 275 *g* for 4 min and 50 µl aliquots from all wells were transferred to fresh 96-well plates prior to addition of 50 µl of substrate buffer to each well. Plates were covered in silver foil to protect them from light and incubated for 30 min at room temperature, prior to addition of 50 µl of the stop buffer (Cytotox 96; Promega). *A*₄₉₂ values were recorded within 1 h of the addition of the stop solution.

Results were calculated as follows: the average absorbance values from the culture medium background were subtracted from all absorbance values except those obtained for the target cell maximum LDH release. In these, the average absorbance values from the volume correction controls were subtracted. Using the corrected values, the following formula was used to compute the percent cytotoxicity for each E:T cell ratio: Cytotoxicity (%) = $\frac{\{(\text{Experimental} - \text{Effector spontaneous}) - \text{Target spontaneous}\}}{\{\text{Target maximum} - \text{Target spontaneous}\}} \times 100$. In all experiments samples were run in triplicate and showed a variance of less than 10%. The data in Tables 1 and 2 represent three separate sets of experiments using animals inoculated with each BTV strain.

Antibody-complement depletion. Anti-L3T4 (CD4) and anti-Lyt2 (CD8) monoclonal antibodies (MAb) (Serotec, Oxford, UK) were used in antibody-complement depletion studies. Virus-stimulated effector cells (50 µl; 1×10^6) were added to 100 µl of antibody (neat) and incubated for 30 min at 4 °C. Cells were washed twice in cold RPMI 1640, resuspended in 100 µl of media and 200 µl of rabbit complement (diluted 1:3; Sigma, UK) and incubated at 37 °C for 1 h. Cells were then washed three times in RPMI 1640 and tested in a non-radioactive cytotoxicity assay at an E:T cell ratio of 40:1. In separate experiments, non-immune spleen cell preparations were treated as above with MAb and complement. When analysed by direct fluorescence microscopy,

less than 3% of the cell populations i.e. CD4 or CD8 were present after treatment with the corresponding MAb.

Statistical analysis. Analysis consisted of fitting all of the factors and their interactions to a series of linear models to explain data variability, using GLIM (Royal Statistical Society; Aitkin *et al.*, 1989). An ANOVA was carried out on the percentage lysis using an arcsine transform with normal errors, with four variables in a fully factorial design, where haplotype (2 levels), serotype (4 levels) and viral protein (10 levels) were the specified factors and E:T cell ratio was a continuous variable.

Results

Lysis of target cells expressing BTV-10 proteins by CTL generated against homotypic and heterotypic BTV serotypes

Initial studies were undertaken to determine the extent of cross-reactivity of BTV-specific CTLs. Lymphocytes derived from mice inoculated with BTV serotype -2, -10, -15 or -17 were assayed for their capacity to lyse target cells infected with homotypic or heterotypic BTV (Fig. 1). The data obtained represent the results of three separate experiments.

Lysis values were assessed for the 40:1 E:T cell ratio value, as analysis by IFAT revealed that only 25–35% of target cells infected *in vitro* showed positive fluorescence at 24 h post-infection (data not shown). Overall, there

Table 1. ANOVA table of GLIM-fitted model (with scaled binomial error structure)

Source	SS*	df†	MS‡	F§
Haplotype	135.3	1	135.3	112.94
Serotype	257.5	3	85.8	71.64
Virus protein	299.4	9	33.3	27.77
Serotype × Haplotype	84.0	3	28.0	23.37
Serotype × Virus protein	291.5	27	10.8	9.01
Virus protein × Haplotype	20.2	9	2.24	NS
Haplotype × Serotype × Virus protein	43.5	27	1.34	NS
Error	191.6	160	1.20	
Total	1323	239		

* Sum of squares

† Degrees of freedom

‡ Mean of squares

§ F values. Data shown in bold type are significant ($P < 0.001$). NS, Not significant.

were no significant differences in the lysis values for all BTV infections combined for the two cell types [$F_{1,59} = 3.52$, NS]. However, homotypic CTLs consistently elicited the highest response in terms of cell lysis. Control target cells infected with either VV-TK⁻ or mock-infected, were not lysed by the effector cell populations (0% lysis).

Lymphocytes derived from the inoculated mice were also assayed for their capacity to lyse target cells infected with recombinant VV expressing specific BTV-10 proteins. The percentage target cell responses were analysed for the following E:T cell ratios i.e. 10:1, 20:1 and 40:1 using normal errors. The percentage cell lysis was found to depend upon the following terms: cell + serotype + protein + ratio + cell.serotype + cell.protein + serotype.protein + cell.ratio + serotype.ratio + protein.ratio + cell.serotype.protein. This model was simplified by grouping

together those proteins which were not significantly different, i.e. the outer capsid proteins VP2 and VP5 and the inner capsid protein VP7 which formed one distinct group (comparing the two models $F_{18,626} = 1.48$, NS). Thus the final model contained the same terms, but the variable protein was a factor with eight group levels instead of ten; these factors accounted for 82% of variance in cell lysis (Tables 1, 2). Percentage lysis values were arcsine transformed and a haplotype + serotype model fitted to give the mean lysis values for each protein or group of proteins (Table 1).

In contrast to target cells infected *in vitro* with BTV serotypes, high levels of positive fluorescence were observed (95% of the cell population) with target cells infected with recombinant VV at 2 h p.i. (data not shown). It was clear that for all of the proteins, lysis values were greatly reduced in the *H-2^d* haplotype mice compared to the *H-2^k* haplotype. The major targets for lysis by CTLs were the non-structural proteins, followed by the minor core proteins, the inner capsid protein VP3 and lastly the outer capsid proteins VP2 and VP5 and the inner capsid protein VP7; control target cells infected with either VV-TK⁻ or mock-infected, were not lysed by the effector cell populations (0% lysis). This trend was consistent in both haplotypes of mice (Table 1). BTV-10, i.e. homotypic CTLs consistently elicited the highest response in terms of cell lysis.

Characterization of cytotoxic activity

Characterization of cytotoxic activity was investigated at the target cell level, by infecting target cells with BTV-10 and generating homotypic immune effector cells. Treat-

Table 2. Cytotoxicity for each BTV serotype protein combination

Percentage lysis means ($\pm 95\%$ confidence limits) at 20:1 E:T cell ratio for each serotype viral protein combination generated using a minimal significant GLIM model with scaled binomial errors. The virus control mean percentage lysis values at a 40:1 E:T cell ratio are also presented from a separate analysis

Protein	<i>H-2^k</i> Type 2	<i>H-2^k</i> Type 10	<i>H-2^k</i> Type 15	<i>H-2^k</i> Type 17	<i>H-2^d</i> Type 2	<i>H-2^d</i> Type 10	<i>H-2^d</i> Type 15	<i>H-2^d</i> Type 17
VP1	32.1 (47-20)	22.3 (40-11)	4.9 (19-1)	31.8 (47-16)	2.2 (8-1)	16.7 (43-5)	0	0
VP2	0	12.0*	0	0	0	8.7 (26-3)	0	0
VP3	10.4 (26-4)	18.2 (47-5)	0	61.6 (87-29)	0	13.5 (37-4)	0	9.1*
VP4	18.6 (37-8)	44.3 (72-20)	13.9 (51-2)	7.2 (28-2)	0	35.7 (67-13)	0	0
VP5	0	12.3*	0	0	0	0	0	0
VP6	9.5 (25-3)	28.9 (61-9)	0	0	0	22.1 (51-7)	0	0
VP7	0	7.0*	0	9.3*	0	5.0 (16-1)	0	0
NS1	14.2 (32-6)	71.9 (90-43)	58.6 (90-19.0)	1.8 (18-1)	0	83.6 (95-58)	0	0
NS2	0	53.3*	65.2*	34.5*	0	44.3 (75-18)	0	0
NS3	15.8 (34-7)	58.1 (82-29)	33.9 (76-8)	21.6 (52-7)	0	49.2 (80-22)	0	0
Virus controls (40:1)								
BTV-10	8.52 (13-6)	18.17 (26-12)	8.02 (12-5)	11.31 (17-7)	5.49 (10-3)	12.17 (23-6)	5.16 (12-2)	7.36 (16-3)
BTV-2	9.52 (14-6)				6.16 (16-2)			
BTV-15			15.64 (23-10)				10.37 (23-4)	
BTV-17				19.53 (28-13)				13.15 (27-6)

* It was not possible to generate confidence intervals for these means in the combination generated from the minimal significant GLIM model specified.

Table 3. Characterization of cytotoxic activity

E:T cell ratio was 40:1 in 4 h assay. The means of triplicate cultures are shown. S.E. did not exceed 2.4%.

Haplotype	BTV-10-stimulated splenocyte treatment	Specific lysis from BTV-10-infected target cells (%)	Inhibition (%)
<i>H-2^d</i>	Complement	31	
	anti-CD8 + Complement	4	87
	anti-CD4 + Complement	31	0
<i>H-2^k</i>	Complement	27	
	anti-CD8 + Complement	5	81
	anti-CD4 + Complement	26	4

ment of the effector cell populations with anti-CD8 antibody and complement reduced the lytic capacity against BTV-10-infected P815 and L929 target cells by 87% and 81%, respectively. However, when effector cell populations were treated with anti-CD4 and complement, no reduction in their cytotoxic capacity was observed (Table 3). Thus a significant decrease in specific lysis of infected target cells compared with controls, i.e. treated only with complement, was observed. Mock-infected cells were only weakly lysed by the effector cell population (< 2%). This demonstrates that the effector cells are conventional $\text{Lyt}2^+ \text{L3T}4^- (\text{CD}8^+ \text{CD}4^-)$ CTLs.

Discussion

BTV-specific $\text{CD}8^+$ CTLs were generated following immunization of two different strains of mice with different BTV serotypes. We found that the non-structural proteins were the predominant antigens, both in the homotypic responses to BTV-10 and, where observed, in the heterotypic responses. Cross-reactivity to individual recombinant proteins was *H-2* dependent. Using target cells infected with recombinant VV synthesizing individual BTV proteins, it was found that homotypic CTLs recognized target cells synthesizing both the structural and non-structural proteins of BTV. The non-structural proteins dominated the CTL response in both BALB/c and CBA/Ca mice, followed by the minor core, inner capsid protein VP3 and finally the inner capsid protein VP7 and the outer capsid proteins VP2 and VP5. Our findings are in accordance with results obtained from studies with flaviviruses (Hill *et al.*, 1992; Lobigs *et al.*, 1994; Parrish *et al.*, 1991; Rothman *et al.*, 1993), murine cytomegalovirus (Del Val *et al.*, 1991) and herpes simplex virus (Banks *et al.*, 1991; Martin *et al.*, 1988) which also found the non-structural proteins to be the dominant source of T cell determinants. The data provide further support for the concept that proteins with an intracellular location are a major source of T cell antigenic peptides (Townsend & Bodmer, 1989). In contrast, CTLs generated in mice orally inoculated with rotaviruses have been reported to

recognize target cells synthesizing the outer protein VP7 better than those synthesizing the outer capsid protein VP4, or the inner capsid protein VP6 (Offit *et al.*, 1991). Furthermore, rotavirus-specific CTLs were only observed when mice were inoculated with recombinant VV synthesizing VP7; inoculation of mice with recombinants synthesizing the other structural and non-structural proteins did not elicit a significant response (Offit *et al.*, 1994).

As shown first by Jeggo & Wardley (1982), we have demonstrated that BTV-specific CTLs cross-react with target cells infected with different BTV serotypes. This was observed for both murine haplotypes studied. Unexpectedly, when VV recombinants synthesizing individual BTV-10 proteins were used to infect target cells, broad cross-reactivity was only observed in mice of the *H-2^k* background. Despite cross-recognition of targets infected with whole BTV-10, mice with the *H-2^d* background failed to cross-react with the individual BTV-10 proteins. Studies with lymphocytic choriomeningitis virus (LCMV) have also demonstrated that CTL responses to a particular virus protein, i.e. LCMV glycoprotein, vary greatly in mice with differing MHC backgrounds (Whitton *et al.*, 1988). However, the patterns of cross-reactivity between different LCMV strains were also dependent on the class I restricting molecules (Ahmed *et al.*, 1984). The converse situation has been observed with measles virus (MV), where CTLs derived from highly susceptible mouse strains did not lyse target cells infected with MV, but weakly lysed cells infected with recombinant VV synthesizing either the N (*H-2^k*), or H (*H-2^b*) proteins of MV (Niewiesk *et al.*, 1993). The authors postulated that the affinity of the immunodominant MV-derived peptide was low and therefore only few MHC-peptide complexes were expressed on the cell surface. Although this was sufficient to induce a low-level CTL proliferation it was too small to induce lysis. Thus, it is possible that the number of MHC class I-peptide complexes needed for lysis varies on the basis of cell type.

We found that the non-structural proteins, which had elicited the strongest homotypic CTL responses in both

haplotypes of mice, also dominated the heterotypic CTL response to individual recombinant proteins in *H-2^k* mice. However, NS2 did not cross-react with serotype 2, suggesting that NS2 allele-specific peptide motifs are not shared throughout the BTV serotypes. Target cells infected with recombinant VV synthesizing the most variable proteins (VP2 and VP5, which are serotype-specific) were not recognized by heterotypic CTLs.

In the case of BTV, where several serotypes of the virus can exist within a given area (Gard & Melville, 1992; Stott *et al.*, 1985b), a vaccine that provides effective cross-protection is paramount (Gibbs, 1993). Thus although second generation vaccines are being developed using recombinant baculovirus technology, their ability to provide effective cross-protection at present may be limited. Sheep vaccinated with virus-like particles which mimic authentic BTV virions, but lack the RNA (French *et al.*, 1990) have been shown to be protected against homologous virus challenge for up to 14 months post-inoculation (Roy *et al.*, 1994a). Evidence was also obtained for cross-protection, although this was dependent on the challenge virus and the amounts of antigen used. Preliminary data have demonstrated that core-like particles synthesized from a dual recombinant baculovirus expressing VP3 and VP7 afforded partial protection against virus challenge in the absence of neutralizing antibodies (Roy *et al.*, 1994b). However, cell-mediated immune responses were not analysed in these experiments and the mechanism of protection remained unresolved.

Recent studies with LCMV, vesicular stomatitis virus and herpes simplex virus type 1 have demonstrated that exogenous antigens can stimulate a CD8⁺ class I-restricted response which affords protection (Bachmann *et al.*, 1994; Tewari *et al.*, 1994). Analysis of virus/CTL interactions at the molecular level and the localization of CTL epitopes within the BTV non-structural proteins would aid in the design of a BTV multicomponent vaccine which could theoretically boost CTL memory. Studies in sheep are required to determine if shared epitopes are selected in different MHC backgrounds.

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