Cytotoxic T cell response to Mengo virus in mice: effector cell phenotype and target proteins

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The Mengo virus specific cytotoxic T lymphocyte (CTL) response was investigated after intraperitoneal infection of mice with the attenuated Mengo virus strain vMC24. A high level of CTL activity was detected in spleen cell cultures obtained from infected C3H/HeJ (H-2k) or C57BL/6 (H-2b) mice after a secondary in vitro stimulation with Mengo virus-infected cells. The CTL activity, which was MHC class I-restricted, was shown to be mediated by CD8+ T cells. Recombinant vaccinia viruses that expressed capsid proteins VP0, VP1 or VP3 were produced and used to identify the protein(s) recognized by the Mengo virus-specific CTLs. In both C3H/HeJ and C57BL/6 mice, analysis of CTL activity against target cells expressing each capsid protein showed that VP0 was the only capsid protein recognized by the CD8+ CTLs. The CTL epitope(s) could be further located in the C-terminal half of VP0, i.e. in capsid protein VP2. Moreover, using unlabelled target cells expressing VP0 as cold competitors, we were able to almost completely inhibit recognition and lysis of Mengo virus-infected cells by specific CD8+ CTLs. Thus, the CTL response directed against VP2 was immunodominant in both C3H/HeJ- and C57BL/6-infected mice.

Introduction

Mengo virus is a member of the Picornaviridae family, genus Cardiovirus. This genus contains two related but distinct groups: the Theiler's murine encephalomyelitis viruses (TMEV) and the viruses of the encephalomyocarditis virus (EMCV) group (EMCV, Mengo, Columbia SK and Maus-Elberfeld). Like all picornaviruses, cardioviruses are small non-enveloped positivesense RNA viruses. Their icosahedral capsid, the three-dimensional structure of which has been elucidated (Luo et al., 1987), is made of 60 copies each of the four structural proteins VP1, VP2, VP3 and VP4. The viral genome is a monocistronic RNA molecule, approximately 7800 nucleotides in length. It encodes a large polyprotein, which is cleaved by autocatalytic processing into the various structural and non-structural viral polypeptides. Viruses of the EMCV group cannot be distinguished from each other on the basis of their antigenicity (they all belong to the same serotype) and are therefore considered as different strains of the same virus (Warren et al., 1949). Their natural host is mice but the host range of these viruses is very wide and includes rodents, domestic pigs, monkeys and even humans. Infections with EMCV are associated with various clinical signs, ranging from a mild febrile illness to paralysis or severe encephalitis (Dick, 1948; Warren, 1965). Numerous variants have been isolated which show different pathologies in mice including parotitis, encephalitis, encephalomyelitis, myocarditis, diabetes and polymyositis (Craighead, 1965, 1975; Miller et al., 1987). Infection of mice with these variants can thus serve as models for human diseases caused by several picornaviruses (Craighead et al., 1990).

The M strain of Mengo virus (Ellem & Colter, 1961) is neurotropic and normally causes a rapid and lethal encephalitis when injected by the intraperitoneal route (i.p.) or the intracerebral route (i.c.) in mice (Colter et al., 1965). Mechanisms of protection and long-lived immunity (Duke et al., 1990) induced by Mengo virus are not well documented. Although it is currently admitted that neutralizing antibodies can mediate protection of the host against subsequent Mengo virus infection (Muir et al., 1991), the mechanisms of virus clearance during primary infection with a sublethal dose of virus are not known. Cellular immune responses such as cytotoxic T cell responses could be involved in virus clearance and elimination of infected cells. On the other hand, these cellular responses could also be involved in the patho-
genesis of Mengo virus infection (Sriram et al., 1989; Topham et al., 1991).

The present report addresses the cytotoxic T lymphocyte (CTL) response to Mengo virus in mice. We investigated the induction of CTL activity after infection of mice with the live attenuated vMC\textsubscript{24} strain of Mengo virus (formerly called vM16; Duke et al., 1990). This strain contains a 24 nucleotide long poly(C) tract in place of the 61 nucleotide long poly(C) tract of the wild-type virus in the 5’ non-coding region of its genome. When injected i.p. or i.c. in mice and in other mammals, vMC\textsubscript{24} shows a markedly reduced pathogenicity, which might be linked to its limited capacity to replicate in the inoculated animals (Palmenberg & Osorio, 1994). However, mice inoculated with vMC\textsubscript{24} develop high titre neutralizing antibodies and eliminate the virus during the second week after inoculation. Consistently, these vMC\textsubscript{21} vaccinated mice are fully protected against a lethal challenge with the normally virulent wild-type Mengo virus or EMCV (Duke et al., 1990).

We describe here evidence for a strong MHC class I-restricted, Mengo virus-specific CD8\textsuperscript{+} CTL response in C57BL/6 and C3H/HeJ mice infected with the attenuated strain vMC\textsubscript{24}. Furthermore, by making use of recombinant vaccinia viruses we were able to locate an immunodominant CTL epitope in the C-terminal part of capsid protein VP2.

Methods

Mice. C57BL/6 and C3H/HeJ female mice were bred in the animal facilities of the Pasteur Institute (Paris, France). Mice used were 7 to 12 weeks old.

Cells. EL4 cells, a C57BL/6 (H-2\textsuperscript{b}) thymoma cell line, were maintained at 37 °C as a stationary suspension culture in R5 medium [RPMI 1640 supplemented with 5% heat-inactivated foetal calf serum (FCS), 100 U/ml penicillin, 100 \mu g/ml streptomycin and 5 \times 10\textsuperscript{-5} m-mercaptoethanol]. L929 cells, an adherent C3H/An (H-2\textsuperscript{a}) fibroblast cell line, were maintained in R5 medium and passaged once a week. The day before use, the cells were trypsinized and transferred to hydrophobic Petri dishes (Plastiques Gosselin, France) to which they do not adhere.

Transfectant Ltk\textsuperscript{-} cells lines expressing the H-2-K\textsuperscript{b} (Daniel-Vedele et al., 1984) or the H-2-D\textsuperscript{b} (Langlade-Demoyen et al., 1988) molecule were kindly provided by P. Langlade-Demoyen (Pasteur Institute, Paris).

Recombinant vaccinia viruses. Recombinant vaccinia viruses (VV) expressing Mengo virus capsid proteins VP0, VP1 or VP3 were constructed by standard procedures. Briefly, sequences encoding VP0, which is the precursor of VP4, and VP2, VP1 or VP3 were obtained by PCR amplification using pMC\textsubscript{24} DNA (formerly called pM16; Duke & Palmenberg, 1989) as a template and primer pairs designed to obtain the sequences of the inserts was verified and shown to contain no mutations as compared to the parental pMC\textsubscript{24} sequence. The VP0 and VP1 cDNA sequences contain a T\textsubscript{a}NT sequence that corresponds to a vaccinia virus early transcription termination signal. These sequences were removed without altering the corresponding protein sequence by appropriate site-directed mutagenesis. Thus, the 183 to 189 nucleotide sequence of VP0 was changed into TTTC\textsubscript{CT}G and the 103 to 109 sequence of VP1 into TT\textsubscript{CT}TCT. Vaccinia virus recombinants were generated by homologous recombination into the Copenhagen strain, essentially as described (Kiely et al., 1986) and plaque purified twice on 143B-tk\textsuperscript{-} cells under bromodeoxyuridine selection (50 \mu g/ml).

Virus stocks were derived from a single plaque isolate, expanded once in CV1 cells and then in HeLa cells at an m.o.i. of 1 p.f.u./cell. The recombinant viruses were named VV-MVP0, VV-MVP1 and VV-MVP3, according to the capsid protein they express. Expression was confirmed by immunoprecipitation of \textsuperscript{\text{35}}S\textsuperscript{methionine-labelled cytoplasmic extracts prepared from CV1 cells infected with the different recombinant vaccinia viruses, using a rabbit antisera raised against formaldehyde-inactivated Mengo virus. Recombinant viruses VV-MVP0-A and VV-MVP0-B that expressed respectively VP4 and aa 1 to 106 of VP2, and aa 87 to 256 of VP2, were obtained in the same way.

Generation of CTL effectors. Mice were inoculated i.p. with the attenuated strain of Mengo virus, vMC\textsubscript{24} (Duke et al., 1990), a generous gift from A. Palmenberg (Univ. of Wisconsin, Madison, Wis., USA). Unless otherwise stated, mice were injected with 10\textsuperscript{5} p.f.u. of virus in PBS. Two weeks later, spleen cells were collected and seeded into upright T75 flasks (Falcon) at 2 x 10\textsuperscript{6} cells/ml in R10 medium (R5 medium with 10% FCS, 1 mM-sodium pyruvate and non-essential amino acids) and were restimulated in vitro for 5 days with vMC\textsubscript{24} infected (m.o.i. 10) syngeneic spleen cells (10\textsuperscript{6} cells/ml), which had been irradiated (2500 rad) 3 h after infection.

Infection and \textsuperscript{\text{51}}Cr labelling of target cells. EL4 and L929 cells were infected with recombinant vaccinia viruses (10 p.f.u./cell) respectively 18 and 3 h before \textsuperscript{\text{51}}Cr labelling or with vMC\textsubscript{24} (10 p.f.u./cell) during \textsuperscript{\text{51}}Cr labelling. To perform the \textsuperscript{\text{51}}Cr labelling, 3 x 10\textsuperscript{6} cells were resuspended in 0.25 ml of R0 medium (RPMI 1640 supplemented with 1 mM-HEPES) and mixed with 100 \mu Ci Na\textsuperscript{\text{51}}CrO\textsubscript{4} (Amersham). After incubation for 90 min at 37 °C, the cells were washed three times in R0 medium and counted using Trypan blue dye exclusion. Control target cells were uninfected cells or cells infected with the tk\textsuperscript{-} vaccinia virus VV-TG186 derived from pTG186 (a kind gift from M. P. Kiely, Transgene, France).

CTL assay. Cytolytic activity of in vitro secondary CTLs was measured using a standard \textsuperscript{\text{51}}Cr release cytotoxicity assay. Twofold serial dilutions of effector cells were prepared and added in triplicate to 10\textsuperscript{4} \textsuperscript{\text{51}}Cr-labelled target cells in round-bottomed (EL4 cells) or flat-bottomed (L929 and Ltk\textsuperscript{-} transfectant cells) 96-well microtitre plates (Costar) in a total volume of 200 \mu l of R5 medium. Spontaneous and maximal release of radioactivity were determined by incubating the target cells (six wells) in R5 medium alone or in 1% Triton X-100, respectively. After incubation for 4 h at 37 °C, the plates were centrifuged at 400 g; half of the supernatant was removed and the radioactivity measured using a gamma counter. Spontaneous release was less than 20% of the maximal release. The percentage specific \textsuperscript{\text{51}}Cr release was calculated as:

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\text{(experimental release—spontaneous release)} \times 100.
\]

(maximal release—spontaneous release)

Depletion with CD4 and CD8 antibodies and Dynabeads. Effector cells (10\textsuperscript{5} cells/ml) were incubated for 1 h at 4 °C in R2 medium (R5 medium with 2% FCS) containing 10 \mu g/ml of rat MAbs GK1.5 (Dialynas et al., 1983) or H35 17.2 (Golstein et al., 1982). They were then washed twice with cold R2 medium, resuspended in cold R0 medium containing...
Results

Induction of specific CD8+ CTLs in Mengo virus-infected mice

The CTL response to Mengo virus was analysed in two strains of mice with different MHC haplotypes, C3H/HeJ (H-2^k) and C57BL/6 (H-2^b) mice.

Animals were infected i.p. either with 10^6 or 10^7 p.f.u. of attenuated Mengo virus vMC24. These doses correspond respectively to suboptimal and optimal doses of virus for the induction of a neutralizing antibody response in mice (Duke et al., 1990). Splenocytes from infected mice were harvested 10 days after inoculation. Mice injected with PBS alone were processed in parallel as uninfected controls. Splenocytes were stimulated in vitro with syngeneic Mengo virus-infected irradiated

spleen cells and the cytolytic activity of the bulk cell cultures was examined 5 days later as described in Methods. A very high level of cytolytic activity was
detected in spleen cell cultures initiated from infected C3H/HeJ mice when Mengo virus-infected cells of the same MHC haplotype (L929 cells) were used as targets (Fig. 1 a). Similarly, H-2b effectors from Mengo virus-infected C57BL/6 mice exhibited a high cytolytic activity against Mengo virus-infected EL4 cells (Fig. 1 b). Stimulated spleen cells did not induce significant lysis of uninfected target cells or of Mengo virus-infected target cells from a different MHC haplotype. No lysis was observed with stimulated splenocytes from control naive mice (Fig. 1). The cytolytic activity detected was therefore specific for Mengo virus determinants and dependent upon prior in vivo antigen exposure of mice. Moreover, in vitro stimulation was required as unstimulated splenocytes did not display any cytolytic activity, indicating that the frequency of activated effector cells present in the spleen 15 days after the infection was not high enough to allow direct detection of CTLs (data not shown).

Essentially the same response was observed whether mice had been inoculated with $10^5$ or $10^6$ p.f.u. of vMC$_{24}$ (Fig. 1). This most likely stems from the fact that the mice were inoculated with a live virus that is able to efficiently replicate.

The effector cells responsible for the cytolytic response to Mengo virus were next characterized by depleting the in vitro stimulated spleen cell cultures of CD4$^+$ or CD8$^+$ T cells using anti-CD4 (MAb GK1.5) or anti-CD8 (MAb H35.17.2) antibodies as described in Methods. Depletion of CD4$^+$ cells did not modify the efficiency of lysis of vMC$_{24}$-infected targets whereas depletion of CD8$^+$ cells resulted in 90% reduction of specific lysis (Fig. 2). The specific H-2b restricted cytolytic activity that could still be detected after depletion of CD8$^+$ cells for the highest effector to target ratios might be due to residual CD8$^+$ CTLs that escaped depletion. Alternatively, it might be attributed to the presence of a small subset of virus-specific CD4$^+$ CTLs among the effector cell population.
Altogether, these data establish that the cytolytic activity induced during primary infection of mice with Mengo virus was mainly mediated by virus specific, MHC-restricted, CD8\(^+\) T lymphocytes, hereafter referred to as CD8\(^+\) CTLs.

The Mengo virus-specific CTLs are likely to be MHC class I-restricted, as expected for CD8\(^+\) effector T cells. To determine which molecule was specifically required for the presentation of Mengo virus CTL epitope(s) in the H2\(^{b}\) context, we used transfectant cell lines expressing the D\(^{b}\) molecule but not L cells expressing the K\(^{b}\) molecule. This demonstrates that the major CTL response against Mengo virus in C57BL/6 mice is H-2\(^{D^{b}}\) restricted.

Identification of capsid protein VP0 as a target for CTLs

Recombinant vaccinia viruses that expressed capsid proteins VP0, the precursor protein of VP4 and VP2 (VV-MVP0), VP1 (VV-MVP1) or VP3 (VV-MVP3) (see Methods) were used to determine which structural protein was recognized by the Mengo virus-specific CD8\(^{+}\) CTLs from C3H/HeJ or C57BL/6 mice. A high level of specific lysis was found against VV-MVP0-infected targets (Fig. 4). In the case of the C57BL/6 mice, no lysis could be detected against control VV-TG186-, or against VV-MVP1- or VV-MVP3-infected targets, whereas, in the case of C3H/HeJ mice, some non-specific lysis was observed against VV-infected targets. This non-specific lysis might result from a greater sensitivity of L929 cells to infection by VV as compared to EL4 cells. In any case, the dominant target antigen among the capsid proteins was VP0 for both the H-2\(^{b}\) and H-2\(^{k}\) Mengo virus-specific CD8\(^{+}\) CTLs.

VP0 contains an immunodominant epitope recognized by H-2\(^{b}\) and H-2\(^{k}\) effectors

It is remarkable that the percentage of specific lysis obtained with target cells expressing VP0 was comparable to that obtained with Mengo virus-infected target cells (compare Figs 1 and 4, which represent data from the same experiment). This could indicate that VP0 contains one or several immunodominant Mengo virus-specific CTL epitope(s).

To test this hypothesis, we determined if VV-MVP0-infected target cells could compete efficiently with Mengo virus-infected target cells. To that end, CTL assays were performed using \(^{51}\)Cr-labelled Mengo virus-infected targets in the presence of unlabelled VV-MVP0-infected cells. The latter acted as cold competitor cells and almost completely inhibited recognition and subsequent lysis of labelled Mengo virus-infected cells by Mengo virus-specific bulk CD8\(^{+}\) CTLs (Fig. 5). Indeed, it could be calculated from this experiment that more than 80% of the Mengo virus-specific cytolytic activity is directed against VP0. This result was obtained both with H-2\(^{b}\) and H-2\(^{k}\) effector cells (Fig. 5). It can thus be concluded that infection of C57BL/6 and C3H/HeJ mice with Mengo virus induces a MHC class I-restricted CD8\(^{+}\) CTL response, which is predominantly directed against capsid protein VP0.
**The immunodominant CTL epitope(s) is (are) located in the C-terminal half of VP2**

To locate more precisely the immunodominant epitope(s) identified in VP0, we constructed two recombinant vaccinia viruses that expressed overlapping parts of VP0: VV-MVP0-A expressed VP4 and aa 1 to 106 of VP2, whereas VV-MVP0-B expressed aa 87 to 256 of VP2 (Fig. 6a). These were used to infect syngeneic cells that served as targets in a CTL assay using H-2\(^b\) or H-2\(^a\) Mengo virus-specific effector cells. As is clearly seen in Fig. 6(b, c), VV-MVP0-B-infected target cells were efficiently lysed by the CTLs from C57BL/6 and C3H/HeJ mice, whereas VV-MVP0-A-infected target cells were not recognized. This indicates that the H-2\(^b\)- and H-2\(^a\)-restricted VP0 CTL epitope(s) is (are) located in the C-terminal part of VP2.

**Discussion**

We have demonstrated in this study that infection with the attenuated Mengo virus strain vMC\(_{24}\) induces a MHC class I-restricted CD8\(^+\) CTL response in two strains of mice (C57BL/6 and C3H/HeJ) that differ in their MHC haplotype. Preliminary results reported by others have shown that a wild-type isolate of Mengo virus could induce a cytotoxic response in C57BL/6 mice, but neither the nature of the effectors, nor the viral epitopes recognized were characterized (Hassin et al., 1985). Other reports have indicated that mice infected with a different cardiovirus, Theiler's virus, developed a CTL response (Lindsley et al., 1991; Pena Rossi et al., 1991). We show here that the specific CTL response elicited by Mengo virus, an EMC-type cardiovirus, is mediated by CD8\(^+\) T cells in infected mice and is restricted by the D\(^b\) class I molecule in C57BL/6 mice. Using recombinant vaccinia viruses, we have been able to locate VP0 CTL epitope(s) in the C-terminal part of capsid protein VP2, whereas none were found in the other capsid proteins (VP4, VP1 and VP3). Moreover, using a cold target inhibition assay, we could show that the CTL epitope(s) located in VP2 is (are) immunodominant.

Although MHC class II-restricted CD4\(^+\) CTL responses have recently been documented for some viruses, for instance for measles virus (Jacobson et al., 1989), viral CTL responses are usually mediated by CD8\(^+\) T lymphocytes that recognize short peptide fragments derived from endogenously synthesized viral proteins presented by MHC class I molecules at the surface of infected cells (Townsend et al., 1986). Recent analyses of naturally occurring self peptides eluted from class I molecules have revealed apparent self peptide motifs (Falk et al., 1991; Jardetzky et al., 1991). Such motifs have been successfully used to identify class I epitopes in various viral proteins (Beauverger et al., 1993; Lobigs et al., 1994). The D\(^b\) specific motif is X\(_2\)NX\(_4\)(M,I,L) and scanning of the Mengo virus VP2 protein sequence (aa 87 to 256) for this consensus motif did not reveal any potential epitope, but numerous peptides fitting the D\(^b\) motif could be found in the non-structural polypeptides or in the N-terminal part of VP0. This indicates that the epitope recognized in the C-terminal part of VP2 constitutes an exception to the D\(^b\) consensus motif. Such exceptions have already been described, like the L\(^a\)-restricted tumour antigen tum-epitope ISTQNNRALVA (Lurquin et al., 1989), which does not fit the L\(^a\) self peptide consensus motif XPX\(_6\)7 (L,M,F) (Corr et al., 1992). Nevertheless, we tested whether VP2 peptides QVQCNASQF, RTQTNRKGPF, MDHQNFQWQTL and NLRTNTTVDL could be recognized by H-2\(^b\)-Mengo virus-specific CTLs. The first two peptides contain a Phe as a hydrophobic anchor at their C terminus, as can be the case for D\(^a\) and L\(^a\) binding peptides (Corr et al., 1992, 1993). The other two VP2 peptides are longer than predicted by the D\(^b\) minimal consensus motif but possess the predicted anchors, Asn at position 4 and Leu at the C terminus, but at position 10 or 11 respectively instead of 9. Such longer CTL peptides have already been described (Kast et al., 1989; Oldstone et al., 1988), but they usually contain amino acid residues such as Pro, or a combination of Pro and Gly, that might contract their spatial length (Falk et al., 1991). None of the four peptides tested were recognized by H-2\(^b\)-Mengo virus-specific CTLs (data not shown), indicating that one of the two anchors should be different from the ones predicted by the self peptide consensus motif for D\(^b\).

The antiviral CTL response for a given restriction element is frequently directed against a single immunodominant peptide, in spite of the presence in the viral proteins of numerous other peptides which fulfil the predicted class I binding motif requirement and which may be presented by class I antigens (Lipford et al., 1993; Lobigs et al., 1994). It is not clear which factors contribute to the selection of immunodominant peptides, but this could reflect differences in processing of individual peptides (Del Val et al., 1991) or in peptide binding affinity to MHC antigens (Jennings et al., 1988). This could also reflect deletions from the T cell receptor repertoire or peripheral regulation of CTL responses (Hämmerling et al., 1993; Hill et al., 1993).

In the case of Mengo virus, more than 80% of the specific CTL activity detectable after a secondary *in vitro* stimulation was directed against the VP0 protein in the two H-2 haplotypes (b and k) tested. This implies that non-structural proteins of Mengo virus are recognized very weakly if at all by Mengo virus-specific CTLs in these haplotypes. However, it has been previously shown...
that in vitro stimulation may select for certain populations of CTLs and obscure other populations (Jennings et al., 1988). Therefore, in our case, VP6-specific CTLs might have been preferentially expanded during in vitro stimulation with Mengo virus-infected cells, despite the presence of precursors specific for other polypeptides, e.g. non-structural polypeptides. We are currently addressing this question by in vitro stimulation of the primed splenocytes with recombinant vaccinia viruses expressing the non-structural polypeptides of Mongo virus.

To date, CTL responses have been characterized after infection with other picornaviruses such as TMEV (Lindsley et al., 1991; Pena Rossi et al., 1991), hepatitis A virus (Vallbracht et al., 1989) and coxsackievirus B3 (Huber & Job, 1983), but the epitopes recognized have not been localized. Although viral pathogenesis seems to be immune mediated for some picornaviruses like coxsackievirus B3 (Guthrie et al., 1984) or the diabetogenic (D) strain of EMCV (Baek & Yoon, 1990; Barger & Craighead, 1991), the role of CTLs in protection or in pathogenesis remains debatable for others like TMEV or most strains of EMCV (Miller & Gerety, 1990; Topham et al., 1991).

Infection of mice with the DA strain of TMEV leads to a persistent demyelinating disease (Lipton, 1975). Virus-specific CTLs arise as a consequence of the infection and can be found in the CNS of mice (Lindsley et al., 1991). Although in most cases CD4+ T cells appear to be the main mediator of CNS demyelination (Gerety et al., 1994; Welsh et al., 1987), CD8+ T cells are involved in the development of the disease in susceptible SJL/J mice (Rodriguez & Sriram, 1988), but can also prevent the establishment of a persistent infection and demyelinating disease in FVB mice (H-2k) made transgenic for the H-2Dk gene (Azoulay et al., 1994).

In contrast to the persistent DA strain of TMEV, wild-type Mengo virus is highly neurotropic and induces a fulminant lethal encephalitis in infected mice. Cellular immune effectors could thus have strikingly different roles in pathogenesis or protection in the two infections. It has been shown that depletion of CD8+ T cells prior to infection of mice with the demyelinating M strain of EMCV drastically reduced the incidence of the disease (Topham et al., 1991). However, depletion after the onset of the disease did not alter the development of the encephalitis or encephalomyelitis. Therefore, in contrast to persistent TMEV infection (Rodriguez & Sriram, 1988), CD8+ T cells appear to be involved in the initial stages of the EMCV-M infection but not in the progression of the disease. As Mengo virus is closely related to EMCV-M but does not induce the same symptoms, it will be of interest to investigate the role of CD8+ T cells during the course of Mengo virus infection, both in pathogenesis and protection from infection. The characterization of a Mengo virus CTL epitope in capsid protein VP2 and the availability of recombinant vaccinia viruses expressing individual Mengo virus proteins provide molecular tools for such a study.

We have recently explored the possibility of using the attenuated strain of Mengo virus as a vector for the expression of foreign antigens and chose to express foreign human immunodeficiency virus type I sequences as a fusion protein with the L polypeptide. Such a recombinant virus was able to elicit a potent CTL response against the foreign CTL epitope inserted (Altmeyer et al., 1994). In order to optimize the design of new recombinant Mengo viruses as live vaccines and to avoid competition between the Mengo virus and inserted foreign CTL epitopes, it is important to delineate the immune responses against the vector itself and particularly to try to understand the rules that govern the immunodominance of the Mengo virus CTL epitopes over the other putative CTL peptides, which could be presented by class I molecules.

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