Equine infectious anaemia virus proteins with epitopes most frequently recognized by cytotoxic T lymphocytes from infected horses

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Efficacious lentiviral vaccines designed to induce cytotoxic T lymphocytes (CTL) in outbred populations with a diverse repertoire of MHC class I molecules should contain or express multiple viral proteins. To determine the equine infectious anaemia virus (EIAV) proteins with epitopes most frequently recognized by CTL from seven horses infected for 0.5 to 7 years, retroviral vector-transduced target cells expressing viral proteins were used in CTL assays. Gag p15 was recognized by CTL from 100% of these infected horses. p26 was recognized by CTL from 86%, SU and the middle third of Pol protein were each recognized by 43%, TM by 29%, and S2 by 14%. Based on these results, it is likely that a construct expressing the 359 amino acids constituting p15 and p26 would contain epitopes capable of stimulating CTL in most horses.

Cytotoxic T lymphocytes (CTL) are associated with the control of human immunodeficiency virus-1 (HIV-1) and other related lentiviruses including equine infectious anaemia virus (EIAV) (McGuire et al., 1994; Ogg et al., 1998; Kuroda et al., 1999). CD8+ T lymphocytes with a CTL phenotype can protect mice from virus challenge in adoptive transfer experiments using CTL clones (Byrne & Oldstone, 1984; Taylor & Askonas, 1986). However, proof of a protective role for CTL against lentiviruses has been difficult to demonstrate. Adoptive transfer of autologous CTL clones results in temporary protective effects in HIV-1-infected humans (Brodie et al., 1999) and transfer of CTL clones to HIV-1-infected human peripheral blood lymphocyte–SCID mice reduces plasma viraemia, but does not eliminate the infection (McKinney et al., 1999). But, in another study, transfer of an expanded autologous CTL clone to HIV-1 Nef to an HIV-1-seropositive individual resulted in enhanced disease due to virus variants lacking the Nef CTL epitope (Koenig et al., 1995). Direct evidence for CD8+ T lymphocyte control of simian immunodeficiency virus (SIV) and chimeric SIV/HIV in infected macaques is provided by in vivo depletion of CD8+ T lymphocytes with monoclonal antibody (Matano et al., 1998; Schmitz et al., 1999). Based on these demonstrations of the protective role of CTL, induction of CTL is an important goal for lentiviral vaccines. Because of the diverse repertoire of MHC class I molecules in outbred populations, vaccines designed to induce CTL to lentiviral-infected cells will likely contain multiple viral proteins or epitopes. This strategy will increase the probability of a protective CTL response in vaccinated individuals.

Horses recently infected with EIAV have CD8+ CTL in peripheral blood mononuclear cells (PBMC) which do not require in vitro stimulation for effector function (McGuire et al., 1994). These CTL recognize epitopes on Env and Gag proteins (McGuire et al., 1994). Later in infection, when the horses have controlled viraemic episodes and are carriers, memory CTL to epitopes on Env and Gag proteins which require in vitro stimulation are present (Hammond et al., 1997; McGuire et al., 1997). These memory CTL have a mean frequency of 293 per million PBMC when measured with targets expressing the infecting EIAV strain (McGuire et al., 1997). Epitopes on Gag proteins that are recognized by CTL from infected horses have been mapped and only p15 matrix and p26 capsid proteins contained epitopes (Zhang et al., 1998). It is not known whether Pol proteins, Tat, Rev or the protein encoded by S2 are recognized by CTL. SU is recognized by CTL (Lonning et al., 1999); however, it is not known if TM is recognized or if...
Table 1. EIAV proteins and polypeptides recognized by CTL from EIAV-infected horses

No CTL response is indicated as —, while a significant response ( > 2.5 SE above control target cells transduced with vLXSN which contained no EIAV insert) is indicated as +. The protein segments in brackets represent further mapping using approximately 100 amino acid Env polypeptides expressed by retroviral vectors defined in Fig. 1. All these experiments were repeated at least twice except for H540 and H532 effectors against S1–3-expressing targets which were done once. Recognition by CTL from H507, H513, H521, H532, and H540 of Gag protein-expressing targets was reported previously (Zhang et al., 1998); in addition to those results, H532 and H540 had developed CTL to p15 in this study.

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* The EIAV proteins were expressed in retroviral vector-transduced autologous EK target cells. None indicates transduction with vLXSN which contained no EIAV insert.

Particular regions of SU are recognized by a high percentage of infected horses. Proteins recognized by CTL from infected horses, particularly carriers, are logical candidates for evaluation in immunization strategies. A research goal is to identify effective ways to induce large numbers of CTL to epitopes on multiple EIAV proteins in naïve horses and to determine the protective effects of such CTL against virus challenge. Therefore, the purpose of this study was to identify EIAV proteins with epitopes most frequently recognized by CTL from infected horses.

CTL assays were first done using autologous target cells infected with EIAV in order to verify that the horses had memory CTL to EIAV proteins at the time of the study. The effector cells in the assay were stimulated PBMC from seven horses infected with EIAV<sub>WSU</sub>. Horses H507, H513, H521, H532 and H540 were ponies infected for 7, 3, 6, 5 and 4 years, respectively, and the virus and dose used to infect these horses has been described previously (McGuire et al., 1994, 1997; Zhang et al., 1998). Arabian breed horse A2140 was infected intravenously for 1 year before the study with 10⁶ TCID₉₀% of EIAV<sub>WSU</sub> and Arabian breed horse A2141 was infected intravenously for 6 months before the study with 10⁶ TCID₉₀% of EIAV<sub>WSU</sub>. The equine leukocyte alloantigen (ELA)-A types of these horses were determined as described (Bailey, 1980) by Pamela J. Henney, Department of Veterinary Science, University of Kentucky, Lexington, KT, USA (Table 1). The ELA-A locus is the best defined polymorphic class I locus (Antczak et al., 1986), though another has been described (Bernoco et al., 1987; Donaldson et al., 1988) and there are non-classical class I molecules transcribed in horse cells (Ellis et al., 1995). However, most MHC class I-restricted CTL killing of EIAV-infected equine kidney (EK) cells targets observed to date correlates with expression of ELA-A molecules (McGuire et al., 1997; Zhang et al., 1998). The PBMC were stimulated in vitro with EIAV<sub>WSU</sub>-infected autologous monocytes, the virus strain used to infect the horses. Initially, PBMC were stimulated twice over a 2 week period with recombinant human IL-2 added in the second week as described (Zhang et al., 1998). Later, a modified protocol with a 1 week stimulation without IL-2 was used, which produced similar results. After isolation of PBMC (Zhang et al., 1998), EIAV<sub>WSU</sub> in 17% foetal bovine serum was added to monocyte cultures in amounts equal to an m.o.i. of 1, assuming that PBMC were 5% monocytes. Virus and PBMC were incubated for 2 h at 37 °C with occasional mixing before centrifugation at 250 g for 10 min. PBMC were resuspended to 2 × 10⁶/ml in RPMI 1640 medium with 10% foetal bovine serum, 20 mM HEPES, 10 µg/ml gentamycin and 10 µM 2-mercaptoethanol; 1 ml was added to each well of
a 24-well plate and incubated for 1 week at 37 °C before use in CTL assays as described (Zhang et al., 1998).

The target cells for the initial CTL assays were autologous or MHC class I ELA-A mismatched EK cells uninfected or infected with EIAV<sub>WSU</sub>. The EK cells were fibroblasts established from kidney biopsies from each horse before EIAV infection (McGuire et al., 1994). These cells express class I, but not class II molecules (McGuire et al., 1997), so only class I-restricted killing by CTL could be detected, although both class I- and class II-restricted killing by CTL from EIAV-infected horses occurs (Hammond et al., 1997). The CTL assays were done using <sup>51</sup>Cr-labelled target cells (Zhang et al., 1998) and an effector:target cell ratio of 20:1. All seven EIAV-infected horses had CTL that lysed autologous EIAV-infected target cells with specific lysis ranging from 17.2 to 65.9%. Significant specific lysis of infected target cells was defined as specific lysis which exceeded that of uninfected target cells by > 2.5 SE (McGuire et al., 1994). The range of specific lysis of uninfected autologous target cells was 0.9 to 9.2%, uninfected mismatched target cells was 1.0 to 6.0%, and EIAV-infected mismatched target cells was 6.6 to 14.2%. Accordingly, most of the CTL killing in each horse was EIAV specific and MHC class I ELA-A-restricted. This kind of killing of EK cells was shown to be caused by CD8<sup>+</sup> T lymphocytes in a previous experiment (McGuire et al., 1994).

To determine the percentage of infected horses with CTL recognizing epitopes on each of the EIAV proteins, CTL activity was evaluated against target cells transduced with retroviral vectors expressing either whole EIAV proteins or polypeptides defined in Fig. 1. A description of retroviral vectors expressing each of the Gag proteins (Zhang et al., 1998) and the construction procedures (Lonning et al., 1999) have been published. The packaging cells and the plasmid pLXSN was obtained from Dusty Miller, Seattle, WA, USA. Eighteen new retroviral vectors were made by inserting genes defined in Fig. 1 into pLXSN, including a Kozak consensus sequence and an initiation codon when needed. The Env and Pol proteins were each expressed as three smaller polypeptides. Then, the three Env polypeptides were each expressed as three smaller polypeptides (Fig. 1). S1, S2 and S3, encoding regulatory and putative regulatory proteins, were individually expressed. The DNA sequence of each cloned gene segment was determined and RT–PCR was used to verify that EK cells transduced with each of the vectors transcribed mRNA of the correct size (Lonning et al., 1999). Transduced EK cells were selected with G418 sulfate before use as target cells. Effector cells were PBMC stimulated as described above and used at an effector:target cell ratio of 20:1. The negative controls for these assays were autologous EK cells transduced with pLXSN and ELA-A mismatched EK cells transduced with the retroviral vector being tested and vLXSN. vLXSN was derived from the same plasmid and packaging cells as other vectors except that no EIAV gene was added (Lonning et al., 1999). As an example, results of mapping with CTL from horse A2140 using targets expressing 14 EIAV proteins and polypeptides are in Fig. 2. Epitopes in p15 and Env polypeptide 1 (EnvT1) were recognized (Fig. 2).

The individual EIAV proteins recognized by CTL from the highest percentage of the seven EIAV<sub>WSU</sub>-infected horses were p15 matrix and p26 capsid proteins (Table 1). Five of these horses were evaluated for CTL to Gag proteins previously (Zhang et al., 1998) and differences are indicated in the legend to Table 1. None of the horses had CTL to Gag p11 or p9 proteins. In contrast, seven (100%) had CTL which lysed EK target cells expressing p15 (Table 1). Six (86%) had CTL recognizing either p26a or p26b, representing the N-terminal and C-terminal halves of p26, respectively.

SU was recognized by CTL from three of the seven (43%) EIAV<sub>WSU</sub>-infected horses while two (29%) had CTL to TM (Table 1). Since CTL for one horse recognized both SU and TM, there were only four horses (57%) with CTL to SU, TM or both. None of the SU or TM epitopes were located in the
same approximately 100 amino acid polypeptide (Table 1). Pol proteins were recognized by CTL from four of the seven (57%) infected horses with three of these recognizing the middle PolT2 polypeptide (Table 1). None recognized the C-terminal third of Pol (PolT3). EIAV has genes for three other proteins, designated S1, S2 and S3. S1 encodes the C-terminal exon (46 of 75 total amino acids) of Tat (Derse et al., 1990). S3 encodes the C-terminal exon (105 of 135 total amino acids) of Rev (Stephens et al., 1990; Rosin-Arbesfeld et al., 1993). S2 encodes the 65 amino acids of a putative regulatory protein of unknown function (Li et al., 1998). When target cells expressing S1, S2 or S3 were examined, only those expressing S2 were lysed by CTL from one horse (14%).

This study evaluated the killing of autologous target cells expressing proteins from the infecting virus strain (EIAV$_{WSU}$) by CTL from horses infected for 6 months to 7 years. This approach cannot identify CTL epitopes that have varied during infection. Instead, it may be biased toward identifying proteins with CTL epitopes that have not changed, as CTL are likely stimulated by low levels of virus, detectable only by RT–PCR in some carrier horses (Langemeier et al., 1996; Oaks et al., 1998). Virus present later in infection that can stimulate CTL which react with the infecting virus is either unchanged at these CTL epitopes or a variant with closely related epitopes. Identification of EIAV proteins with epitopes recognized by CTL from infected horses is important because immunization strategies inducing a CTL response to epitopes on multiple lentiviral proteins will have the best chance of protection against virus challenge. In addition, it is possible that proteins other than those identified by CTL from carrier horses could also induce effective CTL. In any case, the more epitopes inducing a CTL response and requiring change before virus escape, the lower the probability of generating CTL escape variants. Analysis of the data in Table 1 leads to the conclusion that Gag p15 and p26 are the best proteins for inclusion in vaccine strategies to induce CTL to EIAV since CTL from 100% of horses recognized p15 and 86% recognized p26. Therefore, a construct expressing the 359 amino acids constituting p15 and p26 should contain epitopes capable of stimulating CTL in most horses. These Gag proteins are the most conserved in EIAV, although as much as 12% amino acid difference has been demonstrated in p15 and p26 between strains (Zhang et al., 1999). The next best proteins for inclusion in immunization strategies include SU and the middle third of the pol gene, which were each recognized by CTL from 43% of horses. In summary, these results provide information needed for selection of EIAV proteins for use in inducing CTL in horses.

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References


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