Frequency of Memory Cytotoxic T Lymphocytes to Equine Infectious Anemia Virus Proteins in Blood from Carrier Horses

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Horses with equine infectious anemia virus (EIAV) have episodes of viremia and disease; however, most eventually become inapparent carriers. A possible mechanism of control is cytotoxic T lymphocytes (CTL). To evaluate CTL in inapparent carriers with low viral loads, peripheral blood mononuclear cells (PBMC) were stimulated in vitro with autologous EIAV-infected PBMC and human IL-2 to detect memory CTL (CTLm). In initial studies, three carriers had CTLm and one of these had low-level effector CTL (CTLe). The CTLm were restricted by equine lymphocyte alloantigen-A (ELA-A) locus encoded MHC class I molecules on autologous equine kidney (EK) target cells. In addition, EK cells did not express MHC class II molecules. The CTLm frequency in PBMC from five inapparent carriers infected for 22 to 50 months was determined by limiting dilution analysis. PBMC were diluted, stimulated, and tested on EK cell targets infected with EIAV and recombinant vaccinia viruses expressing EIAV Env or Gag/Pr proteins. All five carriers had CTLm to EIAV-infected targets, while four had CTLe to targets expressing Env and four had CTLm to targets expressing Gag/Pr proteins. The CTLm frequency range was 60 to 468 per 10^6 PBMC to EIAV-infected targets, 4 to 286 to Env-expressing targets, and 25 to 190 to Gag/Pr-expressing targets. These results should facilitate the identification of epitopes recognized by predominant CTLm from horses controlling a lentivirus infection.

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INTRODUCTION

Equine infectious anemia virus (EIAV) causes disease in horses with some distinctive features when compared with other lentiviral infections of humans and animals. There is an epidemic of plasma viremia with associated fever, thrombocytopenia, and anemia within 1 to 4 weeks after infection which lasts several days (Kono, 1969; Coggins et al., 1972). Most horses infected with either wild-type strains (Kono, 1969) or strains derived from the tissue culture-adapted prototype strain (Orrego et al., 1982; O’Rourke et al., 1988) terminate the initial disease episode, although some horses infected with wild-type strains may die during the initial disease. Surviving horses usually have episodes of viremia and clinical disease interspersed with quiescent periods of one to several weeks during the first few months after infection (Kono, 1969; Orrego et al., 1982; O’Rourke et al., 1988). Then, the disease episodes become less frequent until the horses are inapparent carriers with low virus loads (Kono, 1969; Issel et al., 1982; Coggins, 1984; Langemeier et al., 1996). Carriers live for years and can be detected by antibodies to EIAV proteins and by blood transmission of EIAV to noninfected horses (Coggins et al., 1972; Issel et al., 1982; Coggins, 1984).

The mechanisms carrier horses use to control EIAV are not known. Infected horses control the initial plasma viremia by specific immune responses since young horses with severe combined immunodeficiency fail to terminate initial viremia, while immunocompetent young horses terminate the initial viremia (Perryman et al., 1988). Whether antibody or other lymphocyte responses are involved is unknown because young horses with severe combined immunodeficiency lack functional T and B lymphocytes (McGuire et al., 1975). Antibodies mediating antibody-dependent cellular cytotoxicity (ADCC) do not appear to be involved in virus control because these antibodies cannot be demonstrated in sera from infected horses (Fujimiya et al., 1979; Tschetter et al., 1997). Neutralizing antibodies are detected following infection with EIAV (Kono, 1969) and the specificity of the neutralizing antibody response broadens during persistent infection (O’Rourke et al., 1989; Rwambo et al., 1990). The delayed appearance of type-specific neutralizing antibodies after the termination of viremia by that virus type (Carpenter et al., 1987; O’Rourke et al., 1989; Rwambo et al., 1990) and the antigenic variation of epitopes recognized by neutralizing antibody (Kono et al., 1973b; Montelaro et al., 1984) may limit the effectiveness of neutralizing antibody in EIAV control. Nevertheless, a protective role for
antibody is indicated by passive transfer of plasma containing neutralizing antibodies from EIAV-infected to noninfected horses (Rushlow et al., 1990). This plasma transfer did not prevent infection of the recipient horses following EIAV challenge, but the transfer delayed seroconversion and in one case delayed clinical disease (Rushlow et al., 1990).

Support for mechanisms of immune control other than antibodies include observations of EIAV-infected horses (Kono et al., 1973a) and vaccinated horses (Issel et al., 1992) resisting challenge in the absence of detectable neutralizing antibody. Active immune responses are implicated in EIAV control by carrier horses since either corticosteroids or cyclophosphamide causes plasma viremia within 1 to 2 weeks after treatment (Kono et al., 1976; Tumas et al., 1994). The virus in plasma following treatment is a neutralizing antibody escape-variant appearing while neutralizing antibody to the variant used for infection is still detectable (Kono et al., 1976). Therefore, neutralizing antibody in plasma probably controls viremia by recognized EIAV variants. New variants, and virus replication to generate new variants, may be controlled by T lymphocyte responses including cytotoxic T lymphocytes (CTL).

Since our long-term goal is to clarify the mechanisms that inapparent carrier horses use to control EIAV replication, we are investigating the hypothesis that MHC class I-restricted virus-specific CD8⁺ CTL are involved. To this end, CTL meeting these criteria were previously demonstrated in peripheral blood mononuclear cells (PBMC) coincident with the initial viremic episode in infected horses (McGuire et al., 1994b). These were effector CTL (CTLe) not requiring in vitro stimulation and were present for at least 3 months after infection. Thereafter, CTLe levels in PBMC declined (unpublished observation). Recently, EIAV-specific memory CTL (CTLm) were demonstrated in PBMC from inapparent carriers using as target cells pokeweed mitogen-stimulated PBMC infected with recombinant vaccinia viruses expressing EIAV proteins (Hammond et al., 1997). One way to further evaluate the role of CTL in controlling viral load is to induce CTL in horses by immunization and to evaluate the effects of EIAV challenge. Since epitopes recognized by predominant CTLm from inapparent carriers may induce the requisite response for evaluation, experiments were done to demonstrate CTLm in carriers to equine kidney (EK) cell targets expressing EIAV proteins, to further indicate that the CTLm were MHC class I-restricted, and to do limiting dilution analysis to determine CTLm frequency to EIAV-infected target cells and to target cells expressing either Env or Gag/Pr proteins.

**MATERIALS AND METHODS**

**EIAV-infected and control horses**

Five horses (mixed breed ponies) infected with EIAV<sub>wsu5</sub> and 16 noninfected horses (mixed breed ponies) were used in these experiments. EIAV<sub>wsu5</sub> is a cell culture-adapted strain isolated after three sequential passages of the prototype strain (Malmquist et al., 1973) in horses and the predominant virus type selected by three limiting dilutions on EK cell cultures (O'Rourke et al., 1988). EIAV<sub>wsu5</sub> causes viremia and clinical disease in young horses (Perryman et al., 1988) and ponies (O'Rourke et al., 1988; McGuire et al., 1994b). Infection of H-507, H-521, and H-525 and the method of obtaining autologous EK cells from kidney biopsies for CTL target cells have been described (McGuire et al., 1994b). Similar procedures were used to obtain EK cells from 18 noninfected horses and to infect 2 of these, H-529 and H-532, with 10⁵ 50% tissue culture infective doses (TCID₅₀) of EIAV<sub>wsu5</sub>. Except for H-507 (McGuire et al., 1994b), the other 4 infected horses had at least one episode of fever, viremia, and thrombocytopenia. The mean platelet count for 16 noninfected horses (mixed breed ponies) in this study was 214,855/μl and thrombocytopenia was defined as <100,000/μl which was 2 SD below the mean. Equine lymphocyte alloantigen-A (ELA-A) locus encoded MHC class I proteins on lymphocytes (Table 1) were identified by microcytotoxicity assays using described serologic reagents (Bailey, 1980, 1983).

**TABLE 1**

<table>
<thead>
<tr>
<th>Horse number (ELA-A haplotype&lt;sup&gt;a&lt;/sup&gt;)</th>
<th>Months after EIAV infection</th>
<th>CTLm per 10⁶ PBMC (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-507 (A7/W11)</td>
<td>50</td>
<td>400 (299 – 535)</td>
</tr>
<tr>
<td>H-521 (A1/W11)</td>
<td>41</td>
<td>468 (314 – 696)</td>
</tr>
<tr>
<td>H-525 (A3/A9)</td>
<td>37</td>
<td>60 (39 – 90)</td>
</tr>
<tr>
<td>H-529 (A1/A5)</td>
<td>22</td>
<td>173 (127 – 234)</td>
</tr>
<tr>
<td>H-532 (A6/A9)</td>
<td>22</td>
<td>362 (271 – 484)</td>
</tr>
</tbody>
</table>

<sup>a</sup> The equine lymphocyte alloantigen (ELA-A) haplotypes were identified by microcytotoxicity assays using described serologic reagents (Bailey, 1980, 1983).

**Assays for CTLe and CTLm**

To measure CTLe, freshly isolated, nonstimulated PBMC (Wyatt et al., 1988) were evaluated using a ⁵¹Cr release assay with a 17-hr incubation period (McGuire et al., 1994b). The formula, % specific lysis = [(E – S)/ (M – S)] × 100 (Siliciano et al., 1985) was used, where E is the mean of six test wells, S is the mean spontaneous release from six target cell wells without effector cells, and M is the mean maximal release from six target cell wells with 2% Triton X-100 in distilled water. The standard error (SE) of percentage specific lysis was calculated using a formula that accounts for the variability of E, S,
and M (Siliciano et al., 1985). Only assays with a spontaneous lysis of <30% were used.

To detect CTLm, 10^7 PBMC were stimulated in 24-well plates with each well containing 1 ml of RPMI 1640 with 10% fetal calf serum, 5 x 10^{-5} M 2-mercaptoethanol, and 20 mM HEPES plus 10^6 irradiated stimulator PBMC which contained EIAVwsu5-infected monocytes. These stimulator cells were prepared by exposing 10^6 autologous PBMC to 3000 rad of gamma irradiation, mixing with 2 ml containing 10^6 TCID_{50}/ml EIAVwsu5, and incubating at 37°C for 1 hr. After 7 days of stimulation, viable lymphocytes were isolated using Histopaque and restimulated in a 24-well plate with each well containing 4 x 10^5 isolated lymphocytes and 10^6 stimulator cells in 1 ml of the above medium plus 20 units/ml recombinant human IL-2 (Gibco, Grand Island, NY), which stimulates equine lymphocytes (Magnarson et al., 1987). Restimulation was done every 3-4 days and lymphocytes stimulated for 14 to 20 days were evaluated for CTL activity using the same assay procedure and calculations described for CTL.

### CTLm frequency in PBMC from carrier horses to EIAV-infected targets

Quantitative estimates of CTLm were derived by limiting dilution analysis of PBMC that were first diluted and then stimulated (Carmichael et al., 1993). Dilutions of PBMC were made in DMEM with 15% fetal calf serum and 15% calf serum (DMEM +) so that 0.1 ml containing 10,000, 5000, 2500, and 1250 PBMC could be added to wells of a 96-well (round-bottom) plate. Three sets of 24 wells for each dilution were stimulated before assaying for CTL activity on three different target cells including EIAVwsu5-infected autologous and ELA-A mismatched EK cells and noninfected autologous EK cells. Stimulation of PBMC was done in each well by adding EIAVwsu5-infected monocytes. These stimulator cells were prepared by exposing 10^6 autologous PBMC to 3000 rad of gamma irradiation, mixing with 2 ml containing 10^6 TCID_{50}/ml EIAVwsu5, and incubating at 37°C for 1 hr. After 7 days of stimulation, viable lymphocytes were isolated using Histopaque and restimulated in a 24-well plate with each well containing 4 x 10^5 isolated lymphocytes and 10^6 stimulator cells in 1 ml of the above medium plus 20 units/ml recombinant human IL-2 (Gibco BRL, Grand Island, NY), which stimulates equine lymphocytes (Magnarson et al., 1987). Restimulation was done every 3-4 days and lymphocytes stimulated for 14 to 20 days were evaluated for CTL activity using the same assay procedure and calculations described for CTL.

CTLm killing and CTLm frequency using cell targets expressing EIAV Env and Gag/Pr proteins

Before using EK cell targets infected with recombinant vaccinia viruses expressing Env or Gag/Pr proteins in limiting dilution assays for CTLm, these target cells were evaluated with stimulated PBMC in CTL assays. One recombinant vaccinia virus was used, VGag/Pr, which expresses the EIAVwsu5 55-kDa Gag precursor and an 82-kDa Gag/Pol fusion protein including protease (PR), which are processed into subviral particles containing p26 (McGuire et al., 1994a). Another recombinant virus was VEnv2, which expresses the EIAVwsu5 Env precursor that is cleaved by cellular proteases into SU and TM proteins (McGuire et al., 1994b). A control recombinant virus, VSC11, which expresses beta-galactosidase, was also used, but EIAV proteins were not used (McGuire et al., 1994b). Finally, recombinant vaccinia virus-infected EK target cells expressing EIAV proteins were used to determine the frequency of CTLm in PBMC from five carrier horses infected for 22 to 50 months. The PBMC were diluted from 32,000 to 4000/well and stimulated, and limiting dilution analysis of CTL activity was done as described in the two preceding paragraphs.
RESULTS

CTLe and CTLm in PBMC from EIAV-infected horses

Prior to assays to measure CTL in PBMC, the five EIAV-infected horses were evaluated for 5 weeks by taking daily body temperatures and determining platelet counts, packed cell volumes, and plasma viremia three times per week. During this period, three horses (H-507, H-521, and H-525), infected for 34, 24, and 22 months, respectively, lacked plasma viremia detectable by assay on EK cell cultures (O’Rourke et al., 1988). They also lacked fever, anemia, and thrombocytopenia and were considered carriers. The two other horses (H-529 and H-532), which were infected for only 6 months, were not considered carriers as both had low-level, but detectable, viremia (10^{-3} and 10^{-2} TCID_{50}/ml, respectively). These two horses had no fever, thrombocytopenia, or anemia except for H-529, which had thrombocytopenia on one occasion.

To measure CTLe, freshly isolated, nonstimulated PBMC were used in the ^{51}Cr release assay. PBMC from carriers H-507 and H-521 did not have CTLe as neither caused significant lysis of autologous EIAV_{wsu5}-infected EK target at 10:1 and 50:1 effector to target (E:T) cell ratios (Fig. 1). However, PBMC from carrier H-525 at a 50:1 E:T cell ratio had low-level CTLe causing significant lysis (12.1%) of infected EK targets (Fig. 1). The percentage specific lysis of autologous infected EK target cells was considered significant when it exceeded the specific lysis of both autologous noninfected EK cells and ELA-A-mismatched, EIAV_{wsu5}-infected EK target cells by three times the standard error. PBMC from H-529, one of the two horses with low-level viremia, caused very low, but significant, lysis (8.6% at a 50:1 E:T cell ratio) of infected targets (Fig. 1). PBMC from H-532, the other horse with low-level viremia, had 13.7 and 22.0% lysis at E:T ratios of 10:1 and 50:1, respectively (Fig. 1).

CTLm were measured following in vitro stimulation of PBMC, and PBMC from carriers (H-507, 521, and 525) caused significant lysis of autologous EIAV_{wsu5}-infected EK target cells at E:T cell ratios of 5:1 and 25:1 with lysis at the 25:1 cell ratio ranging from 24.0 to 34.9% (Fig. 1). Stimulated PBMC from the two horses with low-level viremia also caused significant lysis of autologous infected targets at both E:T cell ratios with 25.8% for H-529 and 54.8% for H-532 at the 25:1 E:T cell ratio (Fig. 1).

Expression of MHC class I on EK target cells and restriction of CTL killing by ELA-A

Previous evidence that EIAV-specific CTLe from infected horses were MHC class I-restricted included failure to kill EIAV-infected target cells from other horses (mismatched) and elimination of CTL activity by removal of CD8+ T lymphocytes with a MAb to equine CD8 (McGuire et al., 1994b). The role of MHC class I-restricted killing of EIAV-infected EK target cells was implicated in the current paper by demonstrating that these cells expressed MHC class I, but not MHC class II molecules. Three MAbs (Davis et al., 1987; Kydd et al., 1994) reacting with monomorphic determinants of equine MHC class I (H58A, PT85A, and B5C) stained 97.4 to 98.4% of the EK cells by fluorescent flow cytometry while three MAbs reacting with monomorphic determinants of equine MHC class II (H34A, TH14B, and TH81A) bound 2.4 to 4.6% of EK cells. Isotype control MAbs bound 1.4 and 2.5% of EK cells.

Killing of infected EK target cells was further confirmed to be MHC class I-restricted by evaluating killing of target cells from horses of known ELA-A types by stimulated PBMC from H-532. These PBMC caused significant lysis of EIAV_{wsu5}-infected autologous EK target cells and of infected EK target cells from horses with one matched ELA-A haplotype, but not of infected target cells from horses with a mismatch of both ELA-A haplotypes (Fig. 2). The percentage specific lysis of mismatched, but infected, target cells was greater than uninfected target cells when A7/w11 and A1/w11 targets were used; however, the percentage lysis did not exceed that occurring with uninfected autologous cells and was not considered significant (see the definition in the legend to Fig. 2). The highest level of EIAV-specific killing occurred when the infected target cells were autologous and killing of infected target cells with one matched ELA-A haplotype was about 50% less (Fig. 2).

CTLm frequency in carrier horse PBMC to EIAV-infected targets

The number of CTLm to EIAV-infected targets was determined by limiting dilution analysis of PBMC from the same five infected horses described above, but it was done 16 months later. The horses were now infected for 22 to 50 months and all were considered carriers as clinical disease (anemia and thrombocytopenia) was not detected for 5 weeks and viremia was not detected by assay on EK cell cultures during the subsequent 4 weeks. The frequency of CTLm in the diluted and stimulated PBMC from these carrier horses to EIAV_{wsu5}-infected autologous EK target cells ranged between 60 and 468 per 10^6 PBMC (Table 1) with a mean frequency of 293 per 10^6 PBMC. There was no lysis in wells containing either
Prior to using EK cell targets infected with recombinant vaccinia viruses expressing EIAV proteins in limiting dilution assays, killing of these targets by stimulated PBMC from H-532 was evaluated. These PBMC caused significant lysis of autologous EK target cells infected with recombinant vaccinia viruses expressing either Env or Gag/Pr proteins when compared to controls of noninfected autologous EK target cells and autologous EK target cells infected with VSC11 (Fig. 3). The specific lysis at an E:T cell ratio of 10:1 was similar with target cells expressing Gag/Pr or Env proteins, but both targets were lysed less than EIAVwsu5-infected target cells (Fig. 3). The relative efficiency of EIAV protein epitope expression by MHC class I molecules on EK target cells infected with either EIAV or recombinant vaccinia viruses expressing EIAV proteins is unknown. The demonstration of CTLm to both Env and Gag/Pr protein epitopes indicated that cell targets expressing these epitopes were suitable for use in limiting dilution analysis.

CTLm frequency in carrier horse PBMC to targets expressing Env or Gag/Pr proteins

The number of CTLm recognizing either target cells expressing Env proteins or Gag/Pr proteins was deter-
with CTLm from two horses with low-level viremia. There was low-level, but significant, specific lysis by CTLe in unstimulated PBMC from one of three EIAV carriers. This latter observation was similar to the detection of in vivo-activated CTL in a person infected with HIV-1 for 15 years and with very low viral load (Harrer et al., 1996). In the case of the carrier horse, the presence of activated CTL may result from low-level virus production undetectable by in vitro assays for infectious virus used here, yet perhaps detectable by RT-PCR assay (Langemeier et al., 1996). Lentiviral particles in the blood, whether infectious or noninfectious, indicate viral protein expression by cells and could provide an antigen stimulus in EIAV carriers both for the CTLe demonstrated in the current paper and for the previously described persistence of antibody to EIAV proteins (Hammond et al., 1997). It was concluded from these results that low-level CTLe could occur in a carrier horse and that CTLm to EIAV-infected HK target cells were easily demonstrated in the three carrier horses evaluated. Moreover, CTLm from a carrier horse recognized epitopes from EIAV Env and Gag/Pr proteins as previously described for CTLe from recently infected horses (McGuire et al., 1994b; Hammond et al., 1997) and for CTLm from carriers (Hammond et al., 1997), allowing target cells expressing these proteins to be used in limiting dilution analysis.

The CTLm lysis of HK target cells infected with EIAV or expressing EIAV proteins was indicated to be MHC class I-restricted by demonstrating that HK target cells expressed MHC class I molecules, but not class II molecules. The CTL-specific lysis of HK target cells was as high as 54.8% (Fig. 1) and occurred in the absence of MHC class II molecules. This indication was further supported by demonstrating lysis of autologous EIAV-infected target cells and of infected target cells from horses with one matched ELA-A molecule, but not of infected target cells from horses with a mismatch of both ELA-A molecules. The data in this paper suggest that the killing observed was MHC class I-restricted, but do not provide direct proof. Previous work demonstrated that depleting CD8+ T lymphocytes removed EIAV-specific CTLe from PBMC from horses with recent EIAV infections (McGuire et al., 1994b). Studies of EIAV-specific CTLm in PBMC from inapparent carriers using pokeweed mitogen-stimulated PBMC targets infected with recombinant vaccinia viruses expressing EIAV proteins demonstrated both MHC class I- and MHC class II-restricted killing, although the MHC class I killing was greater (Hammond et al., 1997). In addition, MHC class I restriction occurs with equine CTL killing of equine herpes virus I-infected target cells (Allen et al., 1995).

The frequency of CTLm in PBMC from carrier horses to EIAV-infected HK target cells ranged from 60 to 468 per 10^6 PBMC. These values are similar to some reported for people infected with HIV-1 (Carmichael et al., 1993), but are lower than those reported for a defined group of

### Table 2

<table>
<thead>
<tr>
<th>Horse number</th>
<th>VSC11 controla</th>
<th>VEnv2b</th>
<th>VGag/Pr,c</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-507</td>
<td>&lt;2</td>
<td>89 (66-118)</td>
<td>59 (44-80)</td>
</tr>
<tr>
<td>H-521</td>
<td>13</td>
<td>286 (205-400)</td>
<td>190 (140-259)</td>
</tr>
<tr>
<td>H-525</td>
<td>&lt;2</td>
<td>4 (2-9)</td>
<td>&lt;2</td>
</tr>
<tr>
<td>H-529</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>25 (17-36)</td>
</tr>
<tr>
<td>H-532</td>
<td>&lt;2</td>
<td>38 (25-59)</td>
<td>37 (24-58)</td>
</tr>
</tbody>
</table>

a Recombinant vaccinia virus expressing β-galactosidase, but no EIAV proteins.
b Recombinant vaccinia virus expressing EIAV Env proteins SU and TM.
c Recombinant vaccinia virus expressing EIAV Gag proteins and protease.

The initial CTL assays in this study evaluated both PBMC stimulated in vitro with EIAV-infected autologous PBMC and nonstimulated PBMC. In vitro stimulation of PBMC from three carrier horses resulted in high levels of specific lysis by CTLm. Similar results were obtained

### DISCUSSION

The initial CTL assays in this study evaluated both PBMC stimulated in vitro with EIAV-infected autologous PBMC and nonstimulated PBMC. In vitro stimulation of PBMC from three carrier horses resulted in high levels of specific lysis by CTLm. Similar results were obtained...
HIV-1-infected, long-term nonprogressors (Rinaldo et al., 1995). For comparison, the mean frequency of anti-EIAV CTLm in five carriers was 293 per 10^6 PBMC while anti-HIV-1 CTLm from seven long-term nonprogressor humans was approximately 1000 per 10^6 PBMC using targets infected with a vaccinia virus expressing Env, Gag, and Pol proteins (Rinaldo et al., 1995). The frequency of CTLm in PBMC from four carrier horses recognizing target cells infected with recombinant vaccinia viruses ranged from 4 to 286 per 10^6 using targets expressing EIAV Env proteins and 25 to 190 per 10^6 using targets expressing EIAV Gag/Pr proteins. There was a direct correlation between the number of CTLm to EIAV-infected target cells and the sum of CTLm to Env and Gag/Pr proteins. However, in 4 of 5 PBMC, the sum reacting to the two proteins was less than the number reacting to EIAV-infected cell targets. This observation may be explained by the presence of CTLm to other EIAV proteins such as Pol and regulatory proteins such as those described for humans infected with HIV-1 (Rinaldo et al., 1995). The reason for EIAV-specific, MHC class I-restricted CTLm in stimulated PBMC in 1 of 16 noninfected horses is unknown; however, a similar observation is described for a healthy non-HIV-1-infected human (Hoffenbach et al., 1989). The CTLm detected in noninfected horse H-504 may have been stimulated by another organism and cross-reacted with an epitope on an EIAV protein. However, these CTLm from H-504 did not recognize an epitope on EIAV Env or Gag/Pr proteins because the CTLm did not kill target cells infected with recombinant vaccinia viruses expressing these proteins.

The data presented in this paper demonstrate that the five EIAV carrier horses examined had easily detectable EIAV-specific, MHC class I-restricted CTLm in their PBMC. This observation and the presence of CTLm as high as 468 per 10^6 PBMC in a carrier horse with a low virus load and infected for over 41 months provide the evidence needed to continue evaluating the role that these CTLm have in EIAV control and the maintenance of the carrier state. The specificity of the CTLm from three carrier horses included both Env and Gag/Pr protein epitopes, while CTLm from two others recognized either Env or Gag/Pr protein epitopes. However, it is likely that the specificity of CTLm extends to other EIAV proteins. The frequency of CTLm demonstrated is suitable for the identification of the epitopes recognized by predominant CTLm from carrier horses that have low virus loads. This will allow testing of the efficacy of identified epitopes for inducing CTL responses by immunization and evaluating the role of induced CTL responses in controlling viremia following EIAV challenge.

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