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Early detection of dominant Env-specific and subdominant Gag-specific CD8+ lymphocytes in equine infectious anemia virus-infected horses using major histocompatibility complex class I/peptide tetrameric complexes

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Abstract

Cytotoxic T lymphocytes (CTL) are critical for control of lentiviruses, including equine infectious anemia virus (EIAV). Measurement of equine CTL responses has relied on chromium-release assays, which do not allow accurate quantitation. Recently, the equine MHC class I molecule 7-6, associated with the ELA-A1 haplotype, was shown to present both the Gag-GW12 and Env-RW12 EIAV CTL epitopes. In this study, 7-6/Gag-GW12 and 7-6/Env-RW12 MHC class I/peptide tetrameric complexes were constructed and used to analyze Gag-GW12- and Env-RW12-specific CTL responses in two EIAV-infected horses (A2164 and A2171). Gag-GW12 and Env-RW12 tetramer-positive CD8+ cells were identified in nonstimulated peripheral blood mononuclear cells as early as 14 days post-EIAV inoculation, and frequencies of tetramer-positive cells ranged from 0.4% to 6.7% of nonstimulated peripheral blood CD8+ cells during the 127-day study period. Although both horses terminated the initial viremic peak, only horse A2171 effectively controlled viral load. Neutralizing antibody was present during the initial control of viral load in both horses, but the ability to maintain control correlated with Gag-GW12-specific CD8+ cells in A2171. Despite Env-RW12 dominance, Env-RW12 escape viral variants were identified in both horses and there was no correlation between Env-RW12-specific CD8+ cells and control of viral load. Although Gag-GW12 CTL escape did not occur, a Gag-GW12 epitope variant arose in A2164 that was recognized less efficiently than the original epitope. These data indicate that tetramers are useful for identification and quantitation of CTL responses in horses, and suggest that the observed control of EIAV replication and clinical disease was associated with sustained CTL recognition of Gag-specific epitopes.

Keywords: EIAV; Viral load; CD8+ lymphocyte frequency; Tetramers; CTL; CTL escape

Introduction

Equine infectious anemia virus (EIAV) is a macrophage-tropic lentivirus that causes persistent infection in horses. Infected horses usually develop recurrent episodes of plasma viremia and associated acute clinical disease (fever, inappetence, lethargy, thrombocytopenia, and anemia) during the first few months of infection. However, in contrast to other lentiviral infections, including human immunodeficiency virus-1 (HIV-1), most horses control EIAV replication within a year and remain persistently infected inapparent carriers (Cheevers and McGuire, 1985; Montelaro et al., 1993; Sellon et al., 1994). Results of EIAV infection in severe combined immunodeficient (SCID) Arabian foals, as well as immune reconstitution in a SCID foal concurrent with EIAV challenge, indicate that this

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control of viral replication is mediated by viral-specific immune responses (Perryman et al., 1988; Crawford et al., 1996; Mealey et al., 2001). Due to robust viral-specific immune responses that contain viral replication and clinical disease, EIAV infection in horses is a useful model system for the study of lentiviral immune control.

Major histocompatibility complex (MHC) class I-restricted viral-specific CD8+ cytotoxic T lymphocytes (CTL) are important for lentiviral immune control, including EIAV. Direct evidence for CD8+ lymphocyte control of simian immunodeficiency virus (SIV) in infected rhesus monkeys is provided by in vivo depletion of CD8+ lymphocytes with monoclonal antibody (Schmitz et al., 1999; Jin et al., 1999). Similar to HIV-1 infection, the initial plasma viremia in acute EIAV infection is often terminated concurrent with the appearance of CTL, but prior to the appearance of neutralizing antibody (McGuire et al., 1990, 1994; Borrow et al., 1994; Pantaleo and Fauci, 1995). EIAV proteins that contain CTL epitopes include Gag, Pol, Env, Rev, and S2 (Lonning et al., 1999; McGuire et al., 2000; Zhang et al., 1998; Mealey et al., 2003; Hammond et al., 1997). Several optimal CTL epitopes have been mapped, including Gag-GW12 and Env-RW12, both of which are restricted by the ELA-A1 haplotype (Mealey et al., 2003). The Env-RW12 epitope occurs within the V3 hypervariable region in the envelope SU, and in one EIAV-infected ELA-A1 horse that experienced early progressive disease, viral variants arose that escaped Env-RW12-specific CTL recognition (Mealey et al., 2003). However, since Env-RW12 occurs within the principle neutralizing domain (PND) of EIAV SU, it is possible that neutralizing antibody was involved in selection of the observed escape viral variants (Mealey et al., 2003). In contrast, viral variants containing amino acid changes within the Gag-GW12 epitope occurred in the same horse, but did not escape CTL recognition (Mealey et al., 2003). Interestingly, the equine MHC class I molecule 7-6, which was derived from a horse with the ELA-A1 haplotype, presents both the Gag-GW12 and the Env-RW12 peptides to Gag-GW12- and Env-RW12-specific CTL, respectively (McGuire et al., 2003). The 7-6 molecule binds the Env-RW12 peptide with higher affinity than the Gag-GW12 peptide (McGuire et al., 2003), and based on chromium-release assays, the Env-RW12 CTL response is immunodominant over the Gag-GW12 response (Mealey et al., 2003).

To date, analysis of EIAV-specific CTL responses has relied on standard chromium-release assays, using equine kidney cells or pokeweed mitogen-stimulated PBMC as targets (Hammond et al., 1998; McGuire et al., 1994). Although chromium-release assays provide important functional information, they are technically-demanding, labor-intensive, and utilize a radioactive isotope. Additionally, the only available method to quantitate equine CTL has been limiting dilution analysis (Kydd et al., 2003; McGuire et al., 1997; Mealey et al., 2001; O'Neill et al., 1999), which requires in vitro stimulation and is known to underestimate CTL frequency (Ogg and McMichael, 1999).

The development of MHC class I/peptide tetrameric complexes has provided a rapid method for direct identification of antigen-specific T lymphocytes in peripheral blood (Altman et al., 1996). Tetramer staining has been useful for analyzing SIV-specific CTL, correlating well with cytotoxic activity, and has allowed quantitation of dominant SIV-specific CTL responses (Egan et al., 1999). Studies using tetramers have confirmed that the emergence of CTL coincides with clearance of virus during SIV infection (Kuroda et al., 1999), and that HIV-specific CTL frequency and plasma viral load are inversely correlated (Ogg et al., 1998), suggesting that CTL play a significant role in controlling HIV infection. However, other work has indicated that the inverse correlation between tetramer-positive CD8+ T cells and viral load does not exist in HIV-1-infected patients with low CD4+ T cell numbers, providing evidence that tetramer-positive cells can have impaired function, verified by measuring IFN-γ production (Kostense et al., 2001). In HIV-1-infected children, CTL frequency as measured by IFN-γ ELISPOT correlates positively with viral load and is lower than that determined by tetramer staining, again indicating that a significant number of HIV-1-specific CD8+ T cells are not functional (Buseyne et al., 2002). Similarly, intracytoplasmic IFN-γ staining has shown that total HIV-1-specific CD8+ responses correlate positively with viral load, suggesting that frequencies of HIV-1-specific CD8+ T cells are not the sole determinant of HIV-1 immune control (Betts et al., 2001). In contrast, standard chromium-release assays have shown an inverse correlation between HIV-1-specific CTL activity and viral load in HIV-1-infected long-term survivors (Betts et al., 1999).

Interpreting the correlations between lentiviral load and lentivirus-specific CTL frequency, whether measured by chromium-release assay, tetramer staining, IFN-γ ELISPOT, or intracytoplasmic IFN-γ staining, remains difficult. Positive correlations likely indicate viral replication driving CTL expansion, or alternatively, that CTL are not effectively controlling viral load. Negative correlations could indicate effective CTL-mediated viral control, or alternatively, virus-mediated immunosuppression. With respect to the latter, transient immunosuppression associated with febrile episodes is known to occur during EIAV infection (Murakami et al., 1999; Newman et al., 1991).

The purpose of the present study was to determine the relationship between CTL response and viral load during early EIAV infection. Two tetramers, 7-6/Gag-GW12 and 7-6/Env-RW12, were constructed to allow direct quantitation of Gag-GW12- and Env-RW12-specific CD8+ lymphocytes during the first 127 days of EIAV infection in two horses (A2164 and A2171) sharing the ELA-A1 haplotype. Chromium-release assays were also performed to confirm functional cytotoxic activity. In addition, neutralizing antibody responses were measured, and plasma virus was