Veterinary Immunology and Immunopathology 161 (2014) 161–169

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Research paper

Epitope shifting of gp90-specific cellular immune responses in EIAV-infected ponies

Chong Liu[a], Sheila J. Cook[a], Jodi K. Craigo[b], Frank R. Cook[a], Charles J. Issel[a], Ronald C. Montelaro[b], David W. Horohov[a,*]

[a] Department of Veterinary Science, University of Kentucky, Lexington, KY, USA
[b] Center for Vaccine Research and Department of Microbiology and Molecular Genetics, University of Pittsburgh, Pittsburgh, PA, USA

ARTICLE INFO

Article history:
Received 26 March 2014
Received in revised form 2 July 2014
Accepted 4 August 2014

Keywords:
EIAV
Lentivirus
Cellular immune responses
Envelope
Immunodominant shifting

ABSTRACT

Unlike other lentiviruses, EIAV replication can be controlled in most infected horses leading to an inapparent carrier state free of overt clinical signs which lasts for many years. While the resolution of the initial infection is correlated with the appearance of virus specific cellular immune responses, the precise immune mechanisms responsible for control of the infection are not yet identified. Since the virus undergoes rapid mutation following infection, the immune response must also adapt to meet this challenge. We hypothesize that this adaptation involves peptide-specific recognition shifting from immunodominant variable determinants to conserved immunorecessive determinants following EIAV infection. Forty-four peptides, spanning the entire surface unit protein (gp90) of EIAV, were used to monitor peptide-specific T cell responses *in vivo* over a six-month period following infection. Peptides were injected intradermally and punch biopsies were collected for real-time PCR analysis to monitor the cellular peptide-specific immune responses *in vivo*. Similar to the CMI response to HIV infection, peptide-specific T cell recognition patterns changed over time. Early post infection (1 month), immune responses were directed to the peptides in the carboxyl-terminus variable region. By six months post infection, the peptide recognition spanned the entire gp90 sequence. These results indicate that peptide recognition broadens during EIAV infection.

1. Introduction

Equine infectious anemia virus (EIAV) has been used as a model for investigating the underlying protective immune mechanisms against lentiviruses (Craigo and Montelaro, 2011). Following infection, most horses control their initial EIAV infection leading to an inapparent carrier state (Hammond et al., 2000; Harrold et al., 2000). Immune responses play an important role in controlling EIAV infection (Mealey et al., 2001; Perryman et al., 1988) since the administration of immunosuppressive drugs to inapparent carriers can induce the recurrence of disease (Craigo et al., 2002). This development of protective immunity is protracted and often requires 6–12 months to become established (Hammond et al., 2000; Harrold et al., 2000). Based on this evidence of protective immunity in EIAV inapparent carriers, it has been proposed that a successful vaccine should induce similar immune responses (Tagmyer et al., 2007). An attenuated strain (D9) of EIAV provided protection against homologous virus challenges (Craigo et al., 2007), but optimum protective immunity was

* Corresponding author at: Maxwell H. Gluck Equine Research Center, Department of Veterinary Science, College of Agriculture, University of Kentucky, Lexington, KY, USA. Tel.: +1 859 257 4757; fax: +1 859 257 8542.
E-mail addresses: David.Horohov@uky.edu, dwhoro2@uky.edu (D.W. Horohov).

http://dx.doi.org/10.1016/j.vetimm.2014.08.001
0165-2427/

Table 1
MHC haplotypes.

Horse ID	Virus	UMN-JH34-2[a]	COR112–COR113–UM011–COR114[b]
G35	$EIAV_{D9}$	219-252-280-172-247	207-254-268-174-235
L126	$EIAV_{D9}$	211-260-268-166-247	211-260-268-166-247
L132	$EIAV_{D9}$	219-252-280-172-247	211-260-268-166-247
F31	$EIAV_{D9}$	205-252-274-168-243	203-260-266-168-249
I28	$EIAV_{D9}$	215-252-260-168-243	211-260-268-166-247
H46	$EIAV_{D9}$	221-268-278-174-241	219-252-280-172-247
D49	$EIAV_{D9}$	211-260-278-174-241	211-258-274-164-237
H38	$EIAV_{D9}$	219-252-280-172-247	223-252-268-166-247
H36	$EIAV_{D9}$	211-260-278-174-241	205-252-274-168-243
H32	$EIAV_{D9}$	211-260-278-174-241	219-252-280-172-247
D55	$EIAV_{D9}$	215-252-260-168-243	211-258-274-164-237
I35	$EIAV_{D9}$	205-262-270-184-245	221-262-270-172-237
J30	$EIAV_{D9}$	197-248-270-184-245	219-244-268-172-249
L124	$EIAV_{D9}$	221-262-270-172-237	221-238-264-180-243
I33	$EIAV_{D9}$	219-236-266-168-249	203-260-266-178-241
L128	$EIAV_{D9}$	219-250-266-170-245	211-262-270-184-245
D64	$EIAV_{PV}$	219-238-266-168-249	211-238-266-178-241

[a] UMN-JH34-2 is an equine MHC microsatellite constellation corresponding to ELA-I.
[b] COR112, COR113, UM011 and COR114 represent microsatellite constellations corresponding to ELA-II haplotypes.

not seen until six months post vaccination. Thus, protective immunity against EIAV infection is a lengthy process that likely involves the development of immunity to viral variants (Hammond et al., 2000). Analyses of longitudinal serum samples from EIAV infected horses displayed an evolution of envelope-specific antibodies responses as measured by changes in avidity, conformational recognition, and neutralization titers in EIAV infected horses (Hammond et al., 1997, 1999).

Cellular immune responses also appear important in controlling EIAV infections as the appearance of EIAV-specific cytotoxic T lymphocytes (CTL) correlates with the control of the initial viremia (McGuire et al., 2000). Additionally, CTL responses are detected in inapparent carriers during the chronic phase of EIAV infection (Chung et al., 2005; Mealey et al., 2003; Zhang et al., 1999). Both T helper (Th) and CTL epitopes have been identified in the EIAV envelope protein of EIAV-infected horses (Tagmyer et al., 2008). While various studies have demonstrated EIAV Gag and Pol specific CTL responses (Lonning et al., 1999; Mealey et al., 2005) and identified broadly reactive T-cell epitopes (Chung et al., 2004, 2005; McGuire et al., 2000), the identification of epitope-specific cellular immune responses that correlate with protection has proven elusive (Mealey et al., 2009).

Not all potential epitopes in an antigenically complex viral antigen can be recognized by CD4 or CD8T cells (Gallimore et al., 1998). Also, not all recognized epitopes elicit equal responses. Typically, T cell responses focus on one or a few epitopes (immunodominant epitopes) and the remainder of the epitopes (immunorecessive epitopes) elicit limited responses (Yewdell and Bennink, 1999). Most acute viral infections involve the early recognition of immunodominant epitopes leading to the eradication of the infection (Chisari and Ferrari, 1995; Perelson et al., 1993). By contrast, there is a shift in T cell recognition from immunodominant to immunorecessive epitopes during chronic viral infections (Fuller et al., 2004; Turner et al., 2005; Wherry et al., 2003). This epitope shifting is evidenced by increases in the specificity, breadth and magnitude of T cell responses over time (Moskophidis et al., 1993). Thus, initial HIV-specific T cell responses are skewed toward immunodominant epitopes in the antigenically variable regions of the virus in the initial stages of the infection, whereas immunorecessive epitopes in the more conserved regions are recognized during the later stages of infection (Goulder et al., 2001; Jamieson et al., 2003; Liu et al., 2013). It has been proposed that protective immunity likely requires T cell recognition of these immunorecessive epitopes in the antigenically conserved regions of HIV (Frahm et al., 2006; Goonetilleke et al., 2009).

Here, we show that EIAV envelope-specific T cell recognition also undergoes a shift in epitope recognition. Initially, the T cell response is directed toward the more variable regions of the viral envelope protein, gp90, and then shifts to more conserved regions later in the infection. This shift in epitope recognition is associated with the control of viral replication and the establishment of the inapparent carrier state.

2. Materials and methods

2.1. Ponies, virus and experimental challenge

Sixteen ponies of mixed age and gender, seronegative for EIAV, were used in this study. All the ponies were also pre-screened by intradermal inoculation of peptide pools A–G prior to EIAV infection (see below) and no responses were detected. MHC haplotypes were determined using polymorphic microsatellites, as previously described (Tseng et al., 2010). Five ponies (G35, L132, H46, H38 and H32) shared the same MHC microsatellite allele 219-252-280-172-247, three of the ponies (L126, L132 and I28) shared the allele 211-260-268-166-247, and another three ponies (D49, H36 and H32) shared the same MHC microsatellite allele 211-260-278-174-241 (Table 1). An inapparent carrier D64 was also included for comparisons. This horse was infected with $EIAV_{PV}$ strain in a previous study (Tagmyer et al., 2007). Throughout this study period,