Transient immune suppression of inapparent carriers infected with a principal neutralizing domain-deficient equine infectious anaemia virus induces neutralizing antibodies and lowers steady-state virus replication

Jodi K. Craigo,1 Caroline Leroux,1† Laryssa Howe,1 Jonathan D. Steckbeck,1 Sheila J. Cook,2 Charles J. Issel2 and Ronald C. Montelaro1

1 Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, USA
2 Department of Veterinary Science, Gluck Equine Research Center, University of Kentucky, Lexington, KY 40546, USA

The genetic variation of equine infectious anaemia virus (EIAV) clearly affects the antigenic properties of the viral envelope; however, effects on immunogenicity remain undefined, although widely assumed. Here, the immunogenicity is reported of a novel, neutralization-resistant, pony-isolate envelope EIAV_PND that contains a 14-residue deletion in the designated principal neutralizing domain (PND) of the gp90 protein. Two ponies inoculated with a chimeric virus, EIAV_PND, containing the EIAV_PND envelope in a reference provirus strain, remained asymptomatic through 14 months post-inoculation, producing high steady-state levels of envelope-specific antibodies but no detectable serum-neutralizing antibodies. Consequent dexamethasone-induced immune suppression produced characteristic EIA that resolved concomitantly with the development of high-titre, strain-specific, neutralizing antibodies and a 100-fold reduction in steady-state virus loads. These results demonstrate: natural variations in the EIAV envelope have profound effects on both antigenic and immunogenic properties; the PND is not required for neutralizing antibody responses; and transient immune suppression can enhance established host immunity to achieve more effective control of steady-state lentivirus replication.

Equine infectious anaemia virus (EIAV) infection of horses results in a dynamic, rapidly progressing disease, which contrasts markedly with the slowly progressive degenerative diseases that characterize other lentivirus infections, including human immuno deficiency virus type 1 (HIV-1) infection of humans (Montelaro et al., 1993). Experimental EIAV infection of horses results in a disease that manifests in three stages: the acute and chronic stages, both accompanied by clinical symptoms (fever, weight loss, thrombocytopenia, anaemia, etc.) and associated viraemia, and the inapparent stage; although lacking outward clinical symptoms, this stage does maintain detectable infecting virus and virus-specific antibodies (Hammond et al., 2000; Harrold et al., 2000; Montelaro et al., 1993). The EIAV system therefore serves as a unique model system for the natural immunological control of virus replication and disease associated with lentivirus infections (Hammond et al., 1997, 2000; Leroux et al., 1997, 2001).

Sequence analysis of EIAV envelope variation during progressive disease cycles demonstrates distinct envelope variants with each wave of viraemia (Leroux et al., 1997, 2001; Lichtenstein et al., 1996; Payne et al., 1987a). The emergence of novel EIAV populations with sequential disease episodes indicates the cyclical nature of chronic disease that results from sequential production and selection of viral antigenic variants temporarily escaping established host immune responses. The principal site of antigenic variation is the envelope surface unit glycoprotein gp90 (Payne et al., 1987b; Rwambo et al., 1990a; Zheng et al., 1997). The genomic variation of gp90 has been analysed in order to define conserved and variable protein domains (Leroux et al., 1997, 2001; Payne et al., 1989), as observed with other animal and human lentiviruses (Greene et al., 1993; Simmonds et al., 1990; Starcich et al., 1986; Suarez & Whetstone, 1995). While the role of envelope variation in altering in vitro antigenic properties (e.g. neutralization sensitivity) has been well documented for over 20 years, there is, to date, no analysis of the effects of EIAV gp90 variation on immunogenicity in experimentally infected equids.

Studies indicate that control of EIAV replication and disease is specifically mediated by host immune responses...
controlling virus infection to subclinical levels and not by attenuation of the virus during persistent infection (Issel et al., 1982; Kono et al., 1976a; Tumas et al., 1994). Immune management of EIAV replication and disease is, evidently, the result of the evolution of both humoral and cellular immune responses (Hammond et al., 1997). The predominant humoral response, including virus-neutralizing antibodies to EIAV infection, is directed against gp90 (Rwambo et al., 1990b). We defined previously three neutralizing epitopes in gp90 (Ball et al., 1992; Hussain et al., 1987, 1988). Epitopes D_NT and E_NT are localized to the V3 domain of gp90, while epitope C_NT is associated with the V5 domain. The V3 region has been designated the principal neutralizing domain (PND) (Ball et al., 1992).

As part of a previous comprehensive investigation of the immune responses to virus infection and viral genomic variation in four ponies experimentally infected with EIAV_PP (Hammond et al., 1997, 2000; Leroux et al., 1997, 2001), we isolated and characterized a novel serum neutralization-resistant variant containing a 14 amino acid deletion in the PND segment of gp90 (EIAV_PP&’PND). The entire envelope of this variant was cloned into a reference strain of EIAV to generate a chimeric clone to further analyse the effects of the PND deletion on in vitro virus replication (Leroux et al., 1997). These studies indicated that the PND deletion did not affect in vitro replication properties as compared to the reference EIAV_PP strain. However, EIAV_PP&’PND was not neutralized in cell culture by homologous or autologous immune serum from the four experimentally infected ponies or other reference-neutralizing immune serum, all displaying high neutralizing activity of the reference strain EIAV_PP (Leroux et al., 1997). In light of the marked neutralization-resistant phenotype of the EIAV_PP&’PND variant, we sought to examine in an experimental infection the immunogenicity of this variant envelope and the role of host immune responses in controlling the replication of EIAV_PP&’PND.

To elucidate the effects of the deleted PND on virus...
replication and immunogenicity during experimental infections, two mixed-breed ponies (animals #599 and #672) were inoculated intravenously with $1 \times 10^3$ TCID$_{50}$ EIAV$_{\Delta PND}$. The infected ponies remained asymptomatic for EIA throughout a 14-month observation period (Fig. 1). Virus replication during the initial acute infection, around 30 days post-infection (p.i.), reached approximately $1 \times 10^4$ plasma RNA copies/ml, then rapidly declined to steady-state levels of about $1 \times 10^4$ copies/ml for the following 13 months. We previously demonstrated that clinical EIA is typically associated with replication levels of at least $1 \times 10^7$ plasma RNA copies/ml (Hammond et al., 2000; Leroux et al., 2001). The apparent lack of disease during the EIAV$_{\Delta PND}$ infection appeared to be due to the control of virus replication to subclinical levels, indicating the ability of the host immune system to effectively target virus in the absence of the immunodominant gp90 PND.

Both ponies developed high-titre, envelope-specific EIAV antibodies within 2 months p.i., seroconverted by 30 days p.i. and maintained a steady, relatively high-titre of envelope-specific serum antibodies ($1 \times 10^6$) for the remainder of the 14-month observation period. The lack of detectable neutralizing antibodies to the infecting EIAV$_{\Delta PND}$ or the reference-EIAV$_{PV}$ strains, however, were not detected in either pony over the 14-month observation period. The apparent lack of disease during the EIAV$_{\Delta PND}$ infection appeared to be due...
strains containing complete gp90 proteins (e.g. EIAV_{PV}), wherein serum-neutralizing antibodies to the infecting virus are typically detected by 2 months p.i. (Hammond et al., 1997, 2000). The lack of neutralizing antibody responses to persistent infection by EIAV_{APND} demonstrated for the first time the immunodominant role of the gp90 PND in eliciting neutralizing antibodies in infected ponies, as designated previously from in vitro neutralization studies (Ball et al., 1992).

To examine further the role of host immune responses in controlling EIAV_{APND} replication, the two ponies were transiently immune-suppressed (420 days p.i.). It has been demonstrated previously that administration of dexamethasone (Baus et al., 1996) to long-term EIAV inapparent carriers results in the overall suppression of the immune system and recrudescence of clinical EIA (Kono et al., 1976b; Tumas et al., 1994). We administered 0.11 mg/kg of dexamethasone per day for 10 days to each infected pony (420–430 days p.i.), as described previously (Tumas et al., 1994). Delayed-type hypersensitivity assays (Baus et al., 1996; Hodgkin et al., 1978) and lymphoproliferation assays of the PBMCs (Hammond et al., 1997) at multiple time-points throughout the dexamethasone treatment indicated effective immune suppression by the 10-day regimen (data not shown).

By 2 days after the last dose of dexamethasone, both ponies developed a classic EIA episode, experiencing a simultaneous drop in platelets and an increase in temperature (Fig. 1). Recrudescence of disease was accompanied by marked increases in plasma viral RNA (Fig. 1). Virus replication increased during the immune-suppression period from steady-state levels of $1 \times 10^4$ RNA copies/ml (420 days p.i.) to a peak of $1 \times 10^8$ copies/ml (432 days p.i.) (Fig. 1). Clinical symptoms resolved by day 434, accompanied by a rapid decline in viral RNA levels, presumably reflecting the return of host immune control. Interestingly, steady-state levels of replication following resolution of the disease cycle were at least 100-fold lower than the steady-state levels observed prior to immune suppression. These observations seem to indicate the enhancement of immune control of the persistent virus infection as a result of transient immune suppression.

Concomitant with the resolution of clinical symptoms and decrease in virus load was a marked increase in envelope-specific antibodies accompanied by the appearance of detectable neutralizing antibodies to the infecting EIAV_{APND} strain (Fig. 2). The envelope-specific serum antibody titre, which had remained at $1 \times 10^3$ for over 1 year, increased to between $1 \times 10^6$ and $1 \times 10^6.5$ following the dexamethasone-induced disease episode (Fig. 2). This increase in antibody titre was accompanied by the development of neutralizing antibody titres (1:350 to 1:500) against the infecting EIAV_{APND} strain (Fig. 2). Serum-neutralization levels observed in the current experimental infections with EIAV_{APND} after immune suppression were similar to responses observed in experimental infection with the reference EIAV_{PV} strain (Hammond et al., 2000). However, neutralizing antibody responses appeared highly type-specific for the APND envelope. Identical serum samples had no detectable neutralizing activity against the reference EIAV_{PV} strain containing prototypic gp90 with a complete PND (Fig. 2). Taken together, these observations indicate the ability of the host immune system to generate effective neutralizing antibody responses to a highly neutralization-resistant EIAV envelope lacking an intact immunodominant PND in the gp90 protein, implying that immunorecessive determinants outside the PND can in fact serve as primary determinants for neutralizing antibodies in the absence of the PND.

The unexpected development of neutralizing antibodies to the EIAV_{APND} strain post-immune suppression could be caused by either quantitative increases in existing antibody responses to the EIAV_{APND} envelope or qualitative changes in antibody specificity triggered by envelope variation post-immune suppression. To examine these two possibilities, we characterized and compared viral envelope genomic quasispecies present immediately prior to immune suppression and the population associated with the febrile episode after immune suppression (Fig. 3). In general, viral envelopes detected pre- and post-immune suppression revealed approximately 3–4% variation from the envelope sequence of the EIAV_{APND} inoculum. Importantly, all envelope clones retained a PND-deleted V3 region, with amino acid variations localized to variable domains of the gp90 defined previously. These data demonstrated a similar evolution rate of EIAV envelopes during persistent infection in the presence or absence of detectable serum-neutralizing antibodies, suggesting there are selective pressures by other immune or non-immune host factors on virus variation. Relative to the issue of the antigen specificity of the neutralizing antibody responses, the envelope sequence data revealed that post-immune suppression virus populations contained distinguishing sequence differences compared to viral envelopes present prior to immune suppression. Thus, it is possible that induction of neutralizing antibodies may be due to both qualitative changes in the viral envelope and quantitative changes in viral antigen load due to immune suppression.

The type-specific nature of the post-immune suppression neutralizing antibody response was unexpected. Given that the gp45 envelope proteins of the two virus strains utilized in the assay (EIAV_{PV} and EIAV_{APND}) are identical (Leroux et al., 1997, 2001), sensitivity to neutralization is evidently related to sequence differences in the related gp90 proteins. A comparison of gp90 sequences of the strains reveals minor differences in the V2, V4, V5 and C6 domains (Fig. 3) in addition to the PND deletion in the V3 region. The V4 and C6 regions of neutralization-sensitive EIAV_{APND} and neutralization-resistant EIAV_{PV} differ by a single residue, repositioning a potential N-linked glycosylation site in the V4 domain and creating one in the C6 domain of their respective gp90 regions. Changes in N-linked glycosylation sites have been demonstrated previously to affect neutralization in other
Fig. 3. Comparison of deduced amino acid gp90 variable region sequences from EIAV isolates before and after the immune suppression of ponies #599 and #672. The entire region of the env gene encoding the surface glycoprotein (gp90) was sequenced from EIAV∆PND and from viral RNA collected both prior to immune suppression (599M, 672P) and during the fever episode (599D, 672D). Clones were sequenced using a Taq Dye Deoxy Terminator Cycle Sequencer kit (Applied Biosystems) and standard internal EIAV primers (Leroux et al., 1997). DNA sequences were resolved with an ABI Prism 373 DNA sequencer (Applied Biosystems). The sequences were analysed using the Genetics Computer Group package analyses software (Group, 1994). Deduced amino acid sequences were aligned and compared to the EIAV∆PND inoculum. The consensus reference EIAVPV sequence is depicted for comparison with the EIAV∆PND inoculum. The first 142 amino acids of gp90 are not displayed in the figure. Amino acid differences between EIAVPV and EIAV∆PND are in white text. Only the amino acid residues different from EIAV∆PND are reported on the alignment. Dots indicate residues identical to the EIAV∆PND sequence; dashes indicate amino acid deletions; black underlined amino acids indicate potential N-glycosylation sites (N\(\angle\)S/T). Newly created potential N-glycosylation sites in the pony isolate sequences as compared to EIAV∆PND are also indicated (\(\_\)). Variable regions described previously (V2–8) are boxed. The principal neutralizing domain (PND) is denoted (\(\%\)). The 14 amino acid deletion is designated in a black box below the V3 region. The Env epitope located in the V3 region is in bold and in larger text in the deletion box and in the EIAV∆PND sequence. The D\(_{RT}\) epitope, partially in the V3 region, is delineated by a shaded box and the C\(_{RT}\) epitope in the V5 region is designated by a clear box. GenBank accession numbers are AF429316–AF429353.
attenuation of EIAV

Control of virus infection could not be attributed to intrinsic mechanisms of host immune control of EIAV replication and the absence of the gp90 PND. The absence of classic EIA in the antibodies) to control a neutralization-resistant EIAV virus in determination in other humoral or cellular immune responses.

Due to introduction of neutralizing antibodies or to modification of serum neutralization sensitivity, they do emphasize managed increases in virus load via removal of antiviral drugs or regulated short-term immunosuppressive therapy allows host immune systems to establish new, more effective responses to established virus infections.

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References


lentiviruses (Cheng-Mayer et al., 1999; Chackerian et al., 1997). While these comparisons cannot definitively identify determinants of serum neutralization sensitivity, they do emphasize the fact that relatively minor variations in gp90 can markedly influence virus neutralization and antigenic properties.

These observations for the first time indicate adaptation of virus-specific host immunity by transient immune suppression of an inapparent EIAV carrier. The basis for this change in antibody specificity is uncertain but raises a number of interesting questions about factors that define steady-state interactions between the immune system and a persistent EIAV infection in inapparent carriers. Interestingly, this inapparent-carrier state was maintained in the absence of detectable neutralizing antibodies, even with an ongoing evolution of gp90 sequences presumably progressively generating de novo antibody responses to evolving virus variants.

Transient immune suppression and associated changes in virus-specific immune responses evidently resulted in a further 100-fold lowering of steady-state virus replication levels (1 × 10⁵ to 1 × 10⁴ RNA copies/ml). However, it remains to be determined if the enhanced suppression of virus replication is due to introduction of neutralizing antibodies or to modification in other humoral or cellular immune responses.

Studies presented here demonstrate the ability of host cellular and humoral immune responses (excluding neutralizing antibodies) to control a neutralization-resistant EIAV virus in the absence of the gp90 PND. The absence of classic EIA in the first year of infection suggests multiple complementary mechanisms of host immune control of EIAV replication and disease that do not require the presence of the gp90 PND. Control of virus infection could not be attributed to intrinsic attenuation of EIAVAPND as immune suppression with dexamethasone produced high levels of virus replication and a characteristic EIA disease cycle. With the removal of dexamethasone, the immune system rapidly recovered with effective resolution of disease and control of virus replication.

Structured treatment interruption (STI) has been recently proposed as a means of managing HIV-1 infection of patients on highly active anti-retroviral therapy (HAART) (Boyle, 2000; Lori et al., 2000; Ruiz et al., 2000). STI involves repetitive on-and-off cycles of HAART, which increases virus replication to stimulate virus immunity and, in particular, the cellular immune response. Initial trials in HIV-1 patients and simian immunodeficiency virus (SIV)-infected monkeys yielded mixed results but indicate a potential to improve virus-specific immunity and lower steady-state virus replication. Effects of transient immune suppression of EIAV-infected inapparent carrier ponies resemble STI studies of HIV-1/SIV infections in that a temporary increase in virus load enhanced virus-specific immunity and reduced virus load. Relevant to our studies with EIAV is a recent report that STI of HIV-1 patients produced increased levels of neutralizing antibodies to primary HIV-1 isolates, in addition to the more frequently observed increase in virus-specific cytotoxic T-cell responses (Montefiori et al., 2001). Taken together, these findings indicate that temporary events in virus load via removal of antiviral drugs or regulated short-term immunosuppressive therapy allows host immune systems to establish new, more effective responses to established virus infections.


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