Short communication

Fine specificity of equine infectious anaemia virus gp90-specific antibodies associated with protective and enhancing immune responses in experimentally infected and immunized ponies

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Equine infectious anaemia virus (EIAV) provides a model for examining the natural immunological control of a persistent lentivirus infection and for evaluating the efficacy of various vaccine strategies. As an initial characterization of antibody responses associated with protective or enhancing immune responses elicited by experimental infections or vaccinations, we have utilized synthetic peptide ELISA to characterize the fine specificity of antibodies to linear determinants of the EIAV surface glycoprotein, gp90. The data indicated that serum antibodies associated with protective or enhancing immune responses differed quantitatively and qualitatively in their pattern of reactivity to gp90 peptides. Protective and enhancing EIAV vaccines could also be distinguished by their ability to evoke anamnestic antibody responses to gp90 peptides. These studies demonstrate for the first time definitive differences in the specificity of protective and enhancing antibody responses to EIAV and emphasize the importance of using native viral glycoprotein immunogens in lentivirus vaccines.

Equine infectious anaemia virus (EIAV), a member of the lentivirus subfamily of retroviruses, causes a persistent infection in horses that is initially characterized by recurring cycles of viraemia. During the first 8–12 months post-infection (p.i.), however, most infected horses evolve broadly protective immune responses that establish a stable inapparent stage of infection (Montelaro et al., 1993). This efficient immunological control observed in EIAV infections is unique among lentivirus infections and thus provides a novel natural model for examining the types of immune responses that can overcome the array of persistence and escape mechanisms intrinsic to lentiviruses.

The EIAV system has also proven useful as a model for examining the efficacy of various vaccine strategies. We have previously described a series of EIAV vaccine trials in Shetland ponies using an inactivated whole virus (wv) vaccine, a subunit vaccine composed of purified viral envelope glycoproteins (gp-enriched) and a vaccine composed of the baculovirus-expressed surface glycoprotein (rgp90) of EIAV (Issel et al., 1992; Wang et al., 1994; Montelaro & Bolognesi, 1995). The results of these studies reveal a remarkable spectrum of vaccine efficacy in preventing EIAV infection and disease. For example, the wv and gp-enriched vaccines elicited sterile protection against infection by homologous EIAV challenge containing up to 10^6 animal infectious doses (ID) of the prototype (Pr) vaccine strain of virus. Both vaccines, however, failed to prevent infection by a heterologous virus challenge with an antigenic variant of EIAV, designated PV (for pony virulent) even at challenge doses of 300 animal ID. The wv vaccine provided complete protection against the development of disease in the wv-immunized ponies after the heterologous virus challenge, whereas the EIAV gp-enriched vaccinated ponies showed mild to severe clinical symptoms. The final EIAV vaccine, rgp90, was unable to elicit protection against infection by either the homologous or heterologous EIAV challenge and resulted in severe enhancement of virus replication and disease after challenge with the PV-EIAV. Thus, these various immunization regimens provide a wide range of protection efficacy that can be exploited to identify immune responses that are characteristic of protection or enhancement.
Fig. 1. EIAV gp90-peptide-specific antibody reactivities of ponies experimentally infected with avirulent and virulent EIAV strains. Serum samples taken from experimentally infected ponies were diluted 1:50 and assayed in peptide-based ELISA assays against a standard panel of gp90-specific synthetic peptides as described by Ball et al. (1992). The location of the peptide segments along the gp90 sequence from the amino to carboxy terminus is indicated along the x-axis, along with an indication of conserved or variable gp90...
In this current study we have examined in detail the evolution of antibody responses to the surface glycoprotein (gp90) of EIAV in experimentally infected ponies and for the first time characterized the nature of antibody responses in ponies immunized with the various vaccines to identify quantitative or qualitative peptide specificities associated with protection against or enhancement of EIAV replication and disease. The rationale for evaluating peptide specificities is based on our previous observations that EIAV gp90 contains a complex array of conserved and variable linear antigenic determinants recognized during persistent infection (Ball et al., 1992) and that all of the defined EIAV-neutralizing determinants are localized to linear peptide segments in the variable sequences of the gp90 protein (Hussain et al., 1988; Ball et al. 1992). In addition, antibodies to various linear peptide determinants of human immunodeficiency virus type 1 (HIV-1) envelope glycoproteins have been shown to contribute a major proportion of immune effector functions, including complement activation, antibody binding, neutralization and enhancement (Eaton et al., 1994; Purtscher et al., 1994; Robinson et al., 1991; Spear et al., 1994). Thus, the pattern of gp90 peptide seroreactivity is used here to address the following questions: (i) is the maturation of immune responses to a protective status in experimentally infected ponies associated with characteristic evolution of peptide-specific antibody responses?; (ii) do protective immune responses generated during persistent infection share a common pattern of gp90 peptide seroreactivity as protective immune responses elicited by experimental vaccines?; and (iii) can protective and enhancing antibody responses to EIAV be distinguished on the basis of gp90 peptide seroreactivity?

To analyse the fine specificity of the gp90-specific antibody responses, we established a panel of 25 overlapping peptides of 20–30 residues, representing the primary sequence of the entire EIAV surface glycoprotein, gp90 (Ball et al., 1992). These peptides were initially used in a standardized peptide ELISA (Grund et al., 1994) to test serum samples from Shetland ponies experimentally infected with either the avirulent Pr strain or the virulent PV strain of EIAV. Under our standard infection conditions, Pr-infected ponies typically establish a persistent inapparent infection associated with relatively low levels of viraemia. In contrast, PV-infected ponies routinely develop symptoms of chronic disease by 4 weeks p.i. and progress to an inapparent stage of infection by 8–12 months p.i. Although infections with each of the two strains of virus display different clinical progressions, both infection models result in an immune status that strictly controls virus replication and prevents disease from challenges with virulent laboratory and field strains of EIAV (Rwambo et al., 1990).

Fig. 1 summarizes the gp90 peptide seroreactivities observed in the experimentally infected ponies at 3 and 17 weeks p.i. After infection by either the Pr (Fig. 1a) or PV (Fig. 1b) strain of EIAV, the early antibody responses appeared to be directed predominantly against the peptide segments from the conserved amino (Cn) and carboxy (Cc) domains of the gp90. With the exception of the V1 peptide constituting the EIAV principal neutralizing domain (PND), there was a marked absence of antibodies reactive with the variable domain (V) peptides of the gp90 in both Pr- and PV-infected ponies. In general, the pattern of peptide seroreactivity was similar between the Pr- and PV-infected ponies at 3 and 17 weeks p.i. Infection with PV-EIAV elicited a substantially stronger antibody response, probably reflecting the higher levels of virus replication observed in PV-infected ponies compared to ponies inoculated with similar doses of Pr-EIAV (Montelaro et al., 1993).

To examine the fine specificity of EIAV gp90-specific antibody responses in long term inapparently infected ponies, serum samples from a representative Pr-EIAV infected pony were taken at 4, 13 and 22 months p.i. and analysed in the standard gp90 peptide assay (Fig. 1c). Compared to the gp90 peptide reactivity profile observed early after Pr-EIAV infection, these later serum samples displayed a general increase in the levels of antibody reactivity to previously seropositive peptides. Furthermore, an increase in antibody reactive with more of the gp90 V domain peptides was observed, in particular to peptide V4 which contains the gp90C neutralization determinant (Ball et al., 1992). Overall, the quantitative levels and patterns of peptide-specific antibody reactivity observed in the Pr-infected ponies at these late times post-infection were similar to the peptide reactivity profiles observed in PV-infected ponies at the early times post-infection.

To compare the gp90 peptide specificity of antibody responses elicited by EIAV wv, gp-enriched and rgp90 vaccines, we analysed serum samples taken from the immunized ponies at the time of virus challenge. The
Fig. 2. For legend see opposite page.
data summarized in Figs 2 and 3 demonstrated that there were both quantitative and qualitative differences in the gp90 peptide-specific antibody reactivities elicited by the different vaccines. In general, the highest levels of peptide-specific antibody responses were observed after immunization with the EIAV gp-enriched vaccine (Fig. 2c, d), followed by the reactivity levels for the wv vaccine (Fig. 2a, b) and then by the rgp90 vaccine (Fig. 3a, b).

Besides the quantitative differences detected among the peptide reactivity profiles associated with each vaccine, there were also qualitative differences in gp90 peptide reactivities that result in characteristic antibody specificity patterns for each vaccine. The largest number of seropositive peptides was observed with immune serum from the gp-enriched vaccinated ponies. The peptide reactivity profile produced by the gp-enriched vaccine resembles the profiles observed for EIAV-infected ponies (c.f. Fig. 1a, b) in that the reactivity was predominantly against peptides in the C region of gp90 and against the PND peptide (V1). However, the gp-enriched vaccine failed to elicit antibody responses to peptide 5, which was highly reactive with serum antibodies from the EIAV-infected ponies. Examination of the gp90 peptide-specific seroreactivity profile for the rgp90-immunized ponies (Fig. 3a, b) revealed a characteristic pattern that differed markedly from the wv and gp-enriched immunized ponies. Especially notable was the absence of antibodies reactive with the gp90 C region peptides (e.g., peptides 11 and 12), peptide 8 and peptide V1, all of which were immunodominant in the other vaccine recipients and in ponies infected with EIAV.

For a viral vaccine to be effective, it must establish immunological memory that responds to subsequent antigen exposure. Therefore, we determined whether the protective properties of the three EIAV vaccines could be correlated with changes in gp90 peptide-specific antibodies in response to virus challenge. Using our standard gp90 peptide ELISA, we analysed serum samples taken from wv and gp-enriched vaccine recipients at 3 weeks post-challenge (p.c.) or from the rgp90 vaccine recipients at 2 and 8 weeks p.c. For each vaccine, sera from immunized ponies that were challenged with either the Pr or PV strain of EIAV were analysed separately to facilitate a discrimination, where applicable, between anamnestic responses in protective and non-protective situations.

The patterns of gp90 peptide seroreactivity observed in wv and gp-enriched vaccine recipients at 3 weeks p.c. with the Pr and PV strains of EIAV are summarized in Fig. 2. The peptide reactivity profiles indicated a marked anamnestic response after Pr or PV EIAV challenge to most of the peptides that did induce an antibody response as a result of immunization and to some peptides (e.g., peptides 4 and 5) that did not appear to induce an antibody response before challenge. These observations suggested that both vaccines were successful in eliciting antibodies to native viral glycoprotein antigenic determinants presented during a challenge exposure to infectious virus. There did not appear to be an obvious correlation between a specific peptide reactivity profile and the outcome of challenge infection with Pr or PV EIAV.

In contrast, the parallel analyses of gp90 peptide-specific responses in the rgp90-vaccinated ponies before and after challenge (Fig. 3) revealed relatively poor anamnestic antibody responses to the gp90 peptide determinants. In the case of the Pr-EIAV challenged ponies (Fig. 3a), there was little increase and often a decrease in antibody reactivity to most of the peptides that were recognized after immunization (e.g. peptides 1, 2, V1 and V2B). Instead, the greatest increases in peptide-specific antibodies were directed against peptides 8 and 12, neither of which displayed seroreactivity with the prechallenge immune serum, but are highly immunogenic during virus infection. The rgp90-immunized ponies challenged with the PV-EIAV failed to develop any detectable peptide-specific anamnestic responses at 2 weeks p.c. (Fig. 3b). However, at 8 weeks p.c. (Fig. 3c) there were relatively high peptide-specific antibody reactivities observed in both the Pr- and PV-challenged immunized ponies, presumably reflecting antibody responses to the high levels of virus replication documented in the rgp90-vaccinated ponies (Wang et al., 1994). These data indicate that the rgp90 vaccine, in contrast to the wv and gp-enriched vaccines, failed to generate the B cell memory required for efficient and effective anamnestic responses upon exposure to infectious virus.

The current serological analyses reveal several new properties of gp90-specific antibody responses during persistent EIAV infection. Firstly, there appears to be a remarkably consistent pattern of gp90 peptide seroreactivity evident through at least the first couple of years.
Fig. 3. EIAV gp90 peptide reactivity of immune serum antibodies in rgp90-vaccinated ponies. Serum samples were taken from the baculovirus rgp90-vaccinated ponies described in Wang et al. (1992) and challenged with either the Pr or PV strain of EIAV. (a) Serum samples taken from vaccinated ponies (n = 4) on the day of challenge (3 weeks p.v.) and at 2 weeks p.c. with Pr-EIAV. (b) Serum samples taken from vaccinated ponies (n = 4) on the day of challenge (3 weeks p.v.) and at 2 weeks p.c. with PV-EIAV. (c) Serum samples taken from vaccinated ponies at 8 weeks p.c. with either Pr (n = 4) or PV (n = 4) EIAV. The EIAV gp90 peptide ELISA conditions and data calculations are as described in Fig. 1.
post-infection, indicating that the gp90 peptide segments that are immunogenic within a few weeks post-infection continue to be predominant linear antigenic determinants in long term infected ponies. Thus, these data suggest that the progression from chronic EIA to the inapparent stage of infection cannot be correlated with an apparent evolution in the specificity of gp90 peptide-specific antibodies. Secondly, the antigenic peptide determinants recognized during persistent EIAV infection are predominantly located in the conserved gp90 sequences, with only the V1 peptide encompassing the gp90 PND displaying substantial seroreactivity in infected ponies. These observations indicate that the variable linear determinants are in general weakly immunogenic and that they may elicit reactive antibodies only after several years of persistent infection, as observed by Ball et al. (1992). Third, the qualitative pattern of peptide seroreactivity was the same for ponies infected with the PV or Pr strain of EIAV. The observed similarity in peptide seroreactivity evidently reflects the similarity between the envelope genes of the PV and Pr strains of EIAV which differ by only about 3% at the amino acid sequence level (D. L. Lichtenstein, C. J. Issel, & R. C. Montelaro, unpublished results). The data do demonstrate common gp90 peptide-specific antibody responses in ponies regardless of the clinical progression of disease. The analyses of the gp90 peptide reactivity of antibody responses elicited by various EIAV vaccines demonstrate the importance of the physicochemical context of the gp90 antigen in determining the quantitative and qualitative properties of antibody responses to the viral gp90. Each experimental vaccine produced a characteristic profile of gp90 peptide seroreactivity. The serum antibodies associated with the protective immune responses elicited by the wv and gp-enriched EIAV vaccines reacted with a profile of gp90 peptides that resembled the seroreactivity patterns observed with EIAV-infected ponies, and these vaccines produced strong anamnestic responses to gp90 peptide determinants following virus challenge. In contrast, the baculovirus rgp90 vaccine generated antibodies with a markedly different gp90 peptide seroreactivity and failed to establish immunological memory as evidenced by the lack of anamnestic responses in the rgp90-vaccinated ponies after virus challenge. Pincus et al. (1993) reported distinct differences in the HIV-1 gp160 peptide-specific antibody responses in two virus-infected laboratory workers compared to individuals immunized with a baculovirus-expressed gp160 vaccine. The inability of the EIAV rgp90 vaccine to elicit antibody responses to peptide determinants present in the native envelope glycoprotein complex of EIAV suggests that the recombinant protein differs markedly from the native viral glycoprotein in immunogenicity and, presumably, in structure. Whether these differences result from protein folding or glycosylation patterns is not certain. However, the variation in recombinant and viral gp90 antigenicity is reminiscent of the differences observed in the antigenic properties of baculovirus-expressed and native viral gp120 of HIV-1 (Abacioglu et al., 1994; Moore et al., 1993a, b; VanCott et al., 1994). Although the use of non-native HIV-1 envelope glycoproteins has been cited as an advantage for an AIDS vaccine (Volvovitz & Smith, 1993), the association of vaccine enhancement of EIAV with serum antibodies that poorly recognize native viral determinants raises additional concerns about the potential harmful effects of using vaccine immunogens that differ substantially from native envelope glycoprotein structure.

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References


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