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Equine monoclonal antibodies recognize common epitopes on variants of equine infectious anaemia virus

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SUMMARY

Equine–murine xenohybridoma cells were produced using SP2/0 murine myeloma cells and splenic lymph node cells obtained from horses infected with 10^6 $TCID_{50}$ of single cloned variants of equine infectious anaemia virus (EIAV). The xenohybridomas secreted equine IgG monoclonal antibodies reactive with EIAV in enzyme immunoassays employing purified virus. Seven antibodies were studied in detail. They bound to viral glycoproteins (gp90 or gp45) in radioimmunoprecipitation assays, and reacted with homologous EIAV as well as five other cloned variants of EIAV. When evaluated against a single cloned variant of EIAV (EIAV-WSU5), two antibodies bound to different epitopes on gp90. The five remaining antibodies reacted with the same or overlapping epitopes on gp45. None of the antibodies exhibited viral neutralizing activity.

Equine infectious anaemia is a persistent viral infection of horses caused by equine infectious anaemia virus (EIAV), a member of the Lentivirinae family of retroviruses (Cheevers & McGuire, 1985, 1988; Parekh, Issel & Montelaro, 1980). Exposure of horses to EIAV is followed by replication of virus, primarily in macrophages, and development of viraemia exceeding 10^4 $TCID_{50}$/ml plasma (McGuire, Crawford & Henson, 1971; O'Rourke, Perryman & McGuire, 1988). Viraemia is accompanied by fever, anaemia, glomerulitis, and tissue infiltration by lymphocytes and monocytes (Cheevers & McGuire, 1985, 1988; McGuire, Henson & Quist, 1969a, b; Banks, Henson & McGuire, 1972). Tissue injury caused directly by the virus has been documented but is less extensive than that which is mediated by the interaction of specific antibodies and/or T lymphocytes with EIAV antigens (Perryman, O'Rourke & McGuire, 1988). Immune responses terminate viraemia, which is then followed by resolution of clinical signs. These horses remain persistently infected, however, as can be demonstrated by transmission of disease through transfer of whole blood to another horse (Issel *et al.*, 1982).

The occurrence of antigenic variants of EIAV has been documented (Kono, Kobayashi & Fukunaga, 1973; Payne *et al.*, 1987; Montelaro *et al.*, 1984; Carpenter *et al.*, 1987; O'Rourke, Perryman & McGuire, 1989). Alterations in *env* gene sequences yield a new variant that is not controlled by existing antibodies and T lymphocytes. This variant replicates and causes disease until viraemia is terminated by immune responses specific for the new variant.

The overall goal of our research is to define immune mechanisms that may prevent initial infection and control persistent infection in horses exposed to EIAV. In this study we produced equine monoclonal antibodies reactive with EIAV and tested the ability of these antibodies to distinguish among biologically cloned variants of the virus.

Six horses were injected intravenously with 10^6 $TCID_{50}$ of one of the following isolates of EIAV: prototype, EIAV-WSU1, EIAV-WSU2, EIAV-WSU3, EIAV-WSU4, or EIAV-WSU5. The viruses were isolated, cloned, and characterized as previously described (O'Rourke *et al.*, 1988, 1989). Splenic lymph nodes were aseptically collected 50–75 days post-injection, and minced to obtain a single-cell suspension. Lymph node cells were fused to murine myeloma SP2/0 cells in the presence of polyethylene glycol, added to 96-well plates, and grown in DMEM containing 20% foetal bovine serum, 1×10^{-4} M hypoxanthine, 4×10^{-4} M aminopterin, and $1{\cdot}6 \times 10^{-5}$ M thymidine, following procedures modified for use in our laboratories (McGuire, Perryman & Davis, 1983; Wyatt, Magnuson & Perryman, 1987). Supernatants from wells containing viable cells 14 days after fusion were screened for reactivity with homologous EIAV antigens in an enzyme immunoassay as follows. Each of the six isolates of EIAV was grown in foetal equine kidney cells and purified by density gradient centrifugation as previously described (O'Rourke *et al.*, 1988). Virus was attached to wells of Immulon I plates (Dynatech Laboratories, Alexandria, VA) as described elsewhere (O'Rourke *et al.*, 1988, 1989). Coated plates were incubated sequentially with equine

Abbreviations: ABTS, 2.2′-azino-di[3-ethyl-benzthiazoline sulphonate]; EIAV, equine infectious anaemia virus; gp90 and gp45, EIAV envelope glycoproteins; mAb, monoclonal antibody(ies); OD, optical density; $TCID_{50}$, 50% tissue culture infectious dose.

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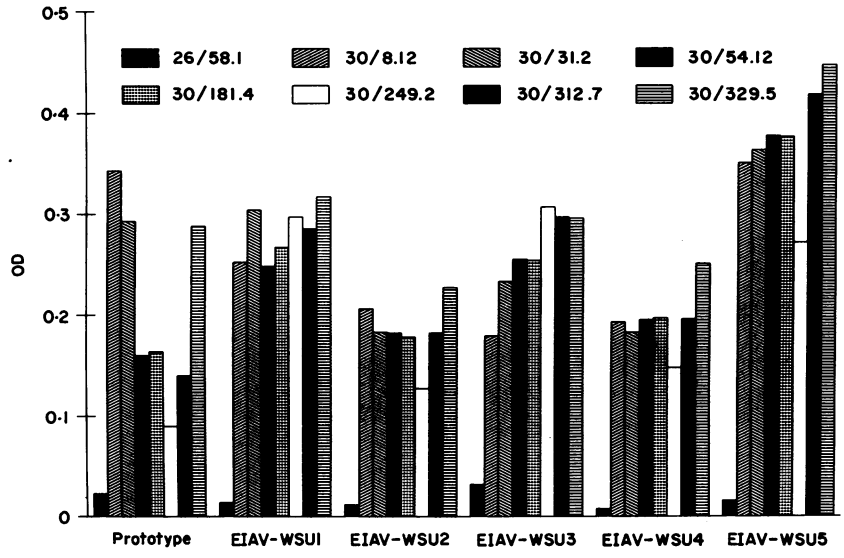

Figure 1. ELISA reactivity of seven anti-EIAV equine mAb and one control equine mAb (26/58·1) with variants of EIAV.

monoclonal antibodies or control media, goat anti-equine IgG conjugated to horseradish peroxidase (Kierkegaard and Perry, Gaithersburg, MD), and 2.2′-azino-di[3-ethyl-benzthiazoline sulphonate] (ABTS; Kierkegaard and Perry). Incubations were performed at 37° for 30 min and plates were washed with 10 mM Tris, pH 7·4, 0·9% NaCl, 0·05% Tween-20. Cells from wells containing antibodies of interest were expanded, cloned by limiting dilution, and cryopreserved. Antigens of EIAV recognized by equine monoclonal antibodies were defined by radioimmunoprecipitation, following established procedures (O'Rourke *et al.*, 1988). Isotypes of equine monoclonal antibodies (mAb) were determined by a subclass-specific assay (O'Rourke *et al.*, 1989) using antisera specific for equine IgM, IgG, and IgG[T] (McGuire & Crawford, 1972).

To determine if mAb recognized distinct or overlapping epitopes, antibody additivity immunoassays were performed using a modification of procedures previously described (Friguet *et al.*, 1983; Hussain *et al.*, 1988). Immulon I plates were coated with 50 ng per well EIAV-WSU5 in 0·01 M carbonate buffer, pH 9·6, for 16 hr at 4°. A saturating quantity of an equine mAb was incubated with EIAV antigen in the plate for 30 min. Unbound antibody was removed by washing prior to addition of a second equine mAb. After subsequent incubation with horseradish peroxidase conjugate of anti-equine IgG followed by ABTS substrate, the reaction intensity was measured spectrophotometrically. The OD in the presence of two EIAV-reactive mAb was compared to OD of reactions with each of the individual equine mAb in combination with control medium. An additivity index was calculated as previously described (Hussain *et al.*, 1988). Those antibody pairs yielding an additivity index $> 50\%$ were considered to recognize different epitopes, while those pairs yielding an index of $< 50\%$ were considered to recognize the same or overlapping epitopes.

Neutralization of EIAV-WSU5 infectivity by mAb was evaluated using a modification of a previously described procedure (O'Rourke *et al.*,1989). Serial threefold dilutions of virus were incubated with an equal volume of medium containing mAb for 30 min at 4°. Infectivity of mAb-treated virus was determined by indirect immunofluorescence.

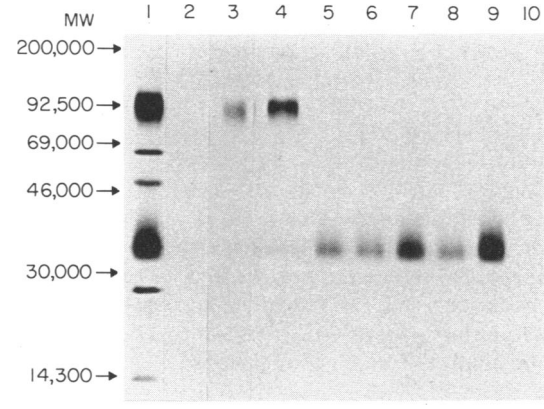

Figure 2. Radioimmunoprecipitation reactions of equine mAb and equine serum with EIAV-WSU5. Lane 1, terminal serum from horse whose splenic lymph node cells were used to produce the mAb designated with the 30/ prefix; Lane 2, pre-infection horse serum; Lane 3, mAb 30/8.12; Lane 4, mAb 30/31.2; Lane 5, mAb 30/54.12; Lane 6, mAb 30/181.4; Lane 7, mAb 30/249.2; Lane 8, mAb 30/312.7; Lane 9; mAb 30/329.5; and Lane 10, negative control mAb 30/287.

Xenohybridoma cell outgrowth occurred in 16–70% of wells from six cell fusion experiments. Equine monoclonal antibodies reactive with EIAV were obtained from four fusions. Following cloning by limiting dilution and cryopreservation in liquid nitrogen, seven stable xenohybridoma cell lines derived from a horse infected with EIAV-WSU5 were selected for detailed study. All antibodies were of the IgG class. None of the seven equine mAb analysed exhibited significant ability to neutralize infectivity of EIAV-WSU5. Figure 1 demonstrates that the antibodies recognized epitopes present on EIAV-WSU5, as well as the EIAV prototype and each of four additional EIAV variants derived from the prototype virus. This suggests the epitopes recognized by the seven mAb are conserved among the variants tested.

EIAV antigens identified by equine mAb were analysed by radioimmunoprecipitation (Fig. 2). Antibodies 30/8.12 and 30/

31.2 reacted with antigen migrating with an apparent molecular weight (MW) of 90,000, previously designated gp90. Antibodies 30/54.12, 30/181.4, 30/249.2, 30/312.7 and 30/329.5 reacted with antigen migrating with an apparent MW of approximately 38,000. This antigen, which was also recognized by serum from the EIAV-infected horse providing the splenic lymph node cells, was previously designated gp45 (Montelaro *et al.*, 1984; O'Rourke *et al.*, 1988). We adhere to this terminology to maintain consistency with previous publications.

The mAb were analysed for their ability to distinguish different epitopes on EIAV-WSU5, the cloned variant used to infect the horse from which the splenic lymph node cells were obtained. Both antibodies reactive with gp90 were additive, with each of the five antibodies reactive with gp45, as expected. None of the five gp45-reactive mAb was additive with any of the four remaining antibodies in the group (additivity indices of 0–31%). This observation suggests the antibodies define overlapping epitopes expressed on viral gp45 (Hussain *et al.*, 1988). These overlapping gp45 epitopes may be immunodominant in horses since five xenohybridoma cell lines producing mAb to them were recovered. Antibodies 30/8.12 and 30/31.2 were additive with each other (additivity index of 95%). Since they react with gp90, they define separate epitopes on this viral envelope glycoprotein, and both epitopes are present on multiple variants of EIAV. Other investigators have employed murine mAb to define distinct, as well as overlapping, epitopes of EIAV envelope gp90 (Hussain *et al.*, 1988).

Monoclonal antibodies of host species origin offer distinct advantages when studying the pathogenesis of lentiviral infections. The antibodies may be used to define immunodominant epitopes recognized by the infected host, and to assess conservation of epitopes among variants of the virus. Use of the strategy presented in this study will provide additional mAb, including neutralizing mAb, for investigation of the pathogenesis, prevention and control of lentivirus infections.

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