Immune Responses Are Required To Terminate Viremia in Equine Infectious Anemia Lentivirus Infection

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Six normal and four immunodeficient horses were injected with a cloned variant of equine infectious anemia virus (EIAV). The six normal horses had detectable EIAV in their plasma by 7 days postinjection. During their primary viremic episode, which was accompanied by fever and anemia, maximum titers of EIAV in plasma ranged from $10^{3.8}$ to $10^{4.8}$ 50% tissue culture infective doses per ml. All six normal horses cleared detectable virus from their plasma by 21 to 35 days after injection. Horses with combined immunodeficiency became viremic by 9 days postinjection and also developed anemia. In contrast to normal horses, foals with combined immunodeficiency did not eliminate the virus from their plasma.

Equine infectious anemia virus (EIAV) is a member of the *Lentivirinae* subfamily of retroviruses. Other members of this group include visna/maedi virus, which induces central nervous system and pulmonary disease in sheep, caprine arthritis-encephalitis virus, which causes arthritis in goats, and human immunodeficiency virus, the causative agent of acquired immunodeficiency syndrome (4, 5, 7, 24-26, 28). Injection of horses with EIAV results in persistent infection, usually accompanied by recurrent episodes of viremia, with resultant fever and anemia. Tissue injury occurs during clinical episodes of disease and consists of reduced erythrocyte production, erythrocyte destruction, glomerulitis, lymphadenopathy, and infiltration of the liver and other organs by macrophages and lymphocytes (1, 2, 8, 13, 15, 16, 20).

Our goal is to define the role of antiviral immune responses in the pathogenesis of lentivirus diseases of animals. A direct way to obtain this information is to study the pathogenesis of disease in animals susceptible to infection but incapable of specific antiviral immune responses. EIAV offers unique opportunity to compare lentivirus infection in immunologically normal and immunodeficient hosts. Horses with combined immunodeficiency (CID) lack functional B and T lymphocytes and cannot manifest antigen-specific immune responses (12, 17, 18, 27). Infection of foals with CID with cloned pathogenic variants of EIAV allows evaluation of the role of specific immune responses in control of viral replication, selection of antigenic variants, and contribution to tissue injury. In this study, we tested the hypothesis that virus-specific immune responses are required to terminate viremia in horses infected with EIAV.

Immunologically normal foals and foals with CID were obtained by selective breeding of Arabian mares and stallions heterozygous for the CID trait (22). Normal and CID-affected foals were left with their dams for 3 weeks and then individually isolated and maintained as previously described (21). At 26 to 30 days of age, six normal and four CID foals were infected by intravenous injection of 10^6 50% tissue culture infective doses (TCID₅₀s) of EIAV-WSU5, a single variant of EIAV cloned three times by limiting dilution. The derivation of this pathogenic variant and its propagation in roller bottle cultures of equine kidney cells have been previously described (19). Two normal foals were

maintained as uninfected controls. All foals were examined daily and considered febrile if their rectal temperatures exceeded 39 degrees C (8). Blood samples were collected three times weekly through 60 days postinfection or less if the animal was euthanized because of advanced disease. Serum was stored at -20°C for analysis of antibodies reactive with the p26 antigen of EIAV (6). Erythrocyte packed cell volumes (PCV) were determined three times weekly. Anti-C3 Coombs tests to determine the presence of complement component C3 on erythrocytes were performed as previously described (14). Plasma was collected aseptically and maintained at -70°C for titration of EIAV by immunofluorescence as previously described (19). In brief, six replicates of serial dilutions of plasma were added to subconfluent monolayers of equine kidney cells in 96-well flat-bottom tissue culture plates (Corning Glass Works, Corning, N.Y.). Cultures were maintained in minimal essential medium supplemented with 5.7 mM sodium bicarbonate, 20 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), and 5% calf serum (Hyclone, Logan, Utah). After 9 to 11 days, infected cells were revealed by direct antiviral immunofluorescent assay. TCID₅₀-per-milliliter endpoints were calculated by the method of Reed and Muench (23). The titer of a standard pool of EIAV was determined 15 times. The mean log₁₀ titer per milliliter was 6.84 (standard deviation, 0.42), and the coefficient of variation was 6.14%.

EIAV infection in normal foals. All six normal foals infected with 10^6 TCID₅₀s of EIAV-WSU5 became febrile and viremic, and the virus was first detected in their plasma at 4 or 7 days postinfection. Maximum titers exceeded 10^4 TCID₅₀s/ml in five of six animals (Fig. 1). Reductions in erythrocyte PCV occurred in all six foals. Erythrocytes were coated with C3 at the time of reduced PCV values. All foals produced antibodies reactive to the p26 antigen of EIAV.

Three patterns of viremia were observed among normal foals infected with EIAV. In the first pattern, foals N-12 and N-13 terminated viremia but became profoundly anemic and died or were euthanized at 35 days postinfection (Fig. 1A). Gross and microscopic tissue changes were compatible with EIAV infection. The second pattern is shown in Fig. 1B. N-8 and N-24 experienced a single viremic episode which began at 7 days postinfection and lasted for 14 to 16 days. Both foals recovered from anemia and were clinically normal at the termination of observations at 60 days postinfection. The

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FIG. 1. Viremia, febrile response, and percent erythrocyte PCV in normal horses after infection with EIAV. Shaded bars mark the days when horses were febrile (temperature, $>39^{\circ}\text{C}$.).

third viremic pattern (Fig. 1C) occurred in foals N-7 and N-25. Both foals were febrile and anemic during the primary viremic episode, which terminated at 21 and 25 days postinfection. These foals experienced one or more additional viremic episodes unassociated with a febrile response, and both were free of clinical signs at the termination of observations at 60 days postinfection, even though N-25 was viremic at that time. Even though three patterns of viremia were observed, all six normal foals were able to terminate the primary viremic episode.

Two normal foals not infected with EIAV were maintained and evaluated by the same protocols used for infected foals. Neither of these foals was anemic or febrile throughout the 60-day observation period. Neither EIAV nor antibodies reactive with the p26 antigen of EIAV were detected in their plasma.

EIAV infection in foals with CID. Foals with CID that were infected with 10^6 TCID₅₀s of EIAV-WSU5 became viremic, and the virus was first detected in their plasma at day 4, 7, or 9 postinfection (Fig. 2). Whereas maximum titers of EIAV in plasma were similar to those observed in the six normal foals, none of the foals with CID terminated the primary viremic episode. None of the foals produced antibodies reactive with the p26 antigen of EIAV.

All four foals with CID that were infected with EIAV developed profound anemia and died or were euthanized at 25 to 35 days postinfection. Anemia is occasionally observed in foals with CID without EIAV infection. To determine whether the anemia in foals with CID in this study was attributable to EIAV, erythrocyte PCVs were compared with those previously measured in age-matched foals with CID that were not infected with EIAV (Fig. 3). For all four infected foals with CID, erythrocyte PCVs in terminal blood samples were more than 2 standard deviations below the mean of age-matched, uninfected foals with CID. Erythro-

cytes from foals with CID that were infected with EIAV-WSU5 did not react in anti-C3 Coombs tests.

Postmortem examination revealed evidence of opportunistic infections. CID-10 was infected with equine adenovirus and had pulmonary abscesses from which *Rhodococcus equi* was cultured. CID-14 was infected with equine adenovirus

FIG. 2. Viremia, febrile response, and percent erythrocyte PCV in immunodeficient horses after infection with EIAV. Shaded bars mark the days when horses were febrile (temperature $>39^{\circ}\text{C}$.).

FIG. 3. Percent erythrocyte PCV (mean minus 2 standard deviations) in 1- to 12-week-old foals with CID. The number of uninfected foals with CID evaluated is listed above each datum point. PCVs in terminal blood samples of four EIAV-infected horses with CID are shown. Symbols: Δ , CID-10; $\square$, CID-14; $\bullet$, CID-20; $\blacktriangle$, CID-47.

and also had extensive necrotic lesions in the adrenal glands and intestinal tract. Bacterial colonies and inclusion bodies indicative of equine adenovirus were observed in the lungs of CID-20. Inclusion bodies were also observed in the lungs of CID-47.

The results of this study show that horses with CID were susceptible to infection with EIAV. The initial kinetics of viremia and the maximal virus titers in plasma were similar to those of age-matched immunocompetent foals. However, foals with CID are unable to diminish or eliminate EIAV from plasma after initial infection. Foals with CID possess large granular lymphocytes inducible in vitro to display natural killer cell activity (11). These foals also possess phagocytically active neutrophils and monocytes, as well as an apparently normal complement system (3, 10). They lack functional T and B lymphocytes and are incapable of specific immune responses. Therefore, the inability to clear EIAV from plasma can be attributed only to absence of specific T- and B-lymphocyte responses to the virus. The results in foals with CID extend the observations of Kono (9), who studied recrudescence of viremia in EIAV-infected horses treated with immunosuppressive drugs.

The observation that EIAV titers in foals with CID did not rise continuously suggests that some control of EIAV infection occurs in these animals. Although the mechanisms are unknown, one possibility is that natural killer cells play a minor role in regulating viremia. Another possibility is that all cells susceptible to infection become infected, thereby limiting viremia.

EIAV-infected foals with CID died earlier than uninfected foals with CID that were previously maintained under similar conditions (17). Whereas intercurrent opportunistic infections were observed in all four foals with CID, it is likely that EIAV infection contributed to the shortened lifespan. This is supported by the observation that two infected normal foals also died after infection. Typical inflammatory lesions of EIAV infection were not observed in foals with CID. The foals were, however, profoundly anemic. Anemia

is a hallmark of EIAV infection and develops as a result of impaired erythrocyte production, as well as erythrocyte destruction (15, 16). Hemolysis occurs both intravascularly and extravascularly and is associated with binding of complement to erythrocyte membranes. Anti-C3 Coombs tests were positive in normal foals infected with EIAV-WSU5 in the current study but negative in infected foals with CID. This observation suggests that suppression of erythropoiesis is an important mechanism of anemia in EIAV-infected foals with CID.

This investigation of immunodeficient horses infected with EIAV provides unequivocal evidence for the role of specific immune responses in controlling viremia caused by a lentivirus. The model can be further exploited to determine the efficacy of components of the specific immune response, especially neutralizing antibodies, in lentivirus control.

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