Demonstration of Antigenic Identity Between Purified Equine Infectious Anemia Virus and an Antigen Extracted from Infected Horse Spleen

HIDEO NAKAJIMA, NEIL L. NORCROSS, AND LEROY COGGINS

Equine Infectious Anemia Research Division, National Institute of Animal Health, Kodaira, Tokyo, 187 Japan, and New York State Veterinary College, Cornell University, Ithaca, New York 14850

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Antigenic relationship between purified equine infectious anemia (EIA) virus and spleen-derived antigen from EIA-infected horses was examined by immunodiffusion. Identical antigenicity of these two antigens has been proven because precipitation lines formed between the two antigens and EIA antiserum connected with each other. The results indicate that the antigenic substance derived from infected spleen is a component of EIA virus.

Equine infectious anemia (EIA) is a viral disease of equidae characterized by persistent infection with the causative agent for the life of the animal, intermittent fever, and anemia. The disease is extremely difficult to diagnose because of the lack of a satisfactory diagnostic means other than the horse inoculation test. Recently, however, an immunodiffusion reaction was independently developed by two separate research groups by using infected horse spleen and purified EIA virus as antigens, respectively (1, 5). Precipitating antibody, which was proved to be specific for EIA, appeared early in the infection and remained for a long period in the serum (1–3, 7). It was detectable in almost all of the infected horses (2, 3, 7), even in horses infected with antigenically distinct strains of EIA virus (5, 7). Therefore, group-specific inner components of the virus rather than type-specific ones seem to be involved in the reaction. The antigenic substance derived from infected horse spleen has been characterized (8).

Based on these data, the immunodiffusion test is now being evaluated as one of the most hopeful procedures for the diagnosis of EIA. However, the relationship of the precipitating antibodies demonstrated by the separate research groups is unknown because of the difference in antigens employed.

The present report describes the antigenic relationship between purified EIA virus and infected spleen-derived antigen against EIA antiserum in an immunodiffusion test.

Purified EIA virus was obtained from horse leukocyte cultures infected with the P337 virus strain, first by ultracentrifugation, then by diethylaminoethyl cellulose chromatography, and finally by cesium chloride equilibrium density gradient centrifugation as described previously (4). The final product was slightly white and turbid, and it had an infective titer of approximately $10^4$ 50% tissue culture-infecting doses per 0.5 ml. Spleen-derived antigen was prepared by finely mincing or homogenizing the spleen obtained from horses.

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**Fig. 1.** Precipitation lines formed between equine infectious anemia (EIA) antiserum (central well) and antigens (peripheral wells). Purified EIA virus (1); spleen-derived antigen (2); uninoculated horse leukocyte culture fluid (3). Two precipitation lines connected, indicating reactions of identity.
infected with the Wyoming virus strain (1). A control positive EIA antiserum that had been matched with the purified EIA virus was obtained later in the infection from an experimental horse (no. 569) infected with the Wyoming virus strain. Immunodiffusion reaction was then carried out between these two antigens and the antiserum by the procedure reported previously (5). A precipitation line was observed between the antiserum and both antigens which formed a line of identity (Fig. 1). No reaction was seen with uninfected horse leukocyte culture fluid and with spleen obtained from healthy horses (8).

The results indicated that the antigenic substance derived from EIA-infected horse spleen is identical with that of EIA virus. It can be concluded, therefore, that each reaction is detecting the same precipitating antibody in EIA-infected horses.

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LITERATURE CITED