Demonstration of an Antigenic Relationship between Hog Cholera and Bovine Viral Diarrhea Viruses by Immunofluorescence

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SUMMARY AND CONCLUSIONS

Specific staining of antigen within bovine embryo kidney tissue culture cells, infected with either Oregon C24V or NADL-MD bovine viral diarrhea virus, was accomplished using fluorescein-conjugated swine anti-hog cholera or bovine antiviral diarrhea globulin. Also specific staining of antigen within pig kidney tissue culture cells, infected with hog cholera virus, was accomplished using the same two types of conjugates. Specificity was confirmed by appropriate controls.

The authors found immunofluorescence to be a convenient and sensitive method for determining an antigenic relationship between hog cholera and bovine viral diarrhea viruses.

Introduction

Previous work, utilizing the agar gel diffusion technique, has suggested a serological relationship between the viruses of hog cholera (swine fever) and those of a mucosal disease complex of cattle. Tissues from cattle clinically infected with mucosal disease and from experimental pigs which had died of hog cholera were used as antigen and reacted against hog cholera and mucosal disease antisera. "A reaction of identity" indicated a serological relationship existed between these two viruses (1,2).

This study was undertaken in an attempt to demonstrate the antigenic relationship between hog cholera and two bovine mucosal-viral diarrhea viruses by immunofluorescence.

Materials and Methods

Fractionation of Antisera. — Antisera were fractionated with one-half volume of saturated ammonium sulfate. The globulin was dialyzed against 0.85% saline until free of sulfate. Globulin was obtained in this manner from nonimmune specific pathogen-free (SPF) swine sera, nonimmune SPF bovine serum, SPF bovine antiviral diarrhea serum (prepared against viral diarrhea virus NADL-MD)[1], randomly selected swine anti-hog cholera sera[2] and SPF swine anti-hog cholera serum.[3] Protein concentrations were determined by the biuret method on a Coleman spectrophotometer at 540 mμ.

Preparation of fluorescent antibodies. — Globulins prepared from the above sera were conjugated with fluorescein isothiocyanate by overnight incubation at 4C after the addition of the powdered dye (.05 mg/mg globulin) to buffered globulin (3). The preparation was freed of unconjugated fluorescein by passage through a Sephedex column[4] previously adjusted to pH 7.2 with .01M phosphate buffered .15M NaCl saline (PBS). Adsorption was carried out overnight at 4 C by the addition of 20 mg (dry weight) of rehydrated rabbit liver powder per mg of globulin to reduce nonspecific fluorescence. The conjugate was cleared of liver powder by centrifugation at 41,000 g for 30 min. followed by overnight dialysis against PBS pH 7.2 and again centrifuged at 41,000 g for 30 min. This resulted in a clear preparation of the desired pH.

Preparation of Slides. — Primary bovine embryonic kidney cells (BEK) grown on coverslips in Leighton tubes were infected with 100 $TCID_{50}$ of either of two bovine viral diarrhea viruses, Oregon C24V or antigenically related NADL-MD. Coverslips were removed at 24 hours or when the first sign of a cytopathic effect was evi-

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[1]Bovine viral diarrhea virus isolated in 1962 at the National Animal Disease Laboratory, Ames, Iowa.

[2]Prepared by Dr. J. P. Torrey, National Animal Disease Lab., Ames, Iowa.

[3]Prepared by Dr. A. W. McClurkin, National Animal Disease Lab., Ames, Iowa.

[4]Sephedex G-25 Coarse, Pharmacia, Uppsala, Sweden.

dent (approximately 36 to 40 hours after infection). Coverslips of noninfected cultures were removed at the same time.

Cultures chronically infected with hog cholera virus (PK-2a) and noninfected control cultures (PK-15), originating from the same pig kidney cell line[5] and an additional noninfected pig kidney cell line (PEK)[6] were grown on coverslips in Leighton tubes. Coverslips were removed when a satisfactory monolayer had formed (approximately 5 days).

The cultures were washed in PBS, dried for 5 min. at room temperature, and fixed in acetone at -60 C for 20 minutes. After fixation the cultures were again washed in

[5]Kindly supplied by Cutter Laboratories, Berkeley, California.
[6]Kindly supplied by Drs. A. W. McClurkin and P. D. Lukert, National Animal Disease Laboratory, Ames, Iowa.

Figure 1A: Bovine kidney monolayer 36 hrs. following inoculation with NADL-MD viral diarrhea virus. Stained with swine anti-hog cholera conjugate.

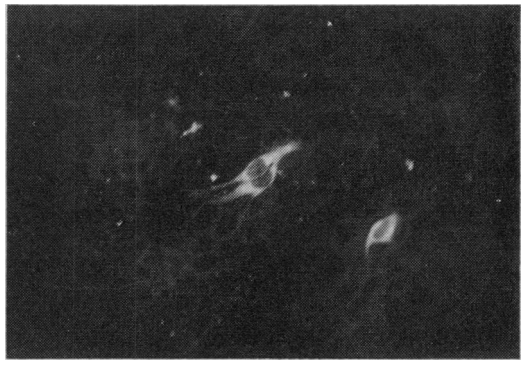

Figure 1B: Bovine kidney monolayer 24 hrs. following inoculation with NADL-MD viral diarrhea virus. Stained with bovine anti-viral diarrhea conjugate.

Figure 2A: Bovine kidney monolayer 24 hrs. following inoculation with C24V viral diarrhea virus. Stained with swine anti-hog cholera conjugate.

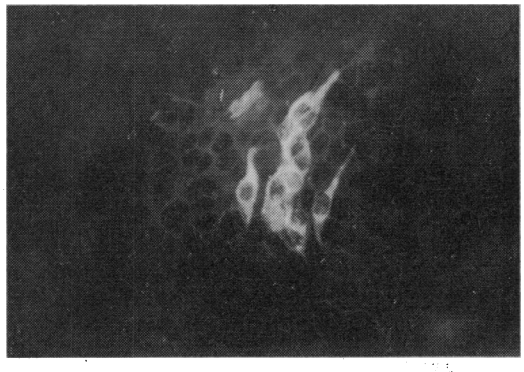

Figure 2B: Bovine kidney monolayer 24 hrs. following infection with C24V viral diarrhea virus. Stained with bovine anti-viral diarrhea conjugate.

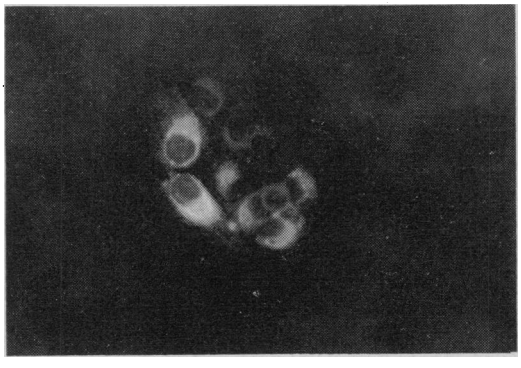

Figure 3A: Swine kidney cell line chronically infected with hog cholera virus. Stained with swine anti-hog cholera conjugate.

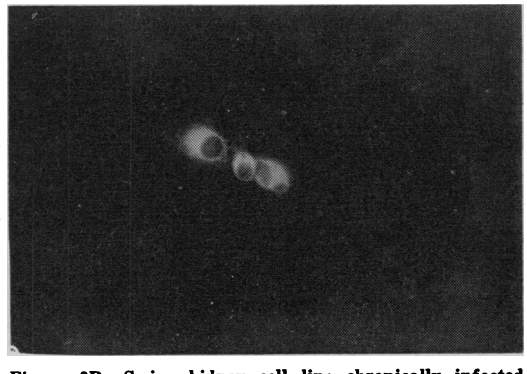

Figure 3B: Swine kidney cell line chronically infected with hog cholera virus. Stained with bovine anti-viral diarrhea conjugate.

PBS and allowed to dry. Slides were stained with appropriate conjugates for a minimum of 1 hour in a moist chamber at 37 C. After incubation the cultures were washed in PBS, dried and mounted in equal volumes of glycerine and double strength PBS pH 7.2.

Microscopy. — Examinations were made with a Leitz fluorescent microscope with a darkfield condenser, illuminated by an Osram lamp HB0200. The primary light filter was a BG12 and the barrier filter was an OG-1.

Results

Staining of bovine viral diarrhea antigen. — Staining of BEK cultures, infected with either C24V or NADL-MD bovine viral diarrhea virus, with fluorescein-conjugated anti-NADL-MD globulin or with swine anti-hog cholera globulin resulted in specific fluorescence in the cytoplasm of infected cells. Antigen was diffusely distributed throughout the cytoplasm of most infected cells (Fig. 1A, 1B, 2A, 2B). Nonimmune SPF swine and bovine conjugates did not stain infected cultures nor did swine anti-hog cholera or bovine anti-NADL-MD conjugates stain noninfected cultures. Staining of C24V or NADL-MD infected cultures was inhibited when unconjugated bovine anti-NADL-MD or swine anti-hog cholera globulin was mixed with bovine anti-NADL-MD or swine anti-hog cholera conjugates before staining. Fluorescence was not inhibited by unconjugated nonimmune SPF swine or bovine globulin when either of these were mixed with conjugated bovine anti-NADL-MD or swine anti-hog cholera conjugates.

Staining of hog cholera antigen. — Staining of PK-2a cultures infected with hog cholera virus with fluorescein-conjugated swine anti-hog cholera globulin or with anti-NADL-MD globulin, resulted in specific fluorescence in the cytoplasm of infected cells (Fig. 3A, 3B). Nonimmune SPF swine and bovine conjugates did not stain infected cultures nor did swine anti-hog cholera or bovine anti-NADL-MD conjugates stain noninfected cultures. Staining of hog cholera infected cultures was inhibited when unconjugated bovine anti-NADL-MD or unconjugated swine anti-hog cholera globulin was mixed with bovine anti-NADL-MD or swine anti-hog cholera conjugates before staining. Staining was not inhibited by unconjugated nonimmune SPF swine or bovine globulin when either of these was mixed with conjugated bovine anti-NADL-MD or swine anti-hog cholera conjugates.

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Some clinical and experimental observations on naturally-occurring pantothenic-acid deficiency in pigs

The availability of a large pig herd, free of enzootic pneumonia and at the same time apparently suffering from pantothenic acid deficiency provided a favourable opportunity to study this disease under field conditions. In the herd, in which the average growth rate was high, signs of pantothenic acid deficiency were: lack of vigour in the new born litters, loss of hair in young growing pigs, scouring and various degrees of neurological involvement. Analysis of the diet showed a deficiency of pantothenic acid. One of the earliest neurological signs was merely a slightly faulty action of the hind legs including a mincing gait. One of the next stages was swaying of the hind quarters. In the main the abnormalities fell into two groups — first, exaggerated actions with the hind legs and secondly, progressive paralysis of the hind legs.

The main gross lesions seen on autopsy were in the alimentary tract. Early cases showed a catarrhal enteritis, longer-standing cases often showed a necrotic enteritis or thickening of lower ileum. Frequently there was gastritis and occasionally peritonitis and bowel rupture. Many of the weaners and young fattening pigs that showed a grossly corrugated or necrotic terminal ileitis and necrotic colitis at autopsy would, with their clinical history of unthriftiness and scouring, have been diagnosed as infective necrotic enteritis or salmonellosis in practice.

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