Detection of Hog Cholera Virus by Its Effect on Newcastle Disease Virus in Swine Tissue Culture

Investigations of hog cholera virus (HCV) have been hampered by the lack of laboratory hosts more convenient than swine. In view of the recent widespread application of tissue-culture technique for virus studies, our efforts to discover such hosts have been concentrated on this technique. Hog cholera virus has been known to multiply in swine tissue culture, but the tissue-culture technique has achieved only limited application for HCV studies because the cytopathogenic changes produced are discernible only by elaborate observation (*1*). In order to discover simple means to determine HCV infection in swine tissue culture, we studied the interaction between HCV and other viruses in this system and found an exalting effect of HCV on Newcastle disease virus (NDV). This effect has subsequently proved to be usable for detection of HCV. This method, designated the END method (*E*xaltation of *N*D*V*), was successfully applied for detecting and measuring HCV and its neutralizing antibodies.

The principle of the method is based on the following findings: (i) Hog cholera virus proliferates in swine testicle cell monolayer culture without any cytopathogenic effect discernible by low-power microscopy. (ii) Under certain experimental conditions (see below), NDV (Miyadera strain) shows little or limited multiplication without any cytopathogenic effect when it is inoculated into the culture within 5 days of cultivation, whereas NDV multiplies well with cytopathogenic effect when it is inoculated into the same culture after 6 to 8 days of cultivation. (iii) In the culture which receives HCV in the initial stage of cultivation, NDV readily proliferates with marked cytopathogenic effect even if it is inoculated as early as the third or fourth day of cell cultivation.

In order to obtain a clear-cut difference in response to NDV between HCV-infected and control cultures, the experimental conditions must be carefully adjusted. In particular, the bovine serum in the culture medium should be carefully selected, because only 4 of 20 serums hitherto tested have been found to be suitable for this purpose, while the remaining sera produced more or less cytopathogenic effect in control as well as in HCV-infected cultures, or only incomplete cytopathogenic effect in HCV-infected culture after NDV infection.

Careful studies of the various factors involved in the phenomenon finally resulted in the following standard procedure: Testicles aseptically removed from piglets weighing about 15 kg are trypsinized, and a 7 percent cell suspension is made in culture medium (10 percent bovine serum and 0.5 percent lactalbumin hydrolyzate in Hanks' balanced salt solution), and distributed in 11- by 150-mm tubes in 0.9-ml amounts; 0.1 ml of HCV material is then added to each tube, and the tubes are then incubated at 37°C in a slanted, stationary position. The medium is changed on the third day. On the fourth day the tubes are inoculated with about 10^6 plaque-forming units of NDV in 1.0 ml of culture medium after culture medium has been removed. The tubes are then incubated at 37°C in a roller drum. The tubes in which a cytopathogenic effect develops are considered as positive in HCV infection and those without development in 3 days are considered as negative. Hemagglutination of fowl erythrocytes is also employed as a criterion, for it becomes positive when the cytopathogenic effect develops. Cell cultures inoculated with either one of HCV and NDV and with no viruses are included in the test as controls.

Using this procedure, we tested various swine materials for the presence of HCV. Of 28 specimens of bloods, serums, and organ tissues obtained from naturally and experimentally (ALD strain) infected swine, 23 gave positive results, while the remaining five, supposedly with low virus contents, were negative. ALD strain, when it was passaged in swine spleen (11 generations) or testicle (25 generations) tissue culture invariably gave positive results. All of 38 specimens obtained from normal swine were invariably negative. The materials which gave positive results became negative after irradiation by ultraviolet light.

The infective titer determined by this method is somewhat lower than that determined by swine inoculation. However, its sensitivity can be enhanced when serial dilutions of the virus material to be tested are first enriched in swine tissue culture and then tested for virus by this method (two-step method). In one of such tests the titer (spleen roller tube culture was used for enrichment) was 0.1 ml of 10^{-5}, while the titer obtained without enrichment was 0.1 ml of 10^{-3} and that for subcutaneous inoculation into swine 1.0 ml of 10^{-5}.

Table 1. Titration by the END method of neutralizing antibodies produced in two swine which received crystal violet vaccine (5 ml for swine No. 50, 50 ml for swine No. 51) and, 3 weeks later, active HCV (ALD strain, 10,000 mld).

Weeks after		Titer*	
Vaccine	Active virus	No. 50	No. 51
0		0	0
3	0	1:2	1:8
4	1	1:1024	1:2048
5	2	1:1024	1:1024

* Serum dilution against 8 ID_{50}'s determined by the END method.

The cytopathogenic effect occurring in HCV-infected culture after NDV infection can be inhibited specifically by antiserum for HCV. One volume of serum dilution (heated at 56°C for 30 minutes) and one volume of virus dilution were mixed, incubated at 37°C for 1 hour, and tested for infectivity by the END method with 0.1-ml amounts. No neutralization was shown with 20 or 21 sera from normal swine purchased from a nonenzootic area, while the remaining one had neutralizing activity in 1 : 2 dilution, but not in 1 : 10 dilution against 100 ID_{50}'s of HCV. On the contrary, swine vaccinated with crystal violet vaccine produced neutralizing antibodies in low titers and rapidly in very high titers after subsequent injection(s) with active HCV. The results of one such experiment are shown in Table 1.

A new in vitro method was developed for detecting and measuring hog cholera virus and its neutralizing antibodies. The method is based on the exalting effect of hog cholera virus on Newcastle disease virus in swine testicle cell monolayer culture (*2*).

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References and Notes

1. D. P. Gustafson and C. M. Pomerat, *Am. J. Vet. Research* 18, 473 (1957).
2. We are greatly indebted to Dr. S. Ishii of the National Institute of Animal Health for his helpful suggestions.

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