Neutralizing Antibody Against Infectious Canine Hepatitis Virus (ICHV) in Veterinary Workers' Sera (37579)

WARREN D. GEHLE, FREDERICK T. LYND, AND KENDALL O. SMITH

Departments of Microbiology and Laboratory Animal Medicine, The University of Texas Health Science Center, San Antonio, Texas 78284

Adenoviruses are among the most numerous and ubiquitous of animal viruses. Although they share group antigens, the many types can be differentiated on the basis of their eliciting specific *neutralizing* antibody. Adenoviruses infecting different animal species have several characteristics in common, including size, morphology, chemical composition, cytopathology and shared antigens (1, 2). Despite these common properties, adenoviruses are usually host specific and are not known to cross species boundaries to cause natural disease (2, 3). This is consistent with the fact that adenoviruses usually replicate poorly in cell cultures of heterologous species (3). We found, for example, that the canine adenovirus, infectious canine hepatitis virus (ICHV), produces an abortive infection in human cell cultures, with low yields of infectious virus (4). Although ICHV cannot replicate well in human cell cultures, large amounts of viral antigens are synthesized (4). Sarma *et al.* (5) reported that ICHV produces tumors when injected into new-born hamsters, animals which do not support significant replication of ICHV.

In a previous study (6), we showed that a substance was present in the sera of many human adults which neutralizes ICHV. This substance, which has the characteristics of antibody, was found in the sera of about three-quarters of the adult population which we sampled. The present study reaffirms this prevalence and provides quantitative data on the concentration of ICHV neutralizing antibody in the sera of veterinary workers, who presumably experience a high risk of contacting ICHV carried by dogs brought to them for treatment.

Methods. The Utrecht stain of ICHV was originally obtained from the American Type Culture Collection and passaged about 10 times in Madin-Darby canine kidney (MD CK) cells. Human adenovirus type 8 (Ad-8) was obtained from the Research Reference Reagents Branch, National Institute of Allergy and Infectious Diseases (NIAID), NIH, and was passaged two times in primary embryonic human kidney cells prior to use. Each of these viruses could be neutralized with specific reference antisera obtained from NIAID.

For infectivity assay of ICHV we used MDCK cells originally obtained from Microbiological Associates, Inc. Human amnion cells (strain FL) from Microbiological Associates, Inc. were used for the assay of Ad-8. Basal Eagle's medium plus 10% fetal calf serum, glutamine and neomycin (20 μg/ml final concentration) was used for cell cultivation and to maintain cells after inoculation. Cells planted in Petri dishes for viral assays were incubated in a 5% CO_2 atmosphere. The methods used for infectivity titrations have already been described (4, 6, 7).

Viral antibody was titrated as reported previously (6, 8). In brief, sera were heated at 56° for 20 min and equal volumes of serial dilutions were mixed with ICHV suspensions containing $10^{4.0}$ infectious units per 0.2 ml or with suspensions containing $10^{4.3}$ infectious units of Ad-8 per 0.2 ml. The mixtures were incubated for one hour at 26°, then inoculated on appropriate cells in plastic Petri plates. After 1 hr, maintenance fluid was added to the dishes and they were incubated, fixed, stained and examined as described above. Positive neutralization in this report

TABLE I. Infectious Canine Hepatitis Virus Neutralizing Antibody in Sera from Veterinary and Nonveterinary Workers.

Serum source	Proportion of sera containing ICHV neutralizing antibody			
	1:2 dilution	%	1:60 dilution	%
Veterinary	20/26	77	24/52	46
Nonveterinary	17/23	74	27/78	35

is defined as suppression of more than 80% infectivity (five-fold decrease in fluorescing cell foci).

Human sera were obtained from veterinarians and veterinary assistants actively working in veterinary clinics or hospitals in the northern section of San Antonio, Texas. Matching nonveterinary workers' sera were obtained from normal individuals undergoing routine serologic tests and from pregnant women receiving pre-natal care in Bexar County, Texas.

All sera which we found to contain ICHV neutralizing antibody were tested at a 1:2 dilution to detect Ad-8 neutralizing antibody.[1] The sera which we found that neutralized *both* viruses were deleted from further consideration in the study and are not described in this paper.

Results. The prevalence of ICHV neutralizing antibody in sera from veterinary and nonveterinary workers is shown in Table I. A high proportion of the adult human population studied had antibody against ICHV, and there was no significant difference in the prevalence of antibody at a 1:2 dilution. Only a slightly higher percentage of veterinary workers' sera were positive at a 1:60 dilution (veterinary workers, 46%; nonveterinary workers, 35%).

Analysis of data other than that presented in the present paper indicated that there might be differences in ICHV antibody prevalence according to age, sex and ethnic classification; therefore, the sera were paired to within three years of age, according to sex and according to whether they had Spanish-American or Anglo-American surnames. Each of 40 positive sera was titrated to determine the concentration of ICHV neutralizing antibody. The result of these titrations is shown (Table II). Veterinary workers' sera generally had higher titers than the control sera. This is further reflected in the calculated geometric mean titers of 1:59 for positive veterinary workers' sera and 1:16 for positive matched control sera.

McNemar's test for paired data was used to evaluate the two samples at an ICHV neutralizing antibody titer of 1:60. The value obtained in this Chi-square test was greater than the value of Chi-square at an α-level of 0.01 for one degree of freedom (Table III). Thus there is a significant difference between the veterinary and nonveterinary workers in the prevalence of high-titered neutralizing antibody to ICHV.

Discussion. The high prevalence of ICHV neutralizing antibody in veterinary workers (77%) and controls (74%) is comparable to the prevalence of antibody to some of the lower human adenovirus types. For example, the prevalence of neutralizing antibody to

TABLE II. Distribution of Infectious Canine Hepatitis Virus Neutralizing Antibody in Positive Test Sera from Veterinary and Nonveterinary Workers

Neutralizing antibody titers	Proportion of positive test sera having titers equal to or greater than indicated (%)	
	Veterinary sera	Nonveterinary sera
1:2	26/26 (100)	14/14 (100)
1:20	21/26 (81)	8/14 (57)
1:60	21/26 (81)	6/14 (43)
1:180	12/26 (46)	4/14 (29)
1:540	3/26 (12)	0/14 (0)

[1] Antibody to human adenovirus types 1-31 does not neutralize ICHV, with the exception of anti-Ad-8. There is a one way cross reaction between ICHV and human Ad-8 *neutralizing* antibody, viz., specific Ad-8 antibody neutralizes ICHV but specific ICHV antibody does not neutralize Ad-8 (6). Because of this, ICHV neutralizing antibody can be distinguished from Ad-8 antibody by testing a serum for neutralizing activity against Ad-8. Antibody to Ad-8 is rare in the North American population and is usually associated with a history of acute keratoconjunctivitis (9).

TABLE III. Infectious Canine Hepatitis Virus Neutralizing Antibody in Matched Sera from Veterinary and Nonveterinary Workers.

		Nonveterinary		
	Neutralizing antibody at 1:60 dilution	No. positive	No. negative	Totals
Veterinary	No. positive	4	15	19
	No. negative	2	9	11
	Totals	6	24	30

McNemar's test: $x^2 = \frac{(|15-2|-1)^2}{15} = 9.6$

$x^2 .01 = 6.63 < 9.6.$

human adenoviruses in the Washington, D.C. population (> 15 years old) was reported as 62% (Ad-1), 80% (Ad-2), 28% (Ad-3), 72% (Ad-4) and 52% (Ad-5), when sera were screened at 1:4 (10). The high frequency of *human* adenovirus antibody occurrence in man might be readily explained by the ease with which the virus spreads from man-to-man and/or the ability of adenoviruses to establish latent infections (2).

The similarly high frequency of ICHV antibody in man can hardly be explained by man-to-man transmission because ICHV produces an abortive infection in human cells (4). One explanation may be that people frequently have contact with virus-shedding pet dogs over a period of several years. Veterinarians give frequent "booster" inoculations of live attenuated ICHV vaccine to dogs, which probably result in long periods of asymptomatic infection. The probability that pet owners and veterinarians will have contact with live virus shed by these animals is enhanced by the fact that ICHV is notably resistant to heat, drying and chemical treatment (12). Often, dogs live in the same quarters occupied by their owners, which practically assures contact with canine viruses that are excreted.

It is difficult to postulate that humans would come in contact with sufficient quantities of *non*infectious ICHV antigen to elicit a significant antibody response, even with prolonged and intimate contact with dogs. The more likely alternative is that humans experience repeated abortive infections with ICHV over a period of years. Although this virus may produce an abortive ("dead end") infection in man, infected cells *in vitro* are known to produce significant quantities of viral antigens which can be readily detected by fluorescence microscopy or complement fixation tests (4). Noninfectious but antigenic material produced during abortive infections may be capable of eliciting an antibody response in individuals receiving a sufficiently large viral inoculum. Thus, infection may serve as a one-step *in vivo* amplification of the relatively small antigenic mass originally contacted, leading to the production of sufficient antigen to stimulate an antibody response. Whether such infections would become latent over a long period of time is presently unknown.

The evidence for repeated human infection with ICHV includes the following: (1) dogs are repeatedly immunized with, and probably shed, live attenuated ICHV in the human environment; (2) veterinarians, who are exposed to large numbers of dogs, have slightly but significantly higher antibody titers than matched controls; (3) prevalence of ICHV antibody in younger children is low but increases to a maximum by their mid-teens (6); and (4) it is difficult to explain the observed maintenance of significant ICHV antibody titers unless there is repeated antigenic stimulation. An alternative explanation for the observed maintenance of ICHV antibody in most adults is the possibility that initial infection leads to viral latency and a consequent slow, prolonged release of viral antigens.

Many adenoviruses, including ICHV, are

oncogenic when experimentally introduced into heterologous animal species, although they do not appear to be oncogenic for the species in which the virus naturally replicates. A recent report (11) presented negative serologic evidence that human, bovine, canine or simian adenoviruses are causes of human cancer. Results with the canine adenovirus (ICHV) were based upon complement fixation tests for specific antibody against ICHV tumor antigen in the sera of cancer patients. The authors of the report (11) suggested caution in the interpretation of the data because of the limitations in the methodology employed. Our findings, indicating that man is frequently infected with ICHV, suggest that the possible consequences of human infections should be studied further.

Summary. A high prevalence of neutralizing antibody to ICHV was found in the sera of veterinary and nonveterinary workers. When sera from veterinary and nonveterinary workers were paired according to age, sex and ethnic classification, veterinary workers were found to have significantly higher antibody titers than nonveterinary controls.

1. Huebner, R. J., Rowe, W. P., Ward, T. G., Parrott, R. H., and Bell, J. A., New Engl. J. Med. **251**, 1177 (1954).

2. Rowe, W. P., and Hartley, J. W., Ann. N.Y. Acad. Sci. **101**, 466 (1962).

3. Klein, M., *in* "Basic Medical Virology" (James E. Prier, ed.). The Williams and Wilkins Company, Baltimore, Md. (1966).

4. Gehle, W. D., and Smith, K. O., Proc. Soc. Exp. Biol. Med. **131**, 87 (1969).

5. Sarma, P. S., Vass, W., Huebner, R. J., Igel, H., Lane, W. T., and Turner, H. C., Nature (London) **215**, 293 (1967).

6. Smith, K. O., Gehle, W. D., and Kniker, W. T., J. Immunology **105**, 1036 (1970).

7. Thiel, J. F., and Smith, K. O., Proc. Soc. Exp. Biol. Med. **125**, 892 (1967).

8. Smith, K. O., Dunlap, R. C., Thiel, J. F., Newman, J. T., and Palmer, A. E., Proc. Soc. Exp. Biol. Med. **133**, 560 (1970).

9. Jawetz, E., Thygeson, P., Hanna, L., Nicholas, A., and Kimura, S., Proc. Soc. Exp. Biol. Med. **92**, 91 (1956).

10. Jordon, W. S., Jr., Arch. Int. Med. **101**, 54 (1958).

11. Gilden, R. V., Kern, J., Lee, Y. K., Rapp, F., Melnick, J. L., Riggs, J. L., Lennette, E. H., Zbar, B., Rapp, H. T., Turner, H. C., and Huebner, R. J., Amer. J. Epidemiol. **91**, 500 (1970).

12. Fastier, L. B., Vet. Res. **70**, 632 (1958).

Received June 6, 1973. P.S.E.B.M., 1973, Vol. 144.