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Immunological Relationship Between Delta Herpesvirus of Patas Monkeys and Varicella-Zoster Virus of Humans

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The Delta herpesvirus (DHV) which produced outbreaks of exanthematous disease in patas monkeys was shown to have a close immunological relationship to varicella-zoster (V-Z) virus of man. Immunization of rhesus monkeys with DHV or V-Z virus resulted in the development of neutralizing antibodies to both viruses and also in the production of complement-fixing antibodies to V-Z virus. Immunoglobulin M neutralizing antibody to V-Z virus was demonstrated in the serum of a rhesus monkey immunized with DHV, suggesting a primary antibody response rather than recall of antibody to a related virus. Convalescent-phase sera from human zoster cases had comparable levels of neutralizing antibody for both DHV and V-Z virus. Patas monkeys involved in an outbreak of DHV infection showed seroconversion to both DHV and V-Z virus by neutralization tests and to V-Z virus by complement fixation tests. The demonstration of the close antigenic relationship between DHV and V-Z virus suggests that DHV may be useful in an animal model system for studies on the latency and reactivation of V-Z virus.

An exanthematous disease with high morbidity and mortality in patas monkeys (*Erythrocebus patas*) caused by a cell-associated herpesvirus was first described by McCarthy et al. (9). A similar epizootic in patas monkeys was observed at the Delta Regional Primate Research Center in 1968. All of the afflicted animals in this outbreak had been inhabitants of the Center for at least 4 years. They were housed in animal quarters together with macaques, baboons, and members of related genera; however, only the patas developed the disease. Details of the pathology, clinical picture, and epidemiology of that epizootic were reported by Riopelle et al. (11). A strongly cell-associated virus, referred to as the Delta herpes virus (DHV), was isolated in primary human embryonic kidney and Vero cell cultures by Ayres (2) from the blood of a moribund patas monkey from the 1968 epizootic. In 1973 a second epizootic of DHV infection occurred in patas monkeys at the Delta Primate Center. Studies on the isolation and characterization of the etiologic agent were reported by Allen et al. (1).

Although Ayres (2) reported no cross-neutralization between DHV and varicella-zoster (V-Z) virus with a neutralization technique using a constant serum concentration and varying dilutions of virus, Allen et al. (1) demonstrated fluorescent antibody staining of V-Z-infected cells with antiserum to DHV.

This report describes the results of comparative studies using a plaque reduction method developed at the Delta Primate Center for assay of neutralizing antibody to DHV and a plaque reduction neutralization test for V-Z virus (13). By neutralization and complement fixation (CF), a very close immunological relationship was demonstrated between the two viruses, suggesting that DHV is indeed a patas monkey varicella virus.

MATERIALS AND METHODS

Viruses. The 1968 strain of DHV at an early passage in Vero cells was sent to K. McCarthy, Department of Bacteriology, University of Liverpool, England. He inoculated the virus into patas monkeys and then reisolated it in Vero cells. It was given the designation Delta Patas I (DPI) virus. After an additional 17 passages in Vero cells, a stock of the virus was returned to A. Felsenfeld. The virus propagated well in Vero cells from several sources, but the best plaques were formed in Vero cells obtained from W. Adrian Chappel, Center for Disease Control, Atlanta, Ga. The medium used for growth of Vero cells, and also for maintenance of the cells during virus propagation, consisted of Eagle minimal essential medium (MEM), prepared in Earle balanced salt solution, supplemented with 10% fetal bovine serum and containing 100 U of penicillin and 100 μg of streptomycin per ml. Stock DHV was harvested from infected Vero cell cultures showing a viral cytopathic effect (CPE) involving 50 to 70% of the cell monolayer. Infected cells were scraped into the medium with a rubber policeman and then sedimented by centrifuga-

tion at 800 rpm for 10 min. Cells from each 16-oz (ca. 480 ml) culture bottle were resuspended in 2 ml of a solution consisting of equal parts of growth medium and 70% sorbitol dissolved in distilled water. The stock virus was stored frozen at −80 C. For subpassage of DHV, the following method was used. A confluent monolayer of uninfected Vero cells from a 16-oz bottle was removed with trypsin. The cell suspension was distributed equally among three 16-oz bottles, and growth medium was added to give a total volume of 50 ml in each bottle. The supernatant fluid was removed from a 16-oz bottle culture of infected Vero cells showing 2 to 3+ viral CPE, and the infected cells were scraped into 4.5 ml of growth medium. The cells were dispersed by vigorous pipetting, and 1.5 ml of the infected cell suspension was added to each of the 16-oz bottles seeded with uninfected cells. After incubation at 37 C for 2 to 4 days, viral CPE involved one-half or more of the cell monolayer.

The Batson strain (14) of V-Z virus was used for the present studies; it was subpassaged as previously described (15) in human fetal diploid lung cell strains, established by J. H. Schieble, by inoculation of cell monolayers with trypsin-dispersed V-Z virus-infected cells. Cell-free virus for use in V-Z neutralization tests (13) was prepared as initially described by Brunell (5) by sonic oscillation of infected human fetal diploid lung cells. Complement-fixing antigen for V-Z virus was produced by a method described elsewhere (14). All tests with V-Z virus were performed at the State of California Department of Health, where DHV had never been handled.

The herpes simplex virus (HSV) strain used for neutralization tests conducted at the Delta Primate Center was originally obtained in 1963 from Walter Reed Army Institute of Research, Washington, D.C. CF tests for HSV were performed with an antigen (12) produced from the MacIntyre strain of type 1 HSV. A few neutralization tests for HSV were performed at the State of California Department of Health using the MacIntyre strain of type 1 HSV and the MS strain of type 2 HSV.

Neutralization test procedures. Plaque reduction neutralization tests for DHV were performed in Vero cells. Test sera were inactivated at 56 C for 30 min, and twofold dilutions were prepared in growth medium. Each serum dilution was mixed with an equal volume of stock virus diluted in growth medium to contain 150 to 200 plaque-forming units per 0.2 ml, and the serum-virus mixtures were incubated at 37 C for 60 min. A suspension of freshly trypsinized Vero cells diluted in growth medium to contain 10^5 cells per ml was added in a volume of 5 ml to 60-mm Falcon culture dishes. Serum-virus mixtures were then inoculated in a volume of 0.4 ml into the cell suspensions, and the culture dishes were incubated at 37 C in a 5% CO_2 atmosphere for 5 days. The cell sheets were stained with a mixture of 3 volumes of 1% methylene blue, 1 volume of 1% basic fuchsin in absolute methanol, and 1 volume of methanol as described by Rangan et al. (10). The DPI strain of DHV formed small plaques ≤ 1 mm in diameter which failed to take the stain. The neutralizing antibody titer was expressed as the highest dilution of serum which reduced the plaque count by 80% or more as compared with the count in the virus controls without serum.

Neutralizing antibody to V-Z virus was assayed by the plaque reduction method developed at the State of California Department of Health, which has been described in detail elsewhere (13). Tests were conducted in parallel without guinea pig complement in the serum-virus mixtures, and with fresh guinea pig serum in the reaction mixtures, as described by Benyesh-Melnick (3) for cytomegalovirus (CMV) neutralization tests. Plaques were counted on days 8 and 9, and the neutralizing antibody titer was expressed in terms of the highest serum dilution producing a 50% or greater reduction in plaque count as compared with counts in the virus controls.

Neutralizing antibody assays for HSV were performed at the Delta Primate Center in tube cultures of primary baby rabbit kidney cells maintained on Eagle MEM with 2% fetal bovine serum. Serum dilutions were assayed against 80 to 100 minimal tissue culture infective doses of virus, and serum-virus mixtures were incubated at 37 C for 60 min before inoculation into cell cultures. Tests were read microscopically 4 to 5 days after inoculation, and neutralizing antibody titers were expressed as the highest serum dilution which prevented the CPE of the test virus dose. HSV neutralization tests performed at the State of California Department of Health were conducted by a plaque reduction procedure (13) in human fetal diploid lung cells using 50% or greater reduction in plaque count as the antibody end point.

Neutralization tests for all viruses were performed using two cell cultures for each serum-virus mixture.

CF tests. Complement-fixing antibody assays for V-Z virus and HSV were conducted by the standard procedure of the State of California Department of Health Virus Laboratory (8).

Sera examined. Patas monkey sera were examined from eight animals associated with the 1968 outbreak of DHV infection at the Delta Primate Center. Four of the animals had clinical symptoms but recovered (no. 57, 538, 2035, 88), and the other four were presumably exposed but showed no clinical symptoms (no. 650, 532, 2037, 1741). Attempts to isolate virus from the monkeys were not successful.

Specific immune sera for DHV were prepared by immunization of two rhesus monkeys. The DPI virus strain was propagated in Vero cells, and the infected cells were washed by centrifugation three times in Eagle MEM without serum. The virus inoculum was prepared in 35% sorbitol in Eagle MEM, and its infectivity titer was 2.5×10^4 plaque-forming units per ml. For the initial immunization, each animal received 0.5 ml of the virus inoculum intratracheally and 1.0 ml of a mixture of equal parts of virus and Freund incomplete adjuvant intramuscularly. At weeks 1 and 2 the animals received 1 ml of the virus without adjuvant by the subcutaneous route. The animals were bled 4 weeks after the initial immunization.

Convalescent-phase sera from human zoster cases were from the State of California Department of Health Virus Laboratory. The patients were confirmed to have V-Z infections by direct fluorescent antibody staining of lesion material (15) or virus isola-

tion, as well as by diagnostically significant increases in V-Z antibody titer.

Specific immune serum for V-Z virus was produced in rhesus monkeys as described elsewhere (15).

Reference antiserum for HSV, produced in guinea pigs against the Mayo 1814 strain, and for simian CMV, produced in rhesus monkeys against the GR 2757 strain, were obtained from the Research Resources Branch, National Institutes of Health.

RESULTS

Neutralization of DHV by V-Z immune sera. Table 1 shows the results of cross-neutralization tests with V-Z immune monkey serum and convalescent-phase sera from human zoster cases against V-Z and DHV viruses. The titers shown for both viruses were obtained without guinea pig complement in the serum-virus mixtures. Comparable neutralization titers were obtained against the two viruses. It is also seen that the V-Z immune monkey had no demonstrable antibody to DHV virus in the pre-immunization serum, but developed antibody over the course of immunization with V-Z virus.

Reference antiserum for HSV with a homologous neutralizing antibody titer of 1:64 against 100 minimal tissue culture infective doses of virus and antiserum for simian CMV with a homologous neutralizing antibody titer of 1:2,148 against 100 plaque-forming units of virus both had neutralizing antibody titers of <1:4 for DPI virus.

Cross-neutralization of DHV and V-Z virus by rhesus immune sera to DHV. Results obtained with DHV immune rhesus sera in neutralization tests against DHV virus, V-Z virus, and HSV, and in CF tests against V-Z and HSV antigens, are presented in Table 2. Neither animal had demonstrable antibody for either DHV or V-Z virus in the pre-immunization sera, but they developed antibody to both viruses over the course of immunization with DHV virus. The neutralizing antibody titers to V-Z virus were markedly enhanced by fresh guinea pig complement, which has also been noted in V-Z neutralization tests on sera from human infections and immune monkey sera (N. J. Schmidt and E. H. Lennette, submitted for publication). The immune serum from monkey no. 4095 was subjected to a sucrose density gradient centrifugation (7), and a low level of immunoglobulin (Ig)M neutralizing antibody to V-Z virus was demonstrable in the 19*S* gradient fraction; this fraction was shown by radial immunodiffusion to be free of IgG. Only one of the monkeys showed complement-fixing antibody to V-Z virus at 4 weeks after the initial immunization. Both animals had antibody to HSV at the time of immunization and, as sometimes occurs in V-Z infections in humans who have had previous infections with HSV (13), the DHV virus elicited a heterotypic complement-fixing antibody titer rise to HSV.

TABLE 1. *Neutralization of DHV by V-Z immune sera*

Serum tested	Neutralizing antibody titer versus:	
	V-Z virus	DHV
V-Z immune monkey S-2372		
Pre-immunization	<4	<4
Immune serum	256	64
Human zoster patient LaMa	256	128
Human zoster patient WiAh	256	128
Human zoster patient OrHa	1024	512

Antibody responses to DHV and V-Z virus of patas monkeys involved in the 1968 outbreak. Table 3 shows the antibody responses to DHV, V-Z virus, and HSV on paired and multiple serum specimens from patas monkeys involved in the 1968 outbreak of DHV infection at the Delta Primate Center. The first bloods were collected on these animals around 15 October 1968, when the outbreak was first recognized. The V-Z neutralizing antibody titers of these animals were either identical or differed by no more than twofold in the presence and absence of complement; titers shown in the table are those obtained in the presence of complement.

Five of the animals showed seroconversion to both DHV and V-Z virus by neutralization and also to V-Z by CF. Two animals which had antibody to DHV in their initial serum specimens, and showed a subsequent fourfold or greater increase in titer, showed stationary or declining neutralizing antibody titers to V-Z virus. Animal no. 88 failed to show complement-fixing antibody to either V-Z virus or HSV, despite the presence of neutralizing antibody to these two viruses. The one animal which failed to develop neutralizing antibody to DHV was also negative for neutralizing and complement-fixing antibody to V-Z virus. The antibody responses to DHV and V-Z virus did not appear to be influenced by prior experience with HSV, as comparable antibody responses occurred in animals with and without HSV antibody.

DISCUSSION

Results obtained in cross-neutralization tests between DHV and V-Z virus, as well as CF tests for V-Z virus on DHV immune sera, indicate a very close immunological relationship between the two viruses. Although neutralizing antibody

TABLE 2. *Cross-neutralization of DHV and V-Z virus by rhesus immune sera to DHV*

Serum tested	Antibody titer versus:					
	DHV	V-Z			HSV	
	Neutralization	Neutralization[a]		CF	Neutralization	CF
		C^-	C^+			
Rhesus no. 4068						
Pre-immunization	<4	<8	<8	<8	ND[b]	8
Immune serum	64	8	32	<8	ND	16
Rhesus no. 4095						
Pre-immunization	<4	<8	<8	<8	64	8
Immune serum	128	8	64[c]	64	128	64

[a] C^-, Complement absent in serum-virus mixture; C^+, complement present.
[b] ND, Not done.
[c] IgM neutralizing antibody titer for V-Z virus = 1:4; IgG titer = 1:32.

TABLE 3. *Antibody responses of patas monkeys involved in the 1968 DHV outbreak*

Animal[a]	Date of bleeding	Antibody titers versus:				
		DHV	V-Z		HSV	
		Neutralization	Neutralization	Complement fixing	Neutralization	Complement fixing
Patas no. 57	10-15-68	<8	<8	<16[b]	128	64
	1-6-69	256	64	64	16	16
Patas no. 538	10-16-68	<8	<8	<8	128	32
	10-30-68	32	<8	ND[c]	ND	ND
	12-4-68	1,024	32	8	128	32
	1-6-69	512	32	ND	ND	ND
Patas no. 2035	10-16-68	<8	<8	<8	64	8
	10-30-68	<8	<8	<8	ND	ND
	12-2-68	1,024	128	16	128	16
	12-19-69	512	64	ND	ND	ND
Patas no. 88	10-8-68	64	256	<8	128	<8
	10-21-68	256	64	<8	ND	<8
	11-25-68	512	16	<8	16	<8
	6-12-69	512	32	<8	ND	<8
Patas no. 650	10-15-68	64	64	64	256	64
	10-29-68	256	64	32	ND	64
	1-6-69	256	32	16	256	16
Patas no. 532	10-15-68	<8	<8	<8	<8	<8
	11-29-68	256	64	16	<8	<8
	6-12-69	1,024	64	16	ND	ND
	12-22-69	1,024	125	ND	ND	ND
Patas no. 2037	10-30-68	<8	<8	<16[b]	<8	<16[b]
	12-3-68	512	64	32	<8	<8
Patas no. 1741	10-15-68	<8	<8	<8	<8	<8
	10-28-68	<8	<8	<8	<8	<8
	1-6-69	<8	<8	<8	<8	<8

[a] No. 57, 538, 2035, and 88, clinically sick but recovered; no. 650, 532, 2037, and 1741, presumably exposed but not showing symptoms.
[b] Serum anticomplementary at 1:8 dilution.
[c] ND, Not done.

titers of DHV immune sera tended to be lower to V-Z virus than to DHV, this may be a reflection of differences in neutralization techniques or volumes of reagents used, rather than an indication of antigenic differences in the viruses.

The fact that immunization or infection with DHV or V-Z virus resulted in seroconversion to both viruses would suggest a primary antibody response rather than recall of antibody elicited by prior infection with a related virus. The finding of a low level of IgM neutralizing antibody to V-Z virus in a rhesus monkey immunized with DHV is further evidence of a primary antibody response. Attempts to demonstrate IgM antibody to V-Z virus in the earliest sera showing antibody from three of the monkeys involved in the 1968 outbreak of DHV infection were unsuccessful (titers < 1:2). However, these sera had been stored for a number of years, and this may have resulted in deterioration of IgM antibody. Further, the exact time of collection of these specimens in relation to onset of infection is not certain, and it may not have been at a time suitable for demonstration of IgM antibody.

DHV was reported by Ayres (2) to show cross-neutralization with the herpesviruses isolated by McCarthy et al. (9) from patas monkeys and by Clarkson et al. (6) from vervet monkeys. It remains to be determined whether it is also related to the herpesvirus isolated by Blakely et al. (4) from a varicella-like disease in macaque monkeys. A relationship of the latter agent to V-Z virus was suggested by the fact that some of the infected animals which showed a significant complement-fixing antibody titer rise to the homologous virus also showed a complement-fixing titer rise to V-Z antigen; however, it was not indicated whether the animals showed seroconversion to V-Z virus or simple elevation of preexisting titers. Immunodiffusion reactions also suggested a possible antigenic relationship between the macaque herpesvirus and V-Z virus.

Although DHV and V-Z virus are closely related immunologically, there are certain biological differences in their behavior. V-Z virus replicates well in human fetal diploid cell strains, including WI-38 cells, and less readily in monkey kidney cell cultures. However, DHV produces little or no CPE in WI-38 cells and replicates optimally in simian cell cultures (1, 2). Attempts to produce disease in patas monkeys with V-Z virus of known infectivity grown in cell culture have been unsuccessful (16); however, susceptibility may be related to whether the animals had experienced previous infection with DHV.

The origin of DHV infections at the Delta Primate Center remains obscure (1, 2, 17). Antibody has been demonstrated in the stumptail monkey and baboon, but not in rhesus, vervet, or newly arrived patas monkeys. The finding of the close relationship of the virus to V-Z virus lends support to the possibility that infection may have been introduced by humans.

The fact that a very close immunological relationship has been demonstrated to exist between DHV and V-Z virus suggests that DHV will prove to be valuable for use in an animal model system for studies on the latency and reactivation of V-Z virus, and possibly as a challenge virus in studies on the immunogenicity of experimental V-Z vaccines.

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