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Serological Investigation of an Outbreak of Simian Varicella in *Erythrocebus patas* Monkeys

NATHALIE J. SCHMIDT,[1]* ANN M. ARVIN,[2] DAVID P. MARTIN,[3] AND ELIZABETH A. GARD[3]

Viral and Rickettsial Disease Laboratory, California Department of Health Services, Berkeley, California 94704[1]; Department of Pediatrics, Stanford University School of Medicine, Stanford, California 94305[2]; and Litton Bionetics, Inc., Kensington, Maryland 20895[3]

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An epizootic of simian varicella occurring in a colony of *Erythrocebus patas* monkeys was studied serologically by using radioimmunoassay and neutralization tests against (i) a virus strain isolated from an animal that died during the epizootic, (ii) a simian varicella virus strain from an earlier outbreak of simian varicella-like disease at another facility, and (iii) human varicella-zoster virus. Serological tests detected more cases of infection among the animals exposed to virus during the epizootic than were evidenced by clinical findings; only 6 of the 26 animals with seroconversion developed a rash. Good correlation was seen between antibody responses demonstrated by radioimmunoassay and by the neutralization tests. Specificity of the radioimmunoassay was evidenced by the complete agreement with neutralization results for 17 animals which failed to show an antibody response over the course of the outbreak and were assumed not to have been infected. Thus radioimmunoassay is a reliable, rapid, and relatively economical method which could be used for serological screening of primates entering experimental colonies to identify those which might be potential sources of outbreaks through activation of latent simian varicella virus infection. Close correlation was seen between antibody responses to the virus strain from the current outbreak and the one from another epizootic, indicating that the two outbreaks were caused by antigenically similar viruses. Animals showing neutralizing antibody responses to the simian varicella viruses also showed responses to human varicella-zoster virus, which further substantiates the close antigenic relationship between human and simian varicella viruses.

An epizootic of simian varicella occurred in 1981 in a colony of *Erythrocebus patas* monkeys. Details of this outbreak are reported elsewhere (5). The aims of the present study were (i) to compare the sensitivities and specificities of radioimmunoassay (RIA) and neutralization tests for detecting infection serologically, (ii) to compare neutralizing antibody responses to the virus strain isolated from the outbreak with responses to another simian varicella virus strain isolated from a 1975 outbreak of varicella-like disease (1), and (iii) to study neutralizing antibody responses to human varicella-zoster virus (VZV) in the primates infected with simian varicella virus.

MATERIALS AND METHODS

Animals studied. Forty-eight adult patas monkeys which survived the epizootic were studied. Eleven of the animals were males and were housed in the animal care area where the outbreak began. Thirty-seven animals were females housed in a room adjacent to the animal care area where the index case occurred. Antibody assays were performed on paired sera. The first sera were collected from asymptomatic animals 12 days after the occurrence of the index case. The second sera were collected approximately 3 months later when the outbreak had subsided. Thirty-one of the animals received antiviral prophylaxis with phosphonoacetic acid or phosphonoformate for the duration of the epizootic (2). Single sera were tested on six male and five female animals that died during the outbreak.

Antibody assays. The RIA for immunoglobulin G (IgG) antibodies to simian varicella virus has been described (2). The antigen was prepared from a virus strain (LBI strain) isolated from a lymph node of an animal that died during the epizootic. This strain was kindly provided by William London (National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, Md.). Assays for neutralizing antibodies to the simian varicella viruses were performed as recently described (6), using the BS-C-1 line of grivet monkey kidney cells, cell-free preparations of the LBI virus strain, and the 592S strain of Delta herpes-virus (DHV). Guinea pig complement at a concentration of approximately 50 hemolytic units was used in the serum-virus mixtures to enhance neutralization. Neutralizing antibody to human VZV was

assayed as described elsewhere (7), also using complement to enhance neutralization.

RESULTS

Table 1 summarizes the serological responses of the 48 patas monkeys which survived the epizootic. Nine of the 11 animals from the male care area where the outbreak originated showed seroconversion to simian and human varicella viruses by all four antibody assays, with the exception of one animal which failed to show an antibody response by RIA. The other two animals from the male care area had low levels of antibody detectable by neutralization, but not by RIA, in their first serum specimens. Both of these animals showed fourfold or greater increases in antibody titer between their first and second serum specimens by all four assay methods. Only 3 of the 11 animals which showed serological evidence of simian varicella virus infection had a rash or other signs of acute infection.

Seroconversion occurred in 17 of 37 animals in the female care area (Table 1). Three of these animals had rash. Three female monkeys showed antibody in their first serum specimens and failed to show fourfold or greater increases in antibody levels in all neutralization assays (Table 2). Seventeen of the 37 female monkeys had no antibody demonstrable by any assay in either their acute- or convalescent-phase sera, and apparently they were not infected during the epizootic.

Sera from six male and five female animals which died during the epizootic were assayed for neutralizing antibodies to the LBI and DHV viruses and by RIA. The sera were collected 2 to 10 days before the animals showed signs of illness. Titers were <1:4 by neutralization and <1:16 by RIA in all cases. Ten of the animals developed a rash before death.

TABLE 1. Serological responses of animals surviving the simian varicella epizootic

Care area	No. of animals studied	Serological response			
		Seroconversion[a]	Antibody in first serum		No antibody response[b]
			Fourfold rise	Twofold rise	
Male	11	9 (3 rash)	2		
Female	37	17 (3 rash)		3[c]	17

[a] Neutralizing antibody titers <1:8 and RIA titers <1:16 in first specimen; antibody present in second specimen.

[b] No detectable antibody in either serum specimen by any assay.

[c] Two animals had antibody present in both specimens by neutralization, but specimens were negative by RIA; one animal showed a fourfold titer rise by RIA.

TABLE 2. Antibody responses of four animals showing discrepant results by neutralization and RIA

Animal	Serum	Neutralization titer to:			RIA titers
		LBI	DHV	VZV	
B8891	Acute	<8	<8	<8	<16
	Convalescent	64	32	32	<16
681J	Acute	32	16	8	<16
	Convalescent	64	32	8	<16
678J	Acute	128	64	32	4,096
	Convalescent	128	64	32	6.5×10^4
656J	Acute	32	32	8	1,024
	Convalescent	64	64	16	4,096

Only four animals showed discrepant results by neutralization tests and RIA (Table 2). One animal showed neutralizing antibody responses to the two simian virus strains and human VZV but did not show an antibody response by RIA. One animal showed stationary or twofold increases in neutralizing antibody titers to the three test viruses but gave negative results in both specimens by RIA. The other two animals showed stationary or insignificant increases in neutralizing antibody titers, but showed fourfold increases in antibody titers determined by RIA.

Table 3 summarizes the antibody levels demonstrated by the three neutralization tests and by RIA. Neutralizing antibody titers against the two strains of simian varicella were not significantly different, but neutralizing antibody titers against human VZV were significantly lower than those against simian varicella virus strains. Titers were markedly higher by RIA than by neutralization.

Figure 1 shows the correlation between antibody titers demonstrated by neutralization against the LBI virus strain and those demonstrated in the other two neutralization test systems and by RIA. There was excellent correlation between neutralizing antibody titers against the two simian varicella strains. There was also a positive correlation between neutralizing antibody titers to simian and human varicella virus-

TABLE 3. Antibody levels in convalescent-phase sera from 26 animals which seroconverted during the epizootic of simian varicella[a]

Test system	Range of titers	Geometric mean titer
Neutralization		
LBI	32–4,096	563
DHV	32–2,048	435
VZV	8–256	70
RIA	<16–$\geq 2.6 \times 10^5$	2.5×10^4

[a] Given as reciprocals of antibody titers.

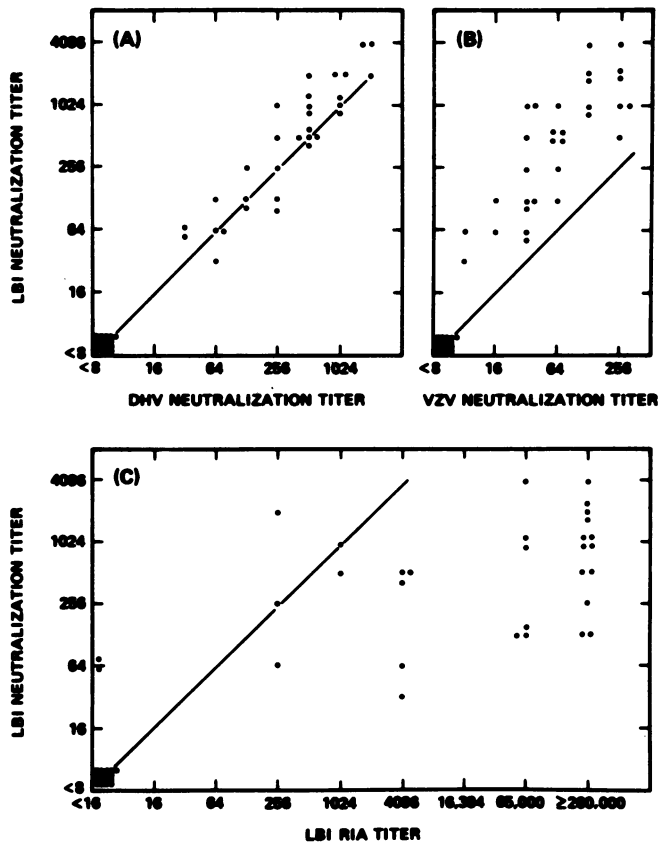

FIG. 1. Correlation of LBI neutralization titers with DHV and VZV neutralization titers and with LBI RIA titers.

es, but titers to the human VZV were lower. There was a wide scattering of RIA titers, but higher RIA titers were associated with higher levels of neutralizing antibody.

DISCUSSION

Serological investigation detected many more cases of simian varicella infection among the *Erythrocebus patas* monkeys exposed to the virus during the epizootic than were evidenced by clinical findings. Only 6 of the 26 animals with seroconversion developed rash. Although many of these animals received antiviral prophylaxis with agents effective against simian varicella in an experimental model, subclinical infection, as documented serologically, was common. Three animals had antibody to simian varicella at the onset of the epizootic. If simian varicella virus causes latent infection which can reactivate, as is true of human varicella virus, serological screening of primates entering experimental colonies could identify those animals which might be a potential source of outbreaks.

The correlation between antibody responses demonstrated by RIA and by neutralization was good, with discrepant results occurring in only four animals (Table 2). In two cases RIA failed to demonstrate antibody in convalescent-phase sera, despite the presence of neutralizing antibody against the three test viruses. In two cases the RIA demonstrated significant antibody titer rises in the face of stationary or insignificant increases in neutralizing antibody titers. Two male animals had antibody by neutralization but not by RIA in their initial sera, with subsequent significant increases in antibody detected by both methods. It is probable that the neutralizing antibody in the acute sera was IgM antibody which was not detected by the RIA for IgG antibody to simian varicella virus. The overall agreement between RIA and neutralization results indicates that the RIA is a highly reliable test for serological evaluation of simian varicella epizootics, which can be performed more economically and rapidly than neutralization tests. The specificity of the RIA was evidenced by the complete agreement with neutralization results for the 17 animals which failed to show an

antibody response over the course of the outbreak and were assumed not to have been infected.

This was the most extensive study which has been performed on neutralizing antibody responses to human VZV in naturally occurring infections with simian varicella virus. Previous studies on sera from a few animals involved in an outbreak of infection with DHV (3) suggested that neutralizing antibody responses to human VZV might occur regularly as a result of infection with simian varicella virus. The present studies on a larger number of animals from another epizootic has confirmed that this is the case. The neutralizing antibody against human VZV found in these animals further substantiates the close antigenic relationship between human and simian varicella viruses.

The close correlation demonstrated between neutralizing antibody titers to the simian varicella virus strain from the current outbreak and the DHV strain from a 1975 outbreak in another animal facility indicates that the two outbreaks were caused by antigenically similar viruses. Other studies (4) have also demonstrated a close antigenic relationship by neutralization and complement fixation tests between simian varicella viruses from diverse outbreaks and various simian species.

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