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Immunogenicity of Wild and Attenuated Varicella-Zoster Virus Strains in Pygmy Marmosets (41677)

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Abstract. Pygmy marmosets were inoculated with the low-passage parental strain or with the attenuated variant of OKA strain of varicella–zoster virus. No clinical signs were observed following inoculation and virus could not be isolated from tissues taken at several times after inoculation. A low-level antibody response developed in all animals. Three months after the first inoculation, all animals were challenged with the low-passage parental strain of virus. Animals primed originally with the parental strain developed higher booster responses than animals primed with the attenuated strain of virus. The results suggest that the parental and attenuated strains of varicella–zoster virus differ in their immunogenicity in pygmy marmosets.

In recent years several attenuated strains of varicella–zoster virus (VZV) have been developed and tested as candidate strains for human immunization (1–4). A major impediment in the development of the vaccine was the lack of a susceptible experimental animal in which the degree of attenuation could be measured. A suitable animal model should also enable studies into the mode of virus transmission, target organs for virus replication, latency, and the mechanism by which latent VZV is reactivated to cause the eruption in herpes zoster.

In the present study we utilized a South American primate, the pygmy marmoset, to determine the degree of replication and antibody response following inoculation with an attenuated and low-passage parental virus strains.

Materials and Methods. *Animals.* Wild-caught pygmy marmosets (*Cebulla pygmaea*) from northeastern Peru were used. These marmosets were in overt good health and adapted to the laboratory for at least 6 months before their use in this experiment. They were clinically normal at the outset of the project.

The animals were housed in two-tiered cages, each containing a compatible male–female pair. A specially prepared diet of ground commercial monkey chow, yogurt, fruit, wax moth larvae, and water was used to maintain the animals.

Viruses. The parental (wild) and the attenuated strains of OKA varicella virus were received from Dr. M. Takahashi (4, 5). The parental strain (OKA-W) was in its sixth passage in human embryonic lung cells; the attenuated strain (OKA-A) had 11 passages in human embryonic lung cells, 12 passages in primary guinea pig embryo cell cultures, and 2 to 5 passages in the humn diploid cell strain MRC-5.

Cell cultures and media. Vero cells at passage level 120 through 140 were supplied by the Tissue Culture Section of the Division of Virology, Office of Biologics, Food and Drug Administration, Bethesda, Maryland. Flow 5000 diploid human cells at passage level 18 were purchased from Flow Laboratories, McLean, Virginia. The cell cultures were grown in Eagle's minimum essential medium (EMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and antibiotics. The serum concentration was reduced to 4% in the maintenance medium.

Preparation of cell-free virus. After additional two to three passages in Flow 5000 cells, cell-free virus was prepared from the cellular phase of infected Flow 5000 cells by sonic treatment as described in detail elsewhere (5, 6). The titer of the two virus preparations used in this experiment was 3.3×10^5 to 5.2×10^5 plaque-forming units (PFU)/ml for the wild virus and 1.0×10^4 to 2.2×10^4 PFU/ml for the attenuated virus.

Antibody assay. Antibody titers were determined by an enhanced plaque neutralization (Nt) test in Vero cell cultures which was approximately 40 times more sensitive than the conventional Nt test (7). Vero cells were

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grown on disposable plastic trays (Costar, Cambridge, Mass.) containing 24 wells with a 16-mm diameter. The cells were seeded at 2.5×10^5 cells/ml, 1.0 ml/well. After 24 hr of incubation at 35°C in a 5% CO_2 atmosphere, the monolayers were confluent and ready for use. Serum dilutions were started at 1:64 because of the prozone effect at lower dilutions (7). Serial serum dilutions were mixed with an equal volume (75 μl) of VZV (OKA-W, passaged additionally two to eight times in Flow 5000 cells) diluted so as to yield approximately 50 PFU/well. After 1 hr of incubation at 35°C, 75 μl of anti-immunoglobulin (AIG) was added to the serum–virus mixture and incubation was continued for another 20 min at room temperature. Then, 0.1 ml of each mixture was inoculated into each of two wells. After adsorption for 1 hr at 35°C, the inoculum was replaced with 1.0 ml of maintenance medium and incubation was continued for 10 days. Cells were stained with 0.02% crystal violet in 10% Formalin for 5 min and plaques were counted with the unaided eye. The antibody titer was defined as the reciprocal of the serum dilution reducing the number of plaques per well by 50%. The titer was calculated according to Karber's method (8).

Anti-immunoglobulin. The IgG fraction of rabbit serum against human IgG (heavy and light chains) (anti-immunoglobulin) was purchased from Cappel Laboratories (Cochranville, Pa.). The concentration of specific antibody as given by the manufacturer was 2.5 to 2.8 mg/ml. The specific titer of the AIG preparation was determined in our laboratory by the Ouchterlony double-diffusion test in 0.5% agarose. Precipitation lines formed at an AIG dilution of 1:8 against pygmy marmoset serum. It was used in the Nt test at a dilution of 1:3.

Virus isolation. Peripheral blood mononuclear cells were separated on Ficoll–Hypaque using heparinized blood samples. Throat swabs were eluted for 1 hr into 1.5 ml of tissue culture medium. Tissues obtained at autopsy were finely minced and washed several times. The materials were inoculated onto freshly prepared Flow 5000 cell monolayers. The cultures were observed for 7 to 10 days, and when no cytopathic effect characteristic of VZV developed, the cultures were trypsinized and subcultured three more times.

Infection of animals. Animals were inoculated with a total of 1.0 ml of cell-free virus suspension subcutaneously in both thighs. The virus dose and experimental design are shown in Table I.

Results. *Inoculation with cell-free varicella–zoster virus.* Twelve marmosets were inoculated with wild, attenuated, or inactivated VZV and challenged 3 months later as indicated in Table I. None of the animals showed signs of disease. Notably absent were rash or signs of discomfort. Attempts to isolate the virus from throat or peripheral blood on Days 5, 10, 14, and 21 after inoculation were negative.

All of the animals developed antibody to VZV 14 to 21 days after virus inoculation (Table II). Measurable antibody titers persisted in the animals for several weeks, and with one exception dropped to undetectable levels 90 days after inoculation. Animal No. 17, which received a high antigen dose of inactivated OKA-W virus, had a short-lived antibody response measurable on day 21 only. The an-

TABLE I. EXPERIMENTAL DESIGN OF STUDY

		Primary infection		Challenge[a]	
Group	Animal No.	Virus strain	Virus dose (PFU/animal)	Virus strain	Virus dose (PFU/animal)
A	7, 8, 13	OKA-W	3.3×10^5	OKA-W	5.2×10^5
B	17	OKA-W, In[b]	3.3×10^5	OKA-W, In	5.2×10^5
C	9, 9A, 10, 14	OKA-W[c]	1.0×10^4	OKA-W	5.2×10^5
D	11, 12, 12A, 16	OKA-A	1.0×10^4	OKA-W	5.2×10^5

[a] The animals were challenged 3 months after primary inoculation.
[b] Heat inactivated at 56°C for 30 min.
[c] The inoculum was a 1:33 dilution of the virus preparation used for group A.

TABLE II. HUMORAL ANTIBODY RESPONSE OF PYGMY MARMOSET INOCULATED SUBCUTANEOUSLY WITH WILD OR ATTENUATED OKA STRAIN OF VZV

Group	Animal No.		Enhanced Nt titer (reciprocal) at days after infection						
			0	5	14	21	28	42	90
A	7		<64[a]	<64	<64	232	143	146	<64
	8		<64	<64	129	399	461	81	<64
	13		<64	<64	<64	120	453	134	67
		GMT[b]	32	32	51	224	309	117	41
B	17		<64	<64	<64	481	<64	<64	<64
C	9		<64	<64	<64	—	—	—	—
	9A		<64	<64	<64	195	154	119	<64
	10		<64	<64	<64	180	300	178	<64
	14		<64	<64	<64	136	453	120	<64
		GMT	32	32	32	170	275	136	32
D	11		<64	<64	<64	137	82	117	<64
	12		<64	<64	<64	190	303	<64	<64
	12A		<64	<64	—	—	—	—	—
	16		<64	<64	<64	192	93	115	<64
		GMT	32	32	32	170	132	76	32

[a] Titers <64 were considered 32 for statistical analysis.
[b] Geometric mean titer.

tibody titers were not significantly different among the three groups of animals infected with live VZV when tested by Student's *t* test.

Animals Nos. 12A and 9 died 10 and 17 days after inoculation, respectively, of reasons unrelated to VZV inoculation. Virus isolation was attempted from several tissues of both animals (lung, spleen, lymph nodes, liver, kidney, brain) with negative results.

Challenge with VZV. Three months after primary inoculation, all animals were challenged with a high dose of live or inactivated OKA-W virus. All animals developed an accelerated antibody response (Table III). This

TABLE III. SECONDARY ANTIBODY RESPONSE OF PYGMY MARMOSET TO CHALLENGE WITH WILD OKA STRAIN OF VZV

Group	Animal No.		Enhanced Nt titer (reciprocal) at days after challenge					
			0	4	7	11	15	42
A	7		<64	<64	241	728	367	140
	8		<64	64	1417	3005	2433	286
	13		67	85	516	2245	901	104
		GMT	41	56	562	1698	933	161
B	17		<64	<64	<64	158	<64	<64
C	9A		<64	72	561	913	976	90
	10		<64	<64	608	680	465	<64
	14		<64	<64	1217	1105	755	124
		GMT	32	42	741	891	692	71
D	11		<64	<64	<64	264	<64	<64
	12		<64	<64	119	887	226	<64
	16		<64	<64	<64	<64	<64	<64
		GMT	32	32	50[a,b]	195	62[a,b]	32[a]

[a] GMT statistically different at $P \leq 0.05$ from GMT in group A by Student's *t* test.
[b] GMT statistically different at $P \leq 0.05$ from GMT in group C by Student's *t* test.

booster response was significantly higher in animals which initially received the wild OKA strain than in animals which initially received the attenuated OKA strain. The antibody response in animal No. 17 receiving inactivated virus was again short-lived, demonstrable on Day 11 after challenge only.

Discussion. Compared to VZV infection in humans, the antibody response to experimental VZV inoculation in pygmy marmosets was of lesser magnitude and shorter duration. Postinfectious enhanced Nt titers in children 1 to 12 months after natural VZV infection were in the range of 1:244 to 1:2905. Enhanced Nt antibody titers in infants 1 month after immunization with the attenuated OKA strain were in the range of 1:355 to 1:516 (7).

A possible explanation for the poor antibody response to VZV in pygmy marmosets may be the generally low immune responsiveness of this species to antigens. Marmosets have been found to respond poorly to several viral and bacterial antigens (13–18). For instance, repeated injections of influenza vaccine were necessary to produce a sustained measurable antibody response in cotton-topped marmosets (16).

On the other hand, it may be that VZV does not infect pygmy marmosets and that the immune response is due to inoculation of nonreplicating viral antigen. The finding that animals inoculated with live virus produced higher antibody titers than the animal inoculated with inactivated virus could be due to heat destruction of antigens eliciting neutralizing antibody. The failure to isolate VZV from lymphocytes or from throat swabs does not rule out the possibility of subclinical infection. Even in humans it is extremely difficult to isolate VZV from these samples in acute infections, except in cases of severely immunocompromised individuals (9–12).

Although there was considerable variation in the responses of individual animals in different groups, animals primed and boostered with the attenuated VZV (group D in Table III) had, as a group, considerably lower antibody titers than animals primed with the wild strain (group C). It would appear that the wild OKA strain primed the animals more effectively than the attenuated strains, either because of better *in vivo* replication or because of broader antigenicity of the nonreplicating strain. As a result, a second inoculation of VZV lead to a better anamnestic response in animals primed with wild VZV than in animals which received the attenuated strain.

Antibody responses to VZV were also observed in experimentally infected patas monkeys and in guinea pigs (19, 20). In guinea pigs an immune response was observed only to the attenuated OKA strain but not to the non-attenuated OKA or another wild VZV strain (20). These results, as well as our own data, suggest that primates and other animal species do respond differently to strains of VZV which have altered pathogenicity and immunogenicity for humans.

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