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Infection of Marmosets with Parainfluenza Virus Types 1 and 3

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Infection of wild marmosets (*Saguinus mystax*) with strains of parainfluenza virus types 1 and 3 resulted in acute respiratory infection. Virus replication in the upper respiratory tract was of a degree similar to that seen in children acutely infected with parainfluenza viruses. Serum antibody developed with both virus types; however, local secretory antibody was not detectable. The infection was transmissible to susceptible animals up to 3 days after inoculation of the primary animal.

Parainfluenza virus types 1 and 3 are important causes of acute respiratory disease in children (4, 9). Parainfluenza virus type 1 is the most frequent cause of acute laryngotracheobronchitis (3), whereas parainfluenza virus type 3 is second only to respiratory syncytial virus as a cause of pneumonia and bronchiolitis in infants less than 6 months of age (2, 3). Prevention with inactivated parainfluenza vaccines has been ineffective (4). Efforts have therefore focused on the use of attenuated live vaccines given intranasally. A number of strains with different degrees of attenuation have been developed, but a strain suitable for human use has not yet been found. Since the target of immunization with parainfluenza vaccines is children in their earliest infancy who more likely than not possess maternal parainfluenza antibody, the degree of attenuation and immunogenicity of the strain will have to be carefully balanced. For this reason, a sensitive experimental host which could serve as an indicator for human attenuation would be highly desirable. This report deals with the susceptibility of a New World monkey species to nonattenuated parainfluenza virus strains.

MATERIALS AND METHODS

Animals. All animals in this study were wild *Saguinus mystax* monkeys which had been in captivity for at least 6 months and weighed 350 to 520 g. Their maintenance and housing were as described previously (8).

Viruses. Parainfluenza virus type 1, strain Sendai, and Parainfluenza virus type 3, strain HA-1, were received from the American Type Culture Collection. Parainfluenza virus type 1 underwent 14 passages in mice, followed by 32 passages in chicken embryos. Parainfluenza virus type 3 underwent eight passages in primary monkey kidney or heart cell cultures, followed by one passage in LLC-MK_2 cells. The viruses received one additional passage in the allantoic sac of embryonated chicken eggs (type 1) or one passage in primary African green monkey kidney cells (type 3) in this laboratory.

Cell cultures. Parainfluenza virus type 1 was titered or isolated from tissues in MA-104 cell cultures. Parainfluenza virus type 3 was titered or isolated in Vero cell cultures (6). The growth and maintenance medium for Vero cells was Eagle minimal essential medium with Earle salts supplemented with 10% fetal bovine serum, 0.03% glutamine, 50 μg of gentamicin per ml, and 0.4 μg of amphotericin B per ml. Solid overlay medium contained 5% rather than 10% fetal bovine serum and 0.5% agarose (Seakem; Marine Colloids, Rockland, Maine). The growth medium used for MA-104 cells was the same as that used for Vero cells. The postinfection maintenance medium for MA-104 cells was Eagle minimal essential medium with Earle salts with 0.5% gelatin (Difco Laboratories, Detroit, Mich.), 0.03% glutamine, antibiotics, and 2 μg of trypsin (1× crystallized; Worthington Diagnostics, Freehold, N.J.) per ml, supplemented when necessary with 0.5 ml of agarose. All cultures were kept in a 5% CO_2 incubator at 34°C.

Experimental protocol. Animals were inoculated with 0.2 ml of virus, using a nebulizer directed into both nares and the pharyngeal area. The animals were observed daily for signs of respiratory infection and behavioral changes. At given intervals, material was taken on cotton swabs from the oropharynx, and blood was drawn from femoral vessels for virus isolation and antibody determination.

Virus isolation. Cotton swabs were eluted for 30 min into 2 ml of Eagle minimal essential medium with Earle salts with double the concentration of antibiotics. Tenfold dilutions were made, and a total of 0.4 ml of each dilution (10^0 to 10^{-5}) was inoculated onto cell monolayers in 4 wells of a 24-well tray (16-mm well diameter). After 1 h of incubation, the inoculum was replaced with 1.0 ml of agarose overlay medium and incubated at 34°C. On day 3, 0.5 ml of agarose medium containing neutral red (1:10,000) was added. On day 4,

TABLE 1. Clinical signs in marmosets infected with parainfluenza virus type 1

Animal no.[a]	Days after exposure on which following symptom was observed:			
	Sneezing	Nasal discharge	Puffy eyes	Coughing
99	5, 7, 9, 15	7, 9	8, 9	
122				
113	5–10, 12, 19	7–9, 12–14, 16, 17	8–10	7, 12
118				
111	4–9, 12, 13, 16, 19	6, 9, 12–15	7–10, 12, 13	8, 14
110	9–11, 13, 14, 16, 17	5, 16, 17	5, 10–14, 17, 19	10, 12, 16, 17
108	5, 7–10, 12, 15, 19	12	7, 8, 10	14
107	16, 17, 19–22	20, 22	19, 20, 22	

[a] Animals no. 99, 113, 111, and 108 were inoculated intranasally with parainfluenza virus. Animals no. 122, 118, 110, and 107 were introduced as susceptible cagemates into cages holding animals no. 99, 113, 111, and 108, respectively. No observations were made on day 8 for animal no. 110, on day 11 for animals no. 99, 113, 111, and 108, on day 15 for animal no. 10, or on day 18 for animals no. 99, 113, 111, 108, and 107.

the overlay was removed, and the number of plaques was counted.

Blood containing 20 U of heparin per ml was diluted 1:4 with phosphate-buffered saline and fractionated on Ficoll-Hypaque. Mononuclear cells were adjusted to 10^6, 10^5, 10^4, 10^3, and 10^2 per ml, and 1 ml of each concentration was inoculated onto one cell monolayer in a 35-mm-diameter petri dish. The medium was changed on days 3 and 5, and on day 6 the cell sheets were examined microscopically for cytopathic effect and hemadsorption with guinea pig erythrocytes.

Antibody measurement. Antibodies were measured by the enhanced virus plaque neutralization (Nt) test described previously (6). Serial dilutions of serum were mixed with an equal volume of virus diluted so as to yield 20 to 30 PFU per well. After this mixture was incubated for 1 h at 37°C, rabbit anti-human immunoglobulin G was added, and the mixture was incubated for 20 min at room temperature. Samples of 0.1 ml were inoculated into each of two wells (16 mm in diameter) on a 24-well disposable plastic tray (Linbro, Hamden, Conn., or Costar, Cambridge, Mass.). After adsorption for 60 min, the inoculum in each well was replaced with 1.0 ml of agarose overlay medium, and the trays were incubated in a humidified 5% CO_2 atmosphere for 4 to 5 days. The virus Nt titer was determined as the dilution of serum reducing the number of viral plaques per well by 50%.

RESULTS

Infection with parainfluenza virus type 1. Four marmosets were infected intranasally, each with 20,000 PFU of virus. Each animal was given a susceptible cagemate at 4 h or 3 or 7 days after infection (see Tables 1 and 2). All inoculated animals and two of the cagemates developed signs of upper respiratory infection between 5 to 20 days after exposure (Table 1) and lost significant amounts of weight within 2 weeks of inoculation or exposure. No patterns in temperature changes were observed. All inoculated animals and the only exposed animal from which throat swabs were taken excreted virus in their throats in high titer (Table 2). Antibodies to type 1 parainfluenza virus appeared, as a rule, 14 to 21 days after infection and reached moderate levels as measured by the conventional plaque Nt test 6 to 8 weeks after infection and declined thereafter (Table 2). Attempts to isolate virus from blood plasma or peripheral mononuclear cells were unsuccessful.

Reinfection with parainfluenza virus type 1. Five animals were infected with a high dose (1.4×10^7 PFU per animal) of parainfluenza virus type 1. All animals secreted virus in upper respiratory passages after primary inoculation (Table 3). After reinfection 9 months later, virus was isolated from the respiratory secretions of one animal only. Eluates of throat swabs were tested for secretory antibody and found to be negative. Serum antibody developed in all animals 14 to 21 days after primary inoculation and dropped to undetectable levels 6 months later (Table 3). After virus challenge, serum titers rose sooner and to higher levels than after primary infection.

Infection with parainfluenza virus type 3. Each of five animals was inoculated intranasally with 3×10^6 PFU of virus. On day 3, a susceptible animal (no. 88) was placed in the cage housing marmoset no. 87. Three of the five inoculated animals and animal no. 88 developed mild, short-lived signs of upper respiratory infection (Table 4). Significant weight losses were recorded during the first 2 weeks of infection. Rectal temperatures fluctuated randomly. Virus was recovered from throat swabs for 7 to 10 days. The extent of virus replication in the upper respiratory area seemed to be lower than that in animals infected with parainfluenza virus type 1. All animals developed specific antibody, including animal no. 88, which failed to yield virus from throat swabs (Table 4). Virus was not isolated from blood plasma or peripheral mono-

TABLE 2. Virus shedding and antibody response in marmosets infected with parainfluenza virus type 1

Animal no.[a]	Treatment	Test[b]	Titer at following time after expt:									
			Day							Mo		
			0	3	7	10	14	21	28	1.5	2	7
99	Inoculated	Virus shedding	0	4.13	4.23	0.4	0	0				
		Serum antibody	<32		<32	<32	<32	669	794	140	<32	40
122	Exposed on day 7	Virus shedding										
		Serum antibody							<32		<32	
113	Inoculated	Virus shedding	0	1.67	2.51	0	0	0				
		Serum antibody	<32		<32	<32	<32	435	1,162	2,752	1,751	
118	Exposed on day 7	Virus shedding										
		Serum antibody							<32		<32	<32
111	Inoculated	Virus shedding	0	3.27	4.18	2.4	0	0				
		Serum antibody	<32		<32	<32	<32	<32	1,056	1,373	3,762	
110	Exposed on day 3	Virus shedding	0	5.0	4.18	4.1	2.1	0				
		Serum antibody			<32	<32	<32	97	1,412	1,340	1,568	128
108	Inoculated	Virus shedding	0	4.41	4.54	2.3	0	0				
		Serum antibody	<32		<32	<32	<32	<32	1,775	512	619	42
107	Exposed at 4 h	Virus shedding										
		Serum antibody	<32						1,440		5,169	1,508

[a] Animals no. 122, 118, 110, and 107 were introduced as susceptible cagemates into cages holding animals no. 99, 113, 111, and 108, respectively.

[b] Virus shedding in throat; infectivity titers given as negative $\log_{10}$. Antibody titers were determined by the enhanced plaque Nt test; empty spaces, no sample.

nuclear cells taken 4, 7, 10, and 14 days after infection. Throat swab fluids taken 4, 7, 10, and 14 days after inoculation were negative when tested for specific immunoglobulin G or immunoglobulin A antibody by the enhanced plaque Nt test.

TABLE 3. Virus shedding and antibody response in marmosets infected and reinfected 9 months later with parainfluenza virus type 1

Animal no.	Test[a]	Titer after following infection at indicated time:													
		Primary								Secondary					
		Day						Mo		Day					
		0	3	7	10	14	21	3	6	0	3	7	10	21	2 mo
49	Virus shedding	−	+	+	+	−				−	−	−	−		
	Serum antibody				<64	1,120	1,729	1,503	<64	<32		123	7,207	5,406	625
62	Virus shedding	−	−	+	+	+				−	−	−	−		
	Serum antibody				<64	856	1,620	917	<64	<32		<32	1,276	1,157	100
69	Virus shedding	−	−	+	−	−				−	−	−	−		
	Serum antibody				400	1,232	2,176	2,764		<32		<32	2,490	7,050	702
85	Virus shedding	−	+	+	+	−				−	−	−	−		
	Serum antibody				450	1,590	2,125	5,058	<64	<32		1,755	6,310	8,741	2,048
92	Virus shedding	−	−	+	+	+				−	+	−	−		
	Serum antibody				<64	<64	1,044	1,460	<64	<32		2,425	8,231	34,797	2,213

[a] Virus shedding in throat; positive (+) or negative (−) virus isolation. Antibody titers were determined by the enhanced plaque Nt test; empty spaces, no sample.

TABLE 4. Infection of marmosets with parainfluenza virus type 3

Animal no.	Test	Titer at following time (days):												
		0	3	4	5	6	7	8	10	14	21	28	42	56
34	Clinical signs[a]			N		S, N	N	S, N						
	Virus shedding[b]		0.4				2.7		1.78	0	0			
	Serum antibody[c]	<64					<64		<64	<64	3,868	1,280	1,019	516
52	Clinical signs			N										
	Virus shedding		2.7				3.0		2.3	0	0			
	Serum antibody	<64					<64		<64	<64	6,690	6,320	5,680	2,218
68	Clinical signs													
	Virus shedding		3.4				2.44		0	0	0			
	Serum antibody	<64					<64		<64	778	7,456	6,008	6,020	963
87	Clinical signs													
	Virus shedding		3.0				2.88		0.4	0.7	0			
	Serum antibody	<64					<64		<64	614	4,004	1,808	1,687	3,962
88[d]	Clinical signs				N	P								
	Virus shedding		0				0		0	0	0			
	Serum antibody	<64					<64		<64	<64	<64	128	800	
103	Clinical signs					N	P							
	Virus shedding		3.0				1.4		0	0	0			
	Serum antibody	<64					<64		6,958	21,015	17,681	6,214	5,236	

[a] N, Nasal discharge; S, sneezing; P, puffy eyes; empty spaces, no signs of disease observed.
[b] Virus shedding in throat; infectivity titers given as negative $\log_{10}$. Empty spaces, no sample.
[c] Antibody titers were determined by the enhanced plaque Nt test.
[d] On day 3 of the experiment, noninfected animal no. 88 was placed in the cage of animal no. 87.

DISCUSSION

Infection of marmosets with parainfluenza virus types 1 and 3 caused a systemic infection with intensive virus replication in upper respiratory passages. The extent of local virus replication was of a magnitude similar to that found in children acutely infected with parainfluenza viruses (5). None of the infections in the marmosets was fatal, although pneumonia and death due to parainfluenza virus type 1 infection has been reported (10). The more benign course of infection with parainfluenza virus type 1 in the present study may have been due to numerous passages of the virus in the allantoic sac of chicken embryos, resulting in its partial attenuation. Parainfluenza virus type 3 caused less symptomatology and replicated less than parainfluenza virus type 1 in marmosets. Nevertheless, serum antibody synthesis after parainfluenza virus type 3 infection was equally high or higher than that after parainfluenza virus type 1 infection. Both viruses were transmissible to susceptible cagemates exposed up to 3 days after infection of the primary animal. The failure to demonstrate local secretory antibody in marmosets before or after intranasal infection was unexpected in view of reports of antibody findings in humans (7, 12). It may be that concentration of the throat swab eluates as done by Hruskova et al. (7) would have yielded positive results.

Marmosets have been shown to be highly susceptible to infection with measles virus, also a member of the paramyxovirus group (1, 8). Infection with attenuated or natural strains of measles virus caused disease of varying severity, ranging from asymptomatic infection to fatality (1). Results of the present study suggest that, unlike measles, parainfluenza infection in marmosets is not, as a rule, fatal. It remains to be seen whether this will hold true of infection with low-passage human (non-Sendai) parainfluenza virus type 1. The degree of attenuation could possibly be determined based on the extent and duration of clinical signs, virus shedding, and the immune response after infection. A distinction of wild and attenuated strains of respiratory syncytial virus along these lines has been achieved in owl monkeys (11).

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