Immunological Studies of Subacute Measles Encephalitis in Ferrets: Similarities to Human Subacute Sclerosing Panencephalitis

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Ferrets inoculated with subacute sclerosing panencephalitis virus strains D.R. and Biken developed a subacute encephalitis. Brain extracts, at neutral pH, from these ferrets showed high measles antibody titers, increased concentrations of immunoglobulin G (IgG), and higher IgG/albumin ratios than those of controls. Although the brain extracts of subacute encephalitic animals showed significant synthesis of measles-specific IgG (20 to 60% of the total IgG) within the central nervous system, the electrophoretic patterns of these extracts did not show oligoclonal bands in the γ -globulin region. Brain residues from most ferrets with subacute encephalitis, when eluted at low pH, demonstrated the presence of bound measles-specific antibodies. Excluding the electrophoresis data, other results are identical to those seen in human subacute sclerosing panencephalitis, indicating that the subacute encephalitis in ferrets may serve as a model for human subacute sclerosing panencephalitis.

In the past 10 years a number of laboratories have studied the possibility of producing subacute sclerosing panencephalitis (SSPE) in animals. Earlier studies from our laboratory (10) have shown that an acute encephalitis can be produced in ferrets by intracerebral inoculation of cell cultures that were infected with measles virus isolated from the brain of an SSPE patient. Recent data (9) have demonstrated that immunization of ferrets with live measles virus vaccine 6 weeks prior to inoculation with an SSPE isolate changes the course of infection from acute to subacute or subclinical in about 50% of the animals. The brain extracts, at neutral pH, of most ferrets with subacute encephalitis showed high measles antibody titers and increased immunoglobulin G (IgG) contents compared to controls. Since these initial results were similar to those observed in human SSPE, the object of the present investigation was to determine whether other features such as high IgG/albumin ratios (6, 12), significant measles-specific IgG content (6), presence of oligoclonal IgG bands (7, 12), and bound measles virus antibody (8, 14), observed in brains from SSPE patients, are also present in brains of ferrets with subacute encephalitis. Results should further help to evaluate whether or not ferret encephalitis is a useful animal model for human SSPE.

MATERIALS AND METHODS

Virus strains. SSPE measles virus strain D.R. was

isolated from the brain of an SSPE patient in our laboratory (10). SSPE strain Biken, passage 44 in Vero cells, was kindly provided by J. Sever and M. Dubois-Dalcq, National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, Md.

Inoculation of ferrets. Three- to 4-month-old male ferrets (Marshall Research Animals, Inc., North Rose, N.Y.) were inoculated intracerebrally with 0.5 ml of infected cell suspension. Six weeks before inoculation with strain D.R. (9), ferrets no. 192, 197, 198, 208, 272, and 328 were immunized subcutaneously with 0.5 ml of live measles vaccine (Attenuvax; Merck, Sharp and Dohme, West Point, Pa.). Ferrets no. 302, 323, and 324 were inoculated with suspensions of Vero cells infected with strain Biken without preimmunization with measles vaccine (11). All of these ferrets developed a subacute infection and were sacrificed from 1.5 to 8.5 months after inoculation. A few ferrets inoculated in the same manner developed an acute infection and were sacrificed 1 to 2 weeks after inoculation. Their brains were used as controls. Brains of measles-immunized ferrets without intracerebral inoculation of SSPE virus and of ferrets inoculated intracerebrally with uninfected Vero cells were also used as controls.

Elution of brain extracts at neutral and low pH. Each brain was thawed, washed with 0.15 M NaCl, thinly sliced, and homogenized with phosphate-buffered saline (pH 7.4), then centrifuged and concentrated as described previously (6). To elute bound antibodies, each brain residue was washed 10 times in 10 volumes of phosphate-buffered saline, and the residue was treated with glycine-hydrochloride buffer at pH 3.0 and 2.5 (8, 14).

Isolation and quantitation of ferret IgG and

albumin. IgG and albumin were isolated from ferret serum by the combination of starch block electrophoresis and Sephadex gel filtration procedures (9). Antisera to isolated ferret IgG and albumin were prepared in rabbits as previously described (9). The quantitation of IgG and albumin was carried out by the radial immunodiffusion method, using agar plates containing specific rabbit antiserum to IgG and albumin, respectively.

Quantitation of measles-specific IgG in brain extracts and sera. The measles-precipitating antigen was made in Vero cell layers infected with the Edmonston strain of measles virus. Ferret brain extracts and sera were suitably absorbed with measles-precipitating antigen as described in studies of human SSPE (7). In brief, 10 mg of measles-precipitating antigen was added to brain extracts, which had IgG concentrations of 1 mg/ml. Each absorbed preparation was examined in a measles hemagglutination inhibition test (7), and if necessary the sample was reabsorbed. The amount of IgG in absorbed and unabsorbed samples was determined by radial immunodiffusion test, and the percentage of measles-specific IgG was calculated as shown in the following equation: Measles-specific IgG as % of total IgG = $[\text{total IgG before absorption } (\mu\text{g/ml})] - [\text{IgG } (\mu\text{g/ml}) \text{ after absorption with MPA}] / \text{total IgG before absorption } (\mu\text{g/ml}) \times 100$.

RESULTS

Measles antibody titers and IgG-to-albumin ratios. As shown in Table 1, the measles virus hemagglutination inhibition antibody titers were significantly increased in brain extracts and sera from ferrets with subacute encephalitis, compared with those from ferrets with acute encephalitis or with no clinical signs of the disease. The IgG-to-albumin ratios were significantly higher in the brain extracts of ferrets with subacute encephalitis than in their sera, indicating an intracerebral synthesis of IgG. No such increase in the ratios was observed in brain extracts from the control groups.

Quantitation of measles-specific IgG. The percentage of measles-specific IgG in brain extracts from ferrets in the subacute encephalitis groups ranged from 20 to 60% of the total IgG (Table 1). These results suggest that a significant amount of measles-specific IgG was synthesized within the central nervous system of the ferrets. The measles-specific IgG contents in sera from 5 of 9 ferrets ranged from 6 to 23% of the total IgG. Measles-specific IgG (% of total IgG) in

TABLE 1. Measles virus antibody titers, IgG contents, IgG/albumin ratios, measles-specific IgG, and presence of bound antibody in brain extracts and sera from subacute encephalitis and control ferrets

Virus strain	Type of encephalitis	Ferret no.	Time of sacrifice (mo.)	Measles HI titers ^a		Brain IgG (% of total protein)	IgG/albumin		Measles-specific IgG (% of total IgG)		Bound antibody in brain
				Brain	Serum		Brain	Serum	Brain	Serum	
SSPE D.R.	Subacute	192	4	128	128	3.1	2.7	0.4	40.1	17.4	+ ^b
		197	4.5	16	32	1.0	1.2	0.9	23.8	ND ^c	ND
		198	1.25	32	128	2.1	2.8	0.6	22.0	ND	+
		208	8	64	32	1.1	1.8	0.6	34.0	ND	+
		272	1.75	800	256	1.7	4.3	0.5	34.2	12.2	+
	(Acute or none)	328	2	1,024	128	3.7	4.4	0.3	34.4	17.0	ND
		Control ^d	0.5-4.5	<8	<8-8	0.1-0.6	0.2-0.6	0.4-0.6	ND	ND	ND
SSPE Biken	Subacute	302	8.5	128	200	2.4	1.6	0.3	43.5	ND	ND
		323	2.5	128	200	1.5	3.6	0.7	43.9	6.0	ND
		324	5.5	4,000	3,000	4.7	7.9	0.7	58.0	23.4	+
	(Acute or none)	Controls ^e	0.5-4	<4	<8	0.1-0.4	0.2-0.3	0.4-0.6	ND	ND	ND
Vero cells	None	Controls ^f	3-4	<4	<8	0.2-0.3	0.2-0.3	0.4-0.5	ND	ND	ND

^a HI, Hemagglutination inhibition.

^b +, Present.

^c ND, Not detectable.

^d Controls consisted of five ferrets inoculated with SSPE strain D.R. Three had acute encephalitis, and two had no clinical signs of the disease.

^e Controls consisted of three ferrets inoculated with SSPE strain Biken. Two had acute encephalitis, and the third had no clinical signs of the disease.

^f Controls consisted of six ferrets inoculated with Vero cells. None of them had any clinical signs of the disease.

brain extracts and sera of control animals was not measurable by the method used, since there was no detectable difference in IgG concentrations before and after absorption with measles-precipitating antigen (Table 1).

Electrophoretic analysis. When concentrated brain extracts from subacute encephalitis and control groups were run in agarose gel electrophoresis, the patterns were almost identical. The extracts from subacute encephalitis ferrets did not show the presence of oligoclonal bands in the γ -globulin region.

Bound antibody. When the brain residues were treated at low pH, the eluates showed significant amounts of bound IgG with specific measles antibody activity in a number of encephalitic ferrets. These results are very similar to those seen in hamsters infected with measles virus (4) and in human SSPE brains (4, 8, 14).

DISCUSSION

A number of investigators (1, 2, 13) have produced subacute encephalitis in animals by intracerebral inoculation with measles virus and have reported high measles antibody titers in the sera and cerebrospinal fluid (1) of these animals during the course of the disease. However, other immunological characteristics of human SSPE have not been studied previously in animal models. In the present study we have demonstrated high IgG-to-albumin ratios, significant proportions of measles-specific IgG, and measles-specific bound antibodies in brains from a number of ferrets with subacute encephalitis. These results are very similar to those observed in human SSPE (7, 12) and indicate that the subacute ferret encephalitis may be a useful model for studying the pathogenesis of SSPE. The results support our previous data (11) that both SSPE strains can be used to produce subacute encephalitis in ferrets, since immunological characteristics of animals inoculated with the D.R. and Biken strains were remarkably similar.

Although most human SSPE studies have compared the IgG-to-albumin ratios in cerebrospinal fluid and serum, we (6) and Vandvik (12) have determined the ratios in brain extracts from SSPE patients and found them to be greatly increased and comparable to the ratios in cerebrospinal fluid. In the present study, autopsy brain material was used to determine IgG-to-albumin ratios in the central nervous system of ferrets with subacute encephalitis. Similar studies have also been done on rabies virus infection in hamsters (3). Since we have found that the IgG-to-albumin ratios were not increased in sera of ferrets with subacute encephalitis, whereas the ratios in their brain extracts

were greatly increased, we have concluded that IgG is produced in the brains of these animals.

Our previous study of ferrets with subacute encephalitis showed that their serum antibody titers against measles virus increased during the subclinical period and that cell-associated SSPE virus was present in their brains at the time of sacrifice, up to 8.5 months after inoculation (11). This indicates that the brains of the ferrets were persistently infected with SSPE virus and that the virus was continuously stimulating the immune system in a similar way as in SSPE patients. Considering the similarities to human SSPE, we were surprised to find that the measles-specific brain IgG from ferrets lacked the oligoclonal bands that are one of the most important characteristics of human SSPE. The reason for the lack of oligoclonal bands is not known, but we speculate that it may have something to do with the way in which the persistent infection in ferrets is initiated, namely by inoculation of large amounts of SSPE measles-virus infected cells directly into their brains. Although it is not known how measles virus spreads to the brains of prospective SSPE patients, one possibility is that it is slowly carried by infected lymphocytes through the blood-brain barrier (5). In the inoculated ferret brains there seems to be a widespread mobilization of the immune response shortly after inoculation, whereas in the human brain the response is probably more restricted in the beginning and increases gradually as the virus spreads through the brain.

Another reason for the lack of oligoclonal bands may be that the duration of the persistent virus infection was too short. In addition, the ferret immunoglobulin system is poorly defined, and it is not known whether ferrets synthesize oligoclonal IgG bands during immunization with virus. Thus, further studies on this and other animal models, particularly the hamster, where IgG is produced locally within the central nervous system, are necessary to understand the mechanism involved in the production of the oligoclonal response.

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