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*IMMUNOLOGICAL RELATIONSHIPS OF MEASLES, DISTEMPER, AND RINDERPEST VIRUSES**

BY DAVID T. IMAGAWA, PIERRE GORET, AND JOHN M. ADAMS

DEPARTMENTS OF PEDIATRICS AND INFECTIOUS DISEASES, SCHOOL OF MEDICINE, UNIVERSITY OF CALIFORNIA AT LOS ANGELES, VETERANS ADMINISTRATION HOSPITAL, LONG BEACH, CALIFORNIA, AND ECOLE NATIONALE VETERINAIRE D'ALFORT, SEINE, FRANCE

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Evidence has accumulated from various laboratories to show an immunological relationship between canine distemper and measles viruses.[1-5] Investigators in Africa and Europe have demonstrated an immunological relationship between viruses of rinderpest and canine distemper.[6-8] Studies reported here present further evidence to substantiate the existence of an antigenic relationship between measles and distemper, and also show the presence of distemper and measles antibodies in rinderpest immune-bovine sera. These results indicate that the viruses share similar antigenic components. Serum samples from various hosts with rinderpest, measles, and distemper were analyzed for measles antibody by neutralization tests in tissue culture and for distemper antibody by neutralization tests in suckling mice.

Materials and Methods.—Serum samples: The normal and antirinderpest bovine sera were received from France. The antisera were prepared either with bovine rinderpest virus or with rabbit adapted rinderpest virus (strain Nakamura III). Normal serum samples were obtained from animals which were neither inoculated nor infected with rinderpest virus. Acute and convalescent sera were obtained from 10 children with clinically diagnosed measles.

Viruses: The Edmonston strain[9] of measles virus, propagated in HeLa cell cultures,[2] was employed for neutralization tests. The canine distemper strain was the mouse-adapted virus developed from the Lederle strain of avianized distemper virus[10] by successive serial brain passages in suckling mice.[2]

Neutralization tests: Measles neutralization tests were carried out in HeLa cell cultures as described in a previous paper.[2] Distemper neutralization tests were performed by mixing approximately 100 LD_{50} of mouse-adapted distemper virus with equal amounts of diluted test sera. Mixtures were incubated at 37°C for $^1/_2$ hr and placed in the refrigerator for an additional $^1/_2$ hr before inoculating 0.02 ml

intracerebrally into 1–12 hr suckling mice. All serum samples were inactivated at 56°C for 1/2 hr before mixing with the virus. Death of animals was recorded for 21 days and neutralization titers calculated by Reed and Muench's method.[11] Titrations of virus were carried out simultaneously with each test. Rinderpest antibody determinations were carried out in France by the method of Huard and his collaborators.[12] The titer was determined with the rabbit-adapted rinderpest virus. By this method, serum from immune animals had a neutralization index log of 4 to 5.

TABLE 1
NEUTRALIZATION TESTS WITH ANTIRINDERPEST AND NORMAL BOVINE SERA

Serum Samples		Antibody Titers		
Type	Description	Measles*	Distemper†	Rinderpest‡
Rinderpest antiserum	Nha Trang			
	No. 1	1:4	1:90	5
	No. 2	1:2	1:26	5
	No. 3	1:12	1:32	4
	Melange	1:12	1:24	4
	Prepared with rabbit adapted rinderpest virus	1:1	1:16	4
	Calf 293 Farcha	1:2	1:2	4
Normal serum	Dakar	0	0	0
	France	0	0	0
	Nha Trang	0	0	0
	Calf 292 Farcha	0	0	0

* Dilution of serum which prevented a cytopathic effect in 50% of HeLa culture tubes against 100–1,000 TCD_{50} of measles virus.
† Dilution of serum which protected 50% of suckling mice from death against approx. 100 LD_{50} of mouse adapted distemper virus.
‡ Neutralization Index Log, method of Huard *et al.*[12]

Results.—Rinderpest antisera: The data presented in Table 1 show that 6 different samples of rinderpest antiserum uniformly contained neutralizing antibodies to measles and distemper viruses. On the other hand, normal sera which had been shown to contain no rinderpest antibody failed to neutralize distemper and measles viruses. The measles titers ranged from 1:1 to 1:12, whereas the distemper antibody titers were generally higher and ranged from 1:2 to 1:90 by the suckling mouse method. However, the antibodies were measured in different neutralization systems.

Distemper antibody titers measured in France on the same serum samples by the chick embryo and ferret distemper neutralization methods also showed the presence of distemper antibody in rinderpest antisera and none in normal sera.[8]

Measles antisera: Ten pairs of acute and convalescent sera from patients with measles showed that all had significant increases in antibody titer to both

TABLE 2
NEUTRALIZING ANTIBODIES IN THE SERA OF PATIENTS WITH MEASLES

	Measles*		Distemper†	
Patient no.	Acute	Convalescent	Acute	Convalescent
91-A	<1:5	1:15	<1:5	1:80
89-A	<1:5	1:30	<1:5	1:20
86-A	<1:5	1:20	<1:5	1:40
81-A	<1:5	1:32	<1:5	1:43
81-B	<1:5	1:10	<1:5	1:15
80-A	<1:5	1:32	1:2	1:64
74-A	<1:5	1:16	<1:4	1:12
71-A	<1:5	1:12	<1:2	1:4
28-A	<1:10	1:20	...	1:20
22-A	<1:10	1:80	...	1:240

* Dilution of serum which prevented a cytopathic effect in 50% of HeLa culture tubes.
† Dilution of serum which protected 50% of suckling mice from death.

measles and distemper. The data in Table 2 show no demonstrable measles antibody in the acute phase sera, whereas the convalescent phase titers ranged from 1:10 to 1:80. Similarily, when the same sera were tested for distemper antibody there were no demonstrable antibody in the acute phase, as compared to titers of 1:4 to 1:240 in the convalescent phase.

Sera from ferrets, guinea pigs, rabbits, and monkeys given multiple injections of tissue culture measles virus contained distemper antibody in low titers as well as antibodies to measles virus. Four ferrets were inoculated intramuscularly with 1.0 ml of a mixture of measles virus and adjuvant[13] prepared as follows: 1 part arlacel A, 9 parts bayol F, and 10 parts measles virus suspension. Four additional inoculations of measles virus (1.0 ml) without adjuvant were given at 3 week intervals. Three weeks after the final injection, the animals were bled for sera. Two rabbits, 2 guinea pigs, and 2 monkeys were given 3 inoculations of 1.0 ml of tissue culture measles virus at intervals of 1 to 3 weeks. Two weeks after the final injection, the animals were bled for sera. The rabbits were injected intravenously, the guinea pigs by the subcutaneous route, and the monkeys by the intramuscular route.

Results of the neutralization tests for measles and distemper antibodies are summarized in Table 3. Sera from animals before injection of measles virus showed

TABLE 3

NEUTRALIZING ANTIBODIES IN THE SERA OF ANIMALS IMMUNIZED WITH MEASLES VIRUS

Animals	Measles*		Distemper†	
	Preimmunization	Postimmunization	Preimmunization	Postimmunization
Ferret				
1	0	1:12	0	1:2
2	0	1:8	0	1:2
3	0	1:3	0	1:4
4	0	1:6	0	1:3
Guinea pig				
1	0	1:96	0	1:8
2	0	1:12	0	1:1
Rabbit				
1	0	1:16	0	1:2
2	0	1:16	0	1:3
Monkey				
1	<1:4	1:24	<1:4	1:16
2	<1:4	1:8	0	1:1

* Dilution of serum which prevented a cytopathic effect in 50% of HeLa culture tubes.
† Dilution of serum which protected 50% of suckling mice from death.

no demonstrable antibodies to measles or distemper viruses, whereas, after injection all showed some level of antibody to both viruses. Control animals inoculated with virus-free tissue culture medium showed no antibody to measles or distemper virus.

Distemper antisera: Animals were immunized with distemper virus and the sera tested for measles and distemper antibodies. Four ferrets were immunized with Lederle's egg-adapted distemper virus and 5 ferrets were immunized with the mouse-adapted distemper virus. These ferrets were subsequently challenged with approximately 2,500 MLD of virulent ferret distemper virus and the sera drawn 4 weeks following the severe challenge.

The data in Table 4 show that neutralization of measles virus occurred in low dilution. Measles neutralization was present only when the distemper antibody

TABLE 4
NEUTRALIZING ANTIBODIES IN THE SERA OF ANIMALS IMMUNIZED WITH DISTEMPER VIRUS

Animals	Measles*		Distemper†	
	Preimmunization	Postimmunization	Preimmunization	Postimmunization
Ferret				
1	0	1:8	0	1:665
2	0	1:1	0	1:128
3	0	1:1	0	1:400
4	0	1:2	0	1:512
5	0	1:4	0	1:512
6	0	1:2	0	1:450
7	0	1:15	0	1:768
8	0	0	0	1:45
9	0	0	0	1:53
Dog				
1	...	1:2	...	1:768
2	...	0	...	1:18

* Dilution of serum which prevented a cytopathic effect in 50% of HeLa culture tubes.
† Dilution of serum which protected 50% of suckling mice from death.

titer was high. Sera in which the distemper antibody responses were 1:128 or greater neutralized measles virus in low dilution. On the other hand, 2 ferrets even though surviving the distemper challenge showed distemper antibody response of only 1:45 and 1:53 and no response to measles antibody. In no instance did preimmunization or normal serum show any inhibition of measles or distemper virus. Serum from a dog previously immunized to distemper likewise showed neutralization of measles virus in low dilution in the presence of high distemper antibody titer. Serum from another immunized dog with low distemper titer showed no measles antibody.

Five guinea pigs were given 3 intramuscular injections of Lederle's egg-adapted distemper virus in 1.0 ml amounts at intervals of 2 weeks. The animals were bled 3 weeks after the final injection. The antibody response to distemper was poor with a range of 1:4 to 1:30, and likewise there was no demonstrable measles antibody.

Discussion.—Immunologic cross reactions between measles and distemper viruses were first described in 1957.[1-3] In the same year, Polding and Simpson[6] and Goret *et al.*[7] presented evidence of an immunologic relationship between canine distemper and rinderpest viruses. Plowright and Ferris[14] suggested a possible link between rinderpest and measles viruses on the basis of cytopathic similarities in tissue cultures. Likewise, they demonstrated that adult human sera neutralized rinderpest virus. In 1953, adult human sera and gamma globulin were shown to contain specific neutralizing antobodies to canine distemper virus.[15] Cytopathic changes produced in dog kidney tissue culture by distemper virus[16] were similar to those caused by measles virus.

Recently Cabasso, Kiser, and Stebbins[17] reported the lack of immunogenic crossing between measles and distemper viruses in dogs and chickens. These varying results may possibly be related to strain differences or technical procedures. Mouse neutralization tests were used in this laboratory whereas Cabasso *et al.* employed chick embryo neutralization methods. It is possible that tests carried out in suckling mice may reveal antibodies that may not be as readily demonstrable by the chick embryo technique. For example, when complement is added to distemper antiserum there is an enhancement of neutralizing property for measles virus.[2]

Studies reported here and those of others[5, 18] indicate that distemper antiserum will fix complement and neutralize measles virus in low dilution when the response to distemper antibody is relatively high. However, measles antibody response was absent or poor in direct relationship to distemper antibody levels. In this same connection, recent measles vaccination studies,[19] employing the Edmonston strain of live virus, showed that the titer responses were of a low order and in approximately half of the subjects no titer could be demonstrated in the post-vaccination sera.

Serum samples from rinderpest immune animals were shown to contain measles and distemper antibodies, whereas, normal beef sera failed to neutralize either virus. These results support the relationship described earlier between distemper and measles[1-5] and subsequently between distemper and rinderpest.[6-8] Further investigations are needed to clarify the exact antigenic interrelationships and their full significance.

Summary.—Neutralization tests on sera from measles, distemper, and rinderpest which were analyzed for measles antibody in tissue culture and for distemper antibody in suckling mice present further evidence of an antigenic relationship among these 3 viruses. Rinderpest antisera contained measles and distemper antibodies, whereas normal beef sera failed to neutralize either virus. Acute and convalescent sera from children with measles showed significant increases to distemper and measles antibodies. Sera from guinea pigs, rabbits, monkeys, and ferrets immunized with measles virus contained distemper antibody in low dilutions. Ferrets immunized with avian or mouse-adapted distemper virus and subsequently challenged with virulent distemper virus demonstrated neutralization of measles virus in low dilution only when the distemper antibody titer was elevated significantly. Measles antibody responses were absent or poor in direct relationship to distemper antibody levels. Sera from guinea pigs following inoculation with distemper virus showed a poor response to distemper and none to measles.

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