Antigenic Variants of Rabies Virus in Isolates from Eastern, Central and Northern Canada

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ABSTRACT

Street rabies virus isolated from 51 specimens from Ontario, Quebec, Manitoba and the Northwest Territories have been typed by a panel of 36 antinucleocapsid monoclonal antibodies. Three main groups were found. The first group comprised those terrestrial mammals originating in Ontario, Quebec and the Northwest Territories. The second group was found in terrestrial mammals from Manitoba. The third heterogenous group was made up of bats from Ontario.

Key words: Rabies, strains, monoclonal antibodies, Canada.

RÉSUMÉ

Après avoir isolé le virus rabique du cerveau de 51 animaux qui provenaient de l'Ontario, du Québec, du Manitoba et des Territoires du Nord-Ouest, les auteurs recherchèrent le type de ces diverses souches, à l'aide de 36 anticorps monoclonaux dirigés contre la nucléocapside virale. Ils obtinrent ainsi trois principaux types de virus rabique: le premier provenait des mammifères terrestres de l'Ontario, du Québec et des Territoires du Nord-Ouest; le second provenait des mammifères terrestres du Manitoba; le troisième provenait des chauves-souris de l'Ontario et il se révéla hétérogène.

Mots clés: rage, souches, anticorps monoclonaux, Canada.

INTRODUCTION

Considerable interest has been raised during the past few years with the use of monoclonal antibodies to distinguish between various fixed and wild strains of rabies virus. It has become evident that there are many strains of street rabies virus, and that "typing" with monoclonal antibodies will be important in studies on the epizootiology of this disease as well as, perhaps, of its treatment.

Since the initial production of a panel of antinucleocapsid antibodies by Wiktor and Koprowski (1), several authors have reported on the typing of various strains or antigenic groupings using this panel (1,2,3,4,5,6,7). Smith *et al* (8) developed a panel of monoclonal antibodies and studied some North American isolates of street virus. They identified several strains apparently characteristic of animal species and/or geographical regions. Most of these authors have stained impressions of brain material either of the original animal or of a mouse passage. Smith *et al* (8) stained both brain impressions and neuroblastoma cells infected with virus. We have succeeded in infecting BHK-21 cells with wild virus and have used this system for typing various isolates of street rabies virus obtained from Ontario, Quebec, Manitoba and the Northwest Territories.

MATERIALS AND METHODS

CELL CULTURES

BHK-21 (C13) (baby hamster kidney) cells were grown in Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum, 10% tryptose phosphate broth, 1% l-glutamine and 50 μg/mL neomycin sulphate. Cells were maintained in 25 cm^2 plastic tissue culture flasks and grown at 37°C in an atmosphere containing 5% CO_2.

VIRUSES

Virus isolates are listed in Table I; all are from field specimens received at our laboratory. Brain tissue was ground in a Ten Broeck grinder and MEM was added to make a 10% (w/v) suspension. This suspension was centrifuged at 1000 g for 10 min and the supernatant was collected and stored at -80°C until used.

ISOLATION PROCEDURES

One mL of BHK-21 cells (at a concentration of 5 x 10^5 cells/mL) containing 50 μg/mL DEAE dextran was mixed with 0.25 mL of virus suspension. After 1 h at room temperature with frequent mixing, the virus-cell suspension was centrifuged at 1000 g for 10 min and the resulting pellet resuspended in 1 mL fresh MEM. Ninety-six well flat-bottom microtiter plates were inoculated with 0.1 mL in each of three wells and the remaining suspension added to 5 mL MEM in a 25 cm^2 flask. All cultures were incubated at 37°C for three days.

Following incubation, the microtiter plate was washed once in 0.01 M phosphate buffered physiological saline (pH 7.2-7.4) and fixed in 75% acetone at room temperature for 30-45 min. After removal of the acetone and air drying, the cultures were stained with a fluorescein-labelled hamster antirabies serum (produced at this laboratory) for 30 min at 37°C. The plates were then washed 2x in buffered physiological saline and a drop of saline:glycerine mixture (1:1) was added to each well. The plates were examined inverted using a Leitz Orthoplan microscope with epi-illumination using a 10 or 20x objective and 10x

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eyepieces. The percentage of cells infected was noted.

The 25 cm^2 flask cultures were trypsinized and resuspended in 2 mL MEM of which 0.5 mL was used to reseed a further flask. At the same time, 96-well microtiter plates were inoculated with a 1:3 dilution of the harvested cells. These were incubated for three days at 37°C.

IMMUNOFLUORESCENT STAINING FOR NUCLEOCAPSID ANTIGEN

When the number of infected cells approached 100% (as determined following staining in the 96-well plate), the flasks were harvested and a 60-well Teresaki plate was inoculated with a 1:3 dilution of the infected cell suspension. Forty wells were inoculated (0.01 mL/well) and the plate was incubated at 37°C for 24 h. The plate was then rinsed in saline, fixed in acetone and air dried as noted above. In the present study, tissue culture fluid from 36 monoclonal antibodies specific for nucleocapsid antigen (9) was used with an antimouse light chain fluorescein-labelled conjugate (Bionetics) to indirectly stain the monolayer in each well. The reaction was read as + = rabies-specific fluorescence, 0 = no fluorescence.

RESULTS

A total of 51 field isolates from various animal species have been examined using antinucleocapsid monoclonal antibodies (Table I). Most virus cultures were stained at least twice. There were some difficulties in the interpretation of fluorescence with certain antibodies. On replicates of the same field specimen, the fluorescence with antibodies 111-2, 111-14, 377-7 and 102-27 could vary from + to 0. Probably the amount of antigen in the cell determined the final reading. Where a reaction to an antibody was variable in replicate tests, we have used the most common reading. This procedure has therefore resulted in the inclusion of a number of isolates into major groupings rather than a larger number of smaller groupings or individuals with only minor (and variable) differences. All other antibodies were uniform in their staining reaction.

The use of a panel of antinucleocapsid monoclonal antibodies has allowed us to separate field virus isolated from various host species into major groups (Table II). One major group is found in those specimens originating in southern and eastern Ontario, western Quebec and the Northwest Territories. We could not detect any differences between the patterns seen in red foxes and striped skunks and those of domestic animals from this area. We specifically examined several cattle, sheep and a dog that were known to have been involved with either rabid foxes or skunks.

The second major group is comprised of those animals which originated in Manitoba. Our finding that antibody 721-2 stained negative in Manitoba specimens and positive in Ontario specimens and that antibody 701-9 stained positive in Manitoba specimens and negative in Ontario isolates clearly separated these two groups.

Our third grouping is a heterogene-

TABLE I. Origin of Field Isolates Examined

Province	Host	Number Examined
Ontario and Quebec (41)	Cattle (*Bos* sp.)	9
	Sheep (*Ovis* sp.)	2
	Goat (*Capra* sp.)	2
	Dog (*Canis familiaris*)	3
	Cat (*Felis domesticus*)	1
	Coyote (*Canis latrans*)	1
	Red fox (*Vulpes vulpes*)	7
	Striped skunk (*Mephitis mephitis*)	7
	Bat[a]	9
Manitoba (8)	Cattle (*Bos* sp.)	2
	Horse (*Equus* sp.)	1
	Raccoon (*Procyon lotor*)	1
	Skunk (*Mephitis mephitis*)	4
NWT (2)	Arctic fox[b] (*Alopex lagopus*)	1
	Red fox[c] (*Vulpes vulpes*)	1

[a]*Eptesicus fuscus* — 8; *Lasiurus borealis* — 1
[b]Eskimo Point, NWT
[c]Mackenzie Delta, NWT

TABLE II. Reaction of Various Wild Isolates from Ontario, Quebec and Manitoba Following Indirect Staining with Antinucleocapsid Monoclonal Antibodies

	Terrestrial Mammals[b]			
Monoclonal No.[a]	Ontario, Quebec, NWT	Manitoba	*E. fuscus*	*L. borealis*
104-4	+[c]	+	0	+
111-2	0	±	0	+
111-14	0	±	0	+
239-10	+	+	0	0
389-1	+	+	0	+
377-7	±	±	+	+
102-27	0	±	0	0
222-9	+	+	0	+
237-3	+	+	0	0
714-3	0	0	+	+
422-5	0	0	0	0
816-1	+	+	0	+
817-5	+	+	0	+
822-7	+	+	0	+
701-9	0	+	0	0
703-8	+	+	0	+
721-2	+	0	+	0
804-9	+	+	0	+
805-3	+	+	0	+

[a]The following antibodies gave a + staining in all specimens: 502-2, 103-7, 206-1, 209-1, 229-1, 590-2, 513-3, 120-2, 364-11, 818-5, 715-3, 801-1, 802-2, 803-6, 806-1, 807-5, 808-2
[b]See Table I
[c]+ = fluorescence; 0 = no fluorescence; ± = variable

ous one containing the two species of bats from Ontario (*Eptesicus fuscus* and *Lasiurus borealis*) so far examined. The patterns obtained from these two species do not resemble either the Ontario or Manitoba groupings of terrestrial mammals.

DISCUSSION

All reports, except for Smith *et al* (8), are based upon the staining and examination of brain impressions either from the host animal or from mouse passages (1,3,4,7). Schneider (4) found major and minor antigenic variants on a worldwide basis. If the results of terrestrial mammals only are considered, Charlton *et al* (3) found differences between Ontario and the two Manitoba isolates examined. However, neither Schneider (4) nor Charlton *et al* (3) used antibodies 701-9 or 721-2 which in the present study have consistently distinguished between the two groups. Smith *et al* (8) also found that isolates from various host species and localities in the United States could be arranged (with the exception of bat isolates) into major antigenic groupings dependent upon geographical locality.

The present study has allowed us to form two major groups with the isolates so far examined. The first is represented by those specimens originating in Ontario, Quebec and the Northwest Territories. This group corresponds to that noted by Smith *et al* (8) who examined isolates from the states of New York and Maine. Red fox and striped skunk rabies is endemic in this area and has been since the mid 1950's when it is believed to have spread southward from the Arctic (10). Our findings in an Arctic fox from Eskimo Point (on Hudson Bay) and a red fox from the Mackenzie Delta, NWT with the same antibody reaction substantiate this view.

The second main group is found in isolates from Manitoba. We had noted previously (10) that striped skunk rabies has been endemic in that area since the late 1950's and early 1960's where it is believed to have spread from North Dakota or Minnesota. This group corresponds to that of skunk rabies in the upper mid-western states of Iowa, Illinois and Minnesota (8).

The two species of bats so far examined do not fall into either of the above groups.

Our experiences (unpublished) with the infection and growth in BHK-21 cells of field isolates further substantiates these findings. We have experienced considerable difficulty in infecting and growing isolates from Ontario and Quebec. We can fairly consistently infect BKH-21 cells but infected cells are usually only 1-5% of the total. On serial passages, infected cells usually reach 15-20% and remain at that level or diminish with further passage. Only occasionally does the percentage of infected cells approach 100. In contrast, most isolates from Manitoba readily infect BHK-21 cells with most infections approaching 50-80% on original isolation. Passage of infected cells readily produces high infection rates.

It would appear therefore that two major antigenic groups can be distinguished among the rabies virus isolates so far studied. One group is found in Ontario, Quebec and the Northwest Territories and is represented in the wild by endemic red fox and striped skunk rabies which has apparently originated in northern Canada. The second group is found in Manitoba where striped skunk rabies is endemic; this being an extension of endemic skunk rabies from the mid-western United States. The bat isolates so far studied do not fall into either grouping.

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