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Use of vaccinia rabies recombinant for oral vaccination of wildlife

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ABSTRACT

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A vaccinia rabies recombinant virus was constructed and shown to induce the synthesis of rabies virus glycoprotein in infected cells and to induce rabies virus neutralizing antibodies and protection in susceptible animals. Active when orally administered, this recombinant is a good candidate for the development of vaccines for wild animal rabies vectors. This recombinant was found stable, safe for target and non-target animal species, and protective for most of the rabies vectors. After extensive experimental studies conducted under controlled conditions, it as used in limited field trials and in an extensive open field trial. The preliminary results confirmed its basic properties and potential for rabies eradication.

INTRODUCTION

Despite the existence of safe and potent inactivated vaccines commonly used for the vaccination of pet, sport and farm animals, rabies remains a subject of great concern in industrialized countries in Europe and North-America. It is also a serious threat in developing countries in Central and South America, Africa and Asia where animal health structures are often lacking. Rabies still prevails due to wild animal vectors and reservoirs which consist of foxes (Europe), raccoons, skunks, bats and foxes (North America), stray dogs (Africa and Asia) and stray dogs and bats (Central and South America).

Attempts to control the disease by vector population reduction have been

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of limited success and are not appropriate to most countries where rabies is endemic. Furthermore, this could endanger the vector animal species. The only effective method would appear to be parental vaccination of vector animals, but since it is impossible to capture those animals, oral administration of the vaccine is the only alternative.

The earliest attempts at oral vaccination were made in North America by Baer et al. (1971), and in Europe by Mayr et al. (1972). Later, live attenuated rabies viruses introduced into various types of baits were used to vaccinate foxes, both in Switzerland and West Germany and more recently in Belgium, France and Luxembourg (Artois et al., 1988; Brochier et al., 1988b; Frisch et al., 1988; Kappeler et al., 1988; Schneider and Cox, 1988). Because inactivated rabies vaccines are ineffective when orally administered, and because of the relatively poor stability and residual pathogenicity of existing attenuated rabies virus strains for some animal species, there is a need for new vaccines. Recombinant DNA technology offers an alternative for the development of such potent, stable and safe vaccines. Using this technology, a recombinant vaccinia virus expressing the immunogenic and protective rabies virus glycoprotein has been constructed and evaluated both in vitro and in vivo.

CONSTRUCTION AND PRELIMINARY EVALUATION OF THE VACCINIA RABIES RECOMBINANT

The cDNA corresponding to the gene coding for the 524 amino acid glycoprotein G of rabies virus strain ERA was cloned (Anilionis et al. 1981) and inserted into the 180 kb double-stranded DNA genome of vaccinia virus strain Copenhagen, under the control of the 7.5 kDa vaccinia protein promoter, in the gene coding for thymidine kinase (TK) (Kieny et al., 1984).

Using immunoprecipitation, immunofluorescence and immunoelectron microscopy, a selected TK^- clone of recombinant virus (VVTGg RAB 26D3 187×P) was shown to induce rabies glycoprotein synthesis in infected cells. Using a panel of monoclonal antibodies, the binding activity of the recombinant-induced glycoprotein was shown to be almost identical to that of ERA rabies virus glycoprotein. Inoculation of mice with the recombinant by scarification or by footpad inoculation, and of rabbits by the intradermal route, resulted in the rapid induction of rabies virus (ERA strain and street virus) neutralizing antibodies. Moreover, inoculation of mice by either scarification or footpad inoculation resulted in full protection against challenge with both rabies and rabies related Duvenhage viruses, while after challenge with Mokola virus no protection was observed (Wiktor et al., 1984; Wiktor et al., 1985).

After demonstrating rabies G glycoprotein synthesis in VVTGg RAB infected cells of various origin, and after showing the induction of rabies virus neutralizing antibodies and protection in laboratory animals, the next step

was to evaluate the safety of the recombinant for target and non target animal species.

SAFETY EVALUATION FOR LABORATORY, DOMESTIC AND WILD ANIMALS

The safety of the recombinant was tested in more than 30 animal species including laboratory, domestic and wild mammals and birds (Brochier et al., 1989) (Table 1). Several routes of administration were used. These included intradermal, subcutaneous and intramuscular as well as the oral route. The oral route was used in non-target as well as target animals to evaluate the safety of the recombinant for species that would compete for bait uptake. Except for intradermal inoculation, neither local nor general reaction (clinical sign or lesion) was observed in animals given a dose of 10^8 $TCID_{50}$ or more of the recombinant. Following intradermal inoculation, and depending upon the animal species, typical poxvirus lesions were seen, the size of which varied according to each individual.

No excretion of the recombinant was shown and no horizontal transmission from vaccinated animals to contact controls was observed, except in a very few cases where vaccinated animals had bitten the controls on returning to their pens immediately after vaccination.

Because of the difficulty of immunosuppressing animals, particularly wild animals, the safety of the recombinant was assessed in nude mice. These did

TABLE 1

Animal species in which safety of the recombinant was assessed

Laboratory animals
mouse, nude mouse,guinea-pig, syrian hamster, cotton rat, rabbit
Domestic animals
cattle, sheep, pig, dog, cat
Wild animals
mammals
target species: red fox (*Vulpes vulpes*), grey fox (*Vulpes argentens*), raccoon (*Procyon lotor*), skunk (*Mephitis mephitis*)
non-target species: white-tailed deer (*Odocoileus virginianus*), wild boar (*Sus scrofa*), eurasian badger (*Meles meles*), bobcat (*Lynx rufus*), common opossum (*Didelphis virginiana*), river otter (*Lutra canadensis*), ferret (*Mustella furo*), wood mouse (*Apodemus sylvaticus*), yellow-necked mouse (*Apodemus flavicollis*), bank vole (*Clethryonomis glareolus*), common vole (*Microtus arvalis*), field vole (*Microtus agrestis*), water vole (*Arvicola terrestris*).
birds
carrion crow (*Corvus corone*), magpie (*Pica pica*), jay (*Garrulus glandarius*), sea gull (*Larus canus*), common buzzard (*Buteo buteo*), kestrel (*Falco tinnunculus*).

not develop any local or general reaction following inoculation into the footpad or by the intraperitoneal route. When intracerebrally administered to mice, the comparison of LD_{50} of the recombinant with that of the parental Copenhagen strain indicated a reduced capability of the recombinant to multiply in the brain. The genetic stability of the construct was checked by 10 passages in vero cell cultures (the cell line used to propagate the virus), and by 7 passages in mice following footpad or intracerebral inoculation. No evidence of genomic breakdown or of change in biological properties was observed. The inability to recover the virus from the tissues of footpad inoculated animals after 7 passages linked to the necessity to perform an amplificative passage on vero cells between the third and fourth passages by footpad and intracerebral inoculation, is further evidence of the safety of the recombinant.

A transient (48 h) and limited persistence (in oral mucosa, soft palate and tonsils) of the virus was shown following oral administration of 10^8 PFU recombinant to foxes (Thomas et al., 1989).

ACTIVITY EVALUATION BY MEANS OF RABIES NEUTRALIZING ANTIBODY TITRATION AND CHALLENGE TESTS

After demonstrating the safety of the recombinant, its activity was evaluated by titration of induced rabies neutralizing antibodies and by challenging vaccinates with virulent rabies virus strains. Among other species, the possibility of effective immunization by oral administration of the recombinant was demonstrated in foxes, raccoons, skunks, dogs and cats (Blancou et al., 1986; Rupprecht et al., 1986; Tolson et al., 1987; Brochier et al., 1988a; Tolson et al., 1988; Blancou et al., 1989).

In foxes (*Vulpes vulpes*) and their cubs given the recombinant by the oral route, antibody production and protection were dose-dependent (Tables 2, 3 and 4). Based on the results obtained from 68 foxes the 100% protective dose (PD 100%) was estimated at about 10^7 PFU. An immunity period of over 18 months was shown in a group of adult foxes (Table 5) and fox cubs (Table 6) vaccinated once and challenged at various times after vaccination.
Following the successful trials to establish the efficacy and safety of the recombinant in foxes and other animals, further trials were started to establish its potential for rabies control in nature.

BELGIUM

An initial, limited field trial of the vaccine was authorized by the Belgium Ministry of Public Health on 23 September 1987. The site chosen was the 27

TABLE 2

Efficacy for foxes

Type of treatment[1]	Neutralizing antibody titers		Protected animals/ challenged[2]
	Day 0	Day 30	
Vaccinated	neg	1.35[3]	2/4
10^4 PFU	neg	neg	
	neg	neg	
	neg	neg	
Vaccinated	neg	0.8	2/4
10^6 PFU	neg	0.8	
	neg	neg	
	neg	neg	
Vaccinated	neg	2.33	4/4
10^8 PFU	neg	2.61	
	neg	2.61	
	neg	2.75	
Vaccinated[4]	neg	1.91	4/4
10^8 PFU	neg	2.33	
	neg	2.61	
	neg	2.75	
Controls	neg	neg	0/4
	neg	neg	
	neg	neg	
	neg	neg	

[1]Vaccination: day 0, direct administration of one dose of vaccine into the oral cavity.
[2]Challenge: day 33, $10^{3.3}$ mouse/intracerebral/LD_{50} of rabies virus strain GS7 by intramuscular route.
[3]Antibody titers determined by rapid fluorescent focus inhibition test (RFFIT) and expressed in International Units per ml of serum (IU/ml).
[4]Scarification of the oral mucosa before administration of the vaccine.

km^2 military field, isolated from public and domestic animals, of Marche-en-Famenne, an area located in the Ardennes province where fox rabies is commonly found.

The vaccine was placed in capsules that were implanted in chicken heads. On 17 and 18 October 1987 a total of 250 baits containing liquid vaccine and tetracycline as biomarker were manually placed over a 6 km^2 area situated in the centre of the military field and surrounded by a non-vaccinated buffer zone. By day 15, the bait uptake was 64%. During the 3-month observation period, no abnormal morbidity or mortality was noted among animals, either in the vaccinated area or in the non-vaccinated buffer zone. Of 145 small mammals trapped in the baited area, only four had eaten baits and none showed any lesion that could be related to poxvirus infection (Pastoret et al., 1988).

TABLE 3

Efficacy for foxes

Type of treatment[1]	Neutralizing antibody titers		Protected animals/ challenged[2]
	Day 0	Day 30	
Vaccinated	neg	0.08[3]	2/4
10^6 PFU	neg	neg	
	neg	neg	
	neg	2.31	
Vaccinated	neg	13.48	4/4
10^7 PFU	neg	1.23	
	neg	neg	
	neg	0.96	
Vaccinated	neg	0.46	4/4
10^8 PFU	neg	6.07	
	neg	1.93	
	neg	0.08	
Contact controls[5]	neg	0.09[4]	1[4]/4
	neg	neg	
	neg	neg	
	neg	neg	
Controls	neg	neg	0/2
	neg	neg	

[1]Vaccination: day 0, direct administration of one dose of vaccine into the oral cavity.
[2]Challenge: day 33, $10^{3.3}$ mouse/intracerebral/LD_{50} of rabies virus strain GS7 by intramuscular route.
[3]Antibody titers determined by rapid fluorescent focus inhibition test (RFFIT) and expressed in International Units per ml of serum (IU/ml).
[4]Animal bitten by its penmate immediately after vaccination.
[5]Animals in the same pen as vaccinated (10^8 PFU) animals.

The recombinant safety thus again having been confirmed, the Belgium Ministry of Public Health on 21 September 1988 gave authorization for an enlarged open field trial. The site chosen was again located in the Ardennes region. Between 24 October and 5 November 1988, a total of 6000 specially developed baits containing liquid vaccine and tetracycline were manually distributed around a 450 km^2 field. A 20 km^2 area within the 450 km^2 was chosen for extensive survey.

Earlier results had shown a vaccine stability in the field of about 3 months. A bait uptake of 68% by day 15, and of 94% by day 30 as recorded. Three species (wild boars, stone martens and stray-cats) competed with foxes for bait uptake. 59% of the foxes captured in the area between 10 November 1988 and 30 March 1989 had eaten one or more baits as indicated by examination

TABLE 4

Efficacy for fox cubs

Type of treatment[1]	Neutralizing antibody titers		Protected animals/ challenged[2]
	Day 0	Day 28	
Vaccinated	neg	9.95[3]	4/4
$10^{7.2}$ PFU	neg	4.92	
	neg	20.90	
	neg	9.95	
Controls	neg	neg	0/2
	neg	neg	

[1]Vaccination: day 0, direct administration of one dose of $10^{7.2}$ PFU of vaccine into the oral cavity.
[2]Challenge: day 33, $10^{3.2}$ mouse/intracerebral/LD_{50} of rabies virus strain GS7 by intramuscular route.
[3]Antibody titers determined by rapid fluorescent focus inhibition test (RFFIT) and expressed in International Units per ml of serum (IU/ml).

TABLE 5

Duration of immunity in adult foxes

Type of treatment[1]	Neutralizing antibody titers					Protected animals/ challenged[2]
	Day 0	Day 30	Day 180	Day 350		
Vaccinated	neg	0.87[3]	0.03	neg		4/4
10^{8} PFU	neg	2.49	neg	0.08		
	neg	0.46	0.01	neg		
	neg	24.5	0.17	0.19		
Controls				neg		0/2
				neg		
	Day 0	Day 30	Day 180	Day 356	Day 545	
Vaccinated	neg	1.36	neg	neg	neg	4/4
10^{8} PFU	neg	7.38	0.94	0.42	0.19	
	neg	0.97	0.04	0.03	0.03	
	neg	1.36	0.19	0.42	0.15	
Controls					neg	0/2
					neg	

[1]Vaccination: day 0, direct administration of one dose 10^{8} PFU of vaccine into the oral cavity.
[2]Challenge: day 350 and day 545 respectively, $10^{3.3}$ mouse/intracerebral/LD_{50} of rabies virus strain GS7 by intramuscular route.
[3]Antibody titers determined by rapid fluorescent focus inhibition test (RFFIT) and expressed in International Units per ml of serum (IU/ml).

TABLE 6

Duration of immunity in fox cubs

Type of treatment[1]	Weight (kg)	Neutralizing antibody titers			Protected animals/ challenged[2]
	Day 0	Day −3	Day 28	Day 180	
Vaccinated $10^{7.2}$ PFU	2.0[3]	neg	3.06	0.73	4/4
	2.0	neg	5.25	3.17	
	1.8	neg	4.45	5.33	
	2.2	neg	5.25	6.07	
	Day 0	Day −3	Day 28	Day 360	
Vaccinated $10^{7.2}$ PFU	2.8	neg	4.45	7.62[4]	2/3
	2.4	neg	2.81	3.81	
	1.3	neg	2.81	5.09	
	1.4	neg	<0.46	<0.46	

[1]Vaccination: day 0, direct administration of one dose of vaccine into the oral cavity.
[2]Challenge: day 180 and day 360 respectively, $10^{3.3}$ mouse/intracerebral/LD_{50} of rabies virus strain GS7 by intramuscular route.
[3]Antibody titers determined by rapid fluorescent focus inhibition test (RFFIT) and expressed in International Units per ml of serum (IU/ml).
[4]Animal accidentally dead on day 1 after challenge.

for tetracycline uptake. No abnormal morbidity or mortality was reported among domestic or wild animals during the 7-month period which followed the distribution of baits. Out of 226 animals (foxes, stone martens, martens, ermines, polecats, stray cats, wildcats, hares, squirrels, hedgehogs, rodents and big game: stags, deers, mouflons, boars) hunted, captured or found dead within the baited area, none showed any lesions that could be related to poxviruses (Brochier et al., unpublished data).

FRANCE

On 20 January 1988 the Commission for Biomolecular Engineering of the Ministry of Agriculture gave authorization to start an initial limited field trial in France. The site was the 53 hectare military field of Mars la Tour (Meurthe et Moselle). It is isolated from the public and from domestic animals.

In November 1988, 174 specially developed baits similar to those used in Belgium, were manually distributed; 187 vaccine-free control baits, also containing the biomarker, were placed in an adjacent area separated from the first area by a buffer zone. Bait consumption was recorded and 90% of the baits had disappeared by day 15.

Wildlife census before and after the vaccination period showed no significant changes in either the vaccinated or the control areas. No increase in morbidity or mortality was noted among the entire fauna. Trapping of carnivores, rodents and birds was carried out in December 1988. Of 77 animals captured, none showed any lesions that could be related to poxvirus infection (Aubert et al., unpublished data).

CONCLUSIONS

The insertion of a cDNA corresponding to the gene coding for the rabies virus glycoprotein into the genome of a vaccinia virus, resulted in a recombinant virus capable of inducing the glycoprotein synthesis in susceptible cells. This glycoprotein cannot be distinguished from the natural one.

When orally or parenterally administered to domestic and wild animals, the recombinant proved to be safe and did not induce any local or general reaction except in some animal species which, following intradermal inoculation, developed a poxvirus-type local reaction. Brief local persistence of the recombinant was observed in the vaccinated animals, but no viral excretion which could be related to transient viraemia was noted.

Except for a few cases when bits occurred immediately after oral vaccination and which could be considered as direct inoculation, no transmission from vaccinated animals to controls was observed, confirming the absence of excretion and diffusion of the virus recombinant. Direct administration of the recombinant into the oral cavity, or through baits, induced, in most animal species tested, a seroconversion to the rabies glycoprotein and a long-lasting and complete protection against rabies challenge in foxes.

Because of its safety, potency and stability, the recombinant appears to be a good candidate for the development of oral vaccines for animal species which constitute the main rabies vectors (foxes, raccoons, skunks, straydogs). Used as a substitute for live vaccines prepared from attenuated rabies virus strains that have residual pathogenicity for animals and humans, it could usefully complement the existing inactivated vaccines specifically intended for domestic animal species, and would represent a new method for the eradication of rabies.

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