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ANTIGENIC VARIATION BETWEEN RABIES VIRUS STRAINS AND ITS RELEVANCE IN VACCINE PRODUCTION AND POTENCY TESTING(*)

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The *Lyssavirus* genus within the Rhabdoviridae consists of rabies virus and the rabies related viruses.[1,2] A tentative subdivision within the group has placed classical rabies strains within serotype 1, the rabies-related viruses being considered as separate serotypes.[3] The rabies-related viruses appear to be confined to Africa and of no epidemiological significance, whereas rabies virus itself has a worldwide distribution.

Only minor antigenic differences have been reported among the classical rabies virus isolates so that it is still the recommended practice to prepare vaccines and antisera for use throughout the world with a few well characterized strains some of which are listed in Table 1.[4] The Pasteur-derived strains are the most widely used in the preparation of vaccines and antisera, while CVS is the standard challenge strain for vaccine potency testing.[5] The Flury strains[6] are given as live veterinary vaccines for the control of rabies in many parts of the world. Strain 675 is a plaque isolate of HEP virus made by Dr. G. Bijlenga of the School of Veterinary Medicine, Lyon, France.

For some years we have been involved in the development of inactivated vaccines for veterinary use. For this purpose we have used both LEP virus and Strain 675 grown in BHK – 21 cells. The vaccines are prepared by inactivating the infected cell supernatants with beta-propiolactone or acetyl ethylene imine.[7-9] We were aware of the small antigenic differences between the Pasteur and Flury strains which had been reported[10] but they were not apparent when we did cross neutralization tests using hyperimmune sera.

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Table 1. Rabies Virus Strains Used for Vaccine and Antiserum Production.

Strain	Origin
Pasteur	Bovine-Europe 1882
Pitman-Moore (P-M)	
CVS	
Flury	Human-U. S. A. 1939
LEP	
HEP	
HEP 675	Plaque isolate from HEP

Our interest in vaccine production led us to consider the methods used to measure vaccine potency. Most authorities will license only those vaccines which reach minimum standards in either the Habel[11] or NIH test.[5] In each test, mice are vaccinated and subsequently challenged with CVS and in both the animals receive more than 1 dose of vaccine (Habel — 6 doses; NIH — 2 doses). Consequently the primary antigenicity of the vaccine is not measured in either test.[12,13] Furthermore, the results depend on the ability of the animals to withstand challenge by the severe and unnatural intracerebral route. For these reasons we suggested an alternative test in which the production of serum neutralizing activity (SNA) after one dose of vaccine was measured.[13,14]

This idea was based on our finding that after a single inoculation the resistance of challenge by mice was related to the SNA developed and that both were dependent (within certain limits) on the dilution of vaccine (amount of antigen) given.[15] In fact, this relationship holds whether one or two doses of vaccines are used and the results of a typical test with a human diploid cell vaccine (HDCV) prepared with P-M virus are given in Table 2. However, as the results show, the single dose test is more stringent: there can be no amplification of potency due to an anamnestic response, so it is to be preferred.

Table 2. Serum Neutralizing Activity and Protection of Mice Against Intracerebral Challenge With CVS After 1 or 2 Intraperitoneal Doses of HDCV.

Vaccine dilution	Protected mice	SNA	Protected mice	SNA
10	20/20[a]	4.2[b]	14/20	3.4
50	13/20	3.7	8/20	2.7
250	4/18	2.3	4/19	1.1
1250	1/20	1.6	1/20	0.9
Inoculation	Day O and day 7		Day O	

[a]Mice challenged with $50ID_{50}$ CVS 14 days after first dose of vaccine.
[b]Depression of virus titer (log_{10}) by 0.015 ml 1/10 pooled from groups of 4 mice 14 days after first dose of vaccine. Sera tested in suckling mice against CVS.

We have not been alone in considering new methods for determining vaccine potency as the numerous papers in the WHO/IABS Symposia on Rabies testify[16-18] and as reported in WHO/*Rab. Res.* 80:7.[19] Nevertheless, there is considerable reluctance to discard challenge tests in potency measurement and the most recent WHO recommendations are that for tissue culture vaccines the NIH test should continue to be used.[20] However, an antibody induction test is considered one possible alternative. It has also been suggested that a variation of the NIH test using a single vaccination should be evaluated.[20] Application of the last type of test to our vaccines confirmed the antigenic differences between the Flury and Pasteur-derived strains[10] and indicated that they might be of greater importance than we had previously considered.

Although our LEP vaccines have given very good results in the Habel test (protective indices > 6.0, G. Turner, personal communication), they provide relatively poor protection in the NIH test. Even the vaccines from the HEP 675 strain, which is characterized by massive production of virus particles, give less protection against CVS challenge than vaccine similarly prepared from the P-M strain. Nevertheless, we were somewhat surprised to find that a single dose of LEP vaccine which protected mice well against homologous challenge was much less effective against a corresponding challenge with CVS. The results of one such test are shown in Table 3. The reduced efficacy of the vaccine against the heterologous strain is reflected in the lower level of SNA against it.

We then examined the neutralizing activity in the sera of mice which had received vaccines prepared from P-M, LEP or HEP 675 strains. In this experiment, the mice were given vaccine on day O and day 7 as in the classical NIH test[5] and bled on day 14. The results in Table 4 show obvious differences in the neutralizing activities against LEP virus and CVS. In each pooled serum neutralizing activity was highest against the homologous

Table 3. Serum Neutralizing Activity and Protection of Mice Against Intracerebral Challenge With Either LEP or CVS Virus After a Single Intraperitoneal Dose of LEP Vaccine.

Vaccine dilution	Protected mice	SNA	Protected mice	SNA
10	8/8[a]	4.5[b]	5/8	2.7
50	4/8	—	2/8	—
250	3/8	—	1/8	—
1250	1/8	—	0/8	—
Control (no vaccine)	1/8	—	0/8	
Challenge virus	LEP		CVS	

[a]Mice challenged with either LEP or CVS virus (Ca. 50 ID_{50}) 14 days after vaccination.
[b]Depression of virus titer (log_{10}) by 0.015 ml 1/10 pooled serum from a group of 8 mice 14 days after vaccination. Sera tested in suckling mice against LEP or CVS virus.

Table 4. Serum Neutralizing Activity in Mice After Intraperitoneal Inoculation on Day O and Day 7 With Either P-M, LEP or HEP 675 Vaccine.

Vaccine dilution	SNA/LEP	SNA/CVS	SNA/LEP	SNA/CVS	SNA/LEP	SNA/CVS
10	3.5[a]	4.3	3.9	2.3	4.9	4.5
50	2.5	4.3	3.3	0.9	3.9	2.3
250	3.3	4.5	3.5	2.1	3.5	1.7
Vaccine	P-M		LEP		HEP	

[a]Depression of virus titer (log_{10}) by 0.015 ml 1/10 pooled sera from groups of 8 mice killed 14 days after the first dose of vaccine. Sera tested in suckling mice against CVS or LEP virus.

strain, undoubtedly the explanation of the greater ability of P-M vaccines to protect against challenge with CVS.

Our results confirm those of earlier workers concerning antigenic differences between rabies virus strains developed from the original Pasteur isolate and those derived from the Flury strain.[10] Similar differences between these and other laboratory strains have also been reported[10] and are being extensively analyzed with batteries of monoclonal antibodies.[21-24] How significant the strain differences may be in terms of resistance to natural infection is not known, but they may account for vaccine failures and have important implications with regard to laboratory safety and in the selection of future vaccine strains.

Our results also raise the question of the suitability of the Pasteur-derived strains to continue as the absolute standards against which all vaccines and antisera are matched, especially in view of the suggestion that they may differ considerably from certain field strains.[3] Furthermore, the design of currently accepted vaccine potency tests is such that only small antigenic differences, possibly of no significance outside the laboratory, may influence their outcome and hence a manufacturer's ability to meet licensing requirements with his products.

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