Short Communications

Attempt to vaccinate orally harbour seals against phocid distemper

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DURING 1987 and 1988, two distinct morbilliviruses were identified as the cause of two outbreaks of serious disease in seals. One of them, phocid distemper virus-1 (PDV-1), killed more than 17,000 harbour seals (*Phoca vitulina*) in the North and Baltic seas in 1988 (Osterhaus and Vedder 1988). PDV-1 is a new member of the genus *Morbillivirus* related to canine distemper virus (CDV) and rinderpest virus (RPV) (Mahy and others 1988, Bostock and others 1990). The second of them, phocid distemper virus-2 (PDV-2) is regarded as a genuine CDV isolate (Visser and others 1990) and was the cause of a severe outbreak among Lake Baikal seals (*Phoca sibirica*) in 1987 (Grachev and others 1989, Osterhaus and others 1989a). A subunit iscom CDV preparation and inactivated whole virus CDV vaccine were proved to protect harbour seals against a PDV-1 challenge (Osterhaus and others 1989b, Visser and others 1989). However, because these vaccines require repeated parenteral administration, their use in the field is fairly limited. Oral immunisation seems therefore more appropriate. This route is currently used to immunise foxes against rabies and has proved efficacious (Brochier and others 1990). Because of the risk of insufficient attenuation for seals, live attenuated CDV vaccines are not considered. The present authors have therefore chosen to use a recombinant vaccinia virus expressing the fusion (F) protein of CDV which provides protective immunity (Norrby and others 1986).

The C-DNA corresponding to the F gene was inserted into the thymidine kinase (TK) gene of vaccinia virus (Copenhagen strain) under the control of a vaccinia virus promoter (R. Drillien and D. Spehner, unpublished data). In a first experiment, five captive male harbour seals were given orally by direct instillation 10^7 plaque forming units (pfu) of the recombinant vaccinia-CDV virus in 1 ml suspension. The fifth seal received an antigen-free preparation. Blood was taken on the day of vaccination as well as four weeks later. An enzyme-linked immunosorbent assay was used for the detection of antibodies to vaccinia virus. Specific antibodies were revealed by purified biotinylated rabbit IgG fraction anti-seal IgG (the rabbit anti-seal sera was kindly provided by Dr S. Carter and Dr J. R. Baker, University of Liverpool). The presence of CDV antibodies was checked in a seroneutralisation test using a standard strain of CDV. Specific antibodies were not detected in any of the sera of the seals.

Too low a dose of the recombinant virus or too low an expression level of the F protein could explain the lack of specific antibodies in the sera. To test these hypotheses, a further experiment was set up with two of the harbour seals involved in the first experiment. These animals received 10^9 pfu of a new recombinant vaccinia-CDV virus. This recombinant strongly expressed the F protein (over to 1 µg per 10^6 cells per 24 hours in vitro) and was able to protect mice against a intracerebral CDV challenge (D. Spehner, unpublished data). Again, blood was taken on the day of vaccination and four weeks later. The presence of antibodies against vaccinia virus or against the F protein of CDV was checked as previously described. In addition, CDV specific antibodies were also detected using an indirect immunofluorescence test on cells infected by CDV. Antibodies against CDV or vaccinia were not detected in either of these tests, although the dose of recombinant vaccinia CDV-virus was the highest dose feasible. The authors therefore suggest that these recombinant viruses failed to multiply in the oral cavity of the seals.

The oral cavity has been shown in foxes to be the primary site of multiplication of vaccinia and recombinant vaccinia-rabies glycoprotein viruses given orally (Thomas and others 1998). Oral administration of the recombinant vaccinia-rabies virus can also face problems in some species like the dog and the cat which are, in this case, difficult to immunise at normal doses (Blancou and others 1989). For these reasons, the present authors did not find it appropriate to make a further attempt with another recombinant virus expressing other CDV or PDV genes, although another poxvirus vector capable of replicating in the oral cavity of seals might prove useful for the vaccination of these animals.

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