# Vaccinia Viruses as Vectors for Vaccine Antigens

Editor
Gerald V. Quinnan, Jr., M. D.

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Proceedings of the Workshop on Vaccinia Viruses as Vectors for Vaccine Antigens, held November 13–14, 1984, in Chevy Chase, Maryland, U.S.A.

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# Vaccinia Viruses as Vectors for Vaccine Antigens

# PREFACE

The use of viruses as vectors for expression of heterologous antigens in mammalian cells *in vivo* exemplifies an exciting recent advance in the application of recombinant DNA technology for vaccine production. In many respects vaccinia virus is highly suited for this purpose. Vaccinia virus vaccines were used extensively world-wide during the smallpox eradication campaign, and were generally very well tolerated, highly immunogenic, easily manufactured and readily applied in mass vaccination efforts. Even with its outstanding performance record, however, the possibility of reintroducing the use of this virus on a wide scale in man or animals raises many public health and ethical concerns. Although very rarely, serious or fatal complications of vaccinia virus infections did occur, and they occurred in contacts of vaccinees as well as in vaccinees themselves. The potential occurrence and significance of similar complications must be evaluated in considering approaches to development of recombinant vaccinia virus vaccines. Even if there were no known risks, the reintroduction of the virus into populations that are not exposed currently should be done only after careful consideration of the benefits to be achieved. Other major questions that must be addressed at the outset include: What strain(s) of vaccinia virus should be used? How should vaccines be manufactured? What tests should be done to assure acceptability of vaccines for use in field trials? What biologic properties of vaccine strains should be evaluated? In whom should vaccine trials be performed?

This book represents the proceedings of a *Workshop on Vaccinia Viruses as Vectors for Vaccine Antigens*, cosponsored by the United States Public Health Service, the World Health Organization, and the National Institute for Biologic Standards and Control, London, held November 13 and 14, 1984, in Chevy Chase, Maryland. The purpose of the Workshop was to begin developing answers to the questions indicated above. The data presented and relevant to these questions emanate both from studies performed during the smallpox eradication campaign and from exciting recent laboratory work on molecular biology, expression of foreign genes, determinants of virulence, and immunology of vaccinia virus infections. It is evident from these data that there are unlikely to be any insurmountable objections to the use of recombinant vaccinia virus vaccines, and that they offer great promise for use as safe and effective immunogens. Work on developing candidate strains for human administration will hopefully progress rapidly. The use of vectors other than vaccinia virus may also prove feasible.

This Workshop was followed by a meeting of consultants to the World Health Organization with the purpose of advising on evidence relevant to the public health, ethical and scientific concerns about these recombinant vaccines, and formulating draft requirements for the use of recombinant vaccinia virus vaccines. These draft requirements will be under continued development for many months. However, the intent of the World Health Organization to publish them should be viewed as an indication of the enthusiasm with which this approach has been greeted. The list of human and veterinary diseases against which vaccines of this type might be used is long and the death and suffering that might be prevented are great. The process of developing any vaccine is an arduous one, and it is never certain at the outset that any specific approach will result in a safe and effective product. It is clear that the long history of vaccinia virus is far from over and there are many opportunities ahead.

The program for this meeting was developed with the assistance of Drs. G.C. Schild, B. Moss, R. Chanock, D.A. Henderson, G. Tourigiani and F. Assaad. We gratefully acknowledge the contributions of Hilda Kopit for meeting coordination, Cathy Hobbs for editorial assistance, and Lois Baker for typing.

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# PART I

## BIOLOGY OF VACCINIA AND OTHER ORTHOPOX VIRUSES

Chairpersons: G. Schild and J. Nakano

# VACCINIA VIRUS

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Vaccinia is a member of the genus Orthopoxvirus. Table I lists the other members, and indicates which are human pathogens. In some cases reliable information is lacking, and ectromelia virus is the only Orthopoxvirus species known not to be a human pathogen. Genetic and serological relationships within the genus are very close and recombinants and hybrids may occur [1].

TABLE I. Orthopoxviruses.

| Species | Reservoir | Other hosts | Human infection |
|---|---|---|---|
| Variola | (Man) | None | (Yes), eradicated |
| Vaccinia | None | See Table III | Yes |
| Cowpox | Rodent? | Cattle, Cats | Yes |
| Monkeypox | ? | Monkeys | Yes |
| Camelpox | Camel | None | No? |
| Raccoonpox | Raccoon | ? | ? |
| Taterapox | Gerbil | ? | ? |
| Ectromelia | Lab. mouse | ? | No |

## Definition of Vaccinia

When compared to e.g. polio or rubella vaccines, any definition of smallpox vaccine is inadequate. This is because the vaccine strains used this century were introduced in the 19th century before licensing procedures were necessary.

The usual working definition of vaccinia virus is that it is a virus of unknown origin, not found naturally, which is maintained in vaccine institutes and research laboratories.

## Origins of Vaccinia

Possible origins of vaccinia virus are listed in Table II and have been discussed at length elsewhere [2]. Derivation has usually been proposed from smallpox and/or cowpox viruses. Some vaccines were probably derived from cowpox in the early 19th century, but there are fundamental reasons for believing that no surviving vaccine was derived

TABLE II. Origins of vaccinia.

1. From smallpox, by arm-to-arm passage.
2. From smallpox, by adaptation to animals.
3. From cowpox.
4. From smallpox and cowpox, by hybridization.
5. From horsepox.


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from smallpox or cowpox viruses. Polio or measles vaccine strains are attenuated variants of the virulent parents, and are very closely related to the parents. In fact, it is sometimes difficult to distinguish between vaccine and wild-type.

The situation with vaccinia is quite different. For vaccinia to have been derived from smallpox or cowpox would require considerable changes in the genome; in fact, the transformation of one virus into another. This is most unlikely. The genomes of vaccinia virus strains are very similar to each other but different from those of smallpox and cowpox viruses [3], and the suggestion that one Orthopoxvirus species may be transformed easily and quickly into another has been discounted [4].

Smallpox vaccines were developed from horsepox virus in the 19th century but horsepox is now extinct. However, it is possible that the clinical suitability of horsepox vaccines led to their retention, and to the rejection of cowpox vaccines. This would explain the survival of a closely related collection of vaccine strains, not found naturally, which were not obviously derived from cowpox or smallpox.

The problem of the origin of vaccinia is not purely academic at a time when we are considering not just reintroducing human vaccination but also considering extending its use to animals. As recently as 1980 it was claimed that vaccinia virus was attenuated smallpox virus, and the death of a fetus, in fact from generalized vaccinia, was cited as evidence that reversion to virulence can and does take place [5]. This suggestion was correctly criticized as absurd [6]. However, at a time when pressure groups are becoming increasingly vocal, we should take every opportunity to establish that, whatever its origins, vaccinia now represents an independent stable species with no tendency to "revert" to a more virulent form.

## Vaccinia as a Typical Poxvirus

Vaccinia virus is easily grown and has been widely used as a typical poxvirus [7]. The assumption that the structure and replication of all Orthopoxviruses is essentially the same is justified, and so data obtained on vaccinia virus could be transferred to smallpox virus.

The complex structure and large size of the virion facilitates analysis by electron microscopy of uncoating, replication and assembly, and the inhibition of cellular protein synthesis in infected cells facilitates the biochemical analysis of these events.

One of the features that attracted molecular biologists to vaccinia virus is the fact that it is a DNA virus which replicates in the cytoplasm. This led to an appreciation of the importance of virion-associated enzymes in poxvirus replication. These factors, and an appreciation of the role played by smallpox vaccination in the control and eradication of smallpox, are more or less responsible for the holding of this Workshop.

## Pathogenesis of Vaccinia

Vaccinia is a dermotropic virus which usually requires inoculation into the superficial layers of the skin in order to infect. Infection is usually localized. However, there are virus strains, originally called rabbitpox but now more properly considered as variants of vaccinia, which produce generalized infection in rabbits, and which may infect by the respiratory route [8].

Infection produces a lesion caused by epidermal hyperplasia and proliferation, and inflammatory infiltration which progresses from a papule through a vesicle and pustule to a crust. A transient viremia probably occurs. Generalized lesions are rare in the immunocompetent person but serious complications can occur in the immunodeficient and eczematous individual [9]. Vaccination induces an adequate humoral and cellular immune response. Studies during the early 1970s showed that an antigen on the envelope of virions released naturally from infected cells was the important inducer of humoral immunity [10].

## Host Range of Vaccinia Virus

Vaccinia has a wide host range (Table III) but we may need to distinguish between hosts which become infected naturally, and those which are susceptible only to experimental infection.

TABLE III. Host range of vaccinia virus.

| | | |
|---|---|---|
| Man[a] | Cow[a] | Buffalo[a] |
| Pig[a] | Camel[a] | Rabbit[a,b] |
| Elephant | Monkey | Sheep |
| Rodents | ? | ? |

a. Naturally-acquired infections occur.
b. Only reported in laboratory animals.

There is no good evidence that vaccinia virus becomes established in animal populations. Smallpox vaccination has been conducted on a massive scale in both developed and developing countries. In addition, particular attention has been paid to possible animal reservoirs of smallpox virus. If vaccinia had any tendency to become established in an animal population it would have certainly been recognized.

Human Vaccinia. By historical precedent and common consent, man is the principal, if artificial, host of vaccinia virus. Other contributors to this Symposium will discuss the morbidity and mortality associated with smallpox vaccine. However, it is important to note that the problem is not confined to complications in vaccinees but also extends to infection in contacts. Avoidable incidents still occur. As recently as April 1983 a young girl in Nevada was vaccinated mistakenly and transmitted infection to seven friends at a slumber party [11].

Smallpox vaccine was intended to prevent smallpox, and there is doubt about its ability to provide long-term protection against revaccination. On revaccination the lesion is usually more superficial and transient than a primary vaccination, and complications are virtually non-existent. Nevertheless, an infection does occur on revaccination and may be transferred to eyes, genitals, etc., or to contacts.

Now that smallpox has been eradicated it might be reasonable to regard any transfer of vaccinia to a contact as a complication of the original vaccination.

Bovine Vaccinia. Cowpox is the Orthopoxvirus usually associated with bovine infection. However, although bovine cowpox does occur, it is rare and the virus is probably not enzootic in cattle [12]. Vaccinia virus infects cattle producing lesions indistinguishable from those