33

Vaccinia Virus and Other Poxviruses as Live Vectors

Bernard Moss
Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, U.S.A.

VACCINIA VIRUS: THE SMALLPOX VACCINE

On May 14, 1796, Edward Jenner inoculated eight-year old James Phipps with cowpox virus, obtained from an infection on the hand of Sarah Nelmes, a milkmaid. This simple procedure was shown to provide complete protection against smallpox, and formed the basis for its ultimate eradication (1). The prophylactic effect of vaccination was due to the close genetic and antigenic relationships between variola virus, the causative agent of smallpox, and its more benign relatives, cowpox virus and vaccinia virus. The latter virus may have been isolated from an infected horse and, presumably because of its milder reactivity, was substituted for cowpox virus (2). Vaccinia virus was economical to produce, active in low amounts, heat stable, resistant to freeze drying, simple to administer, relatively safe, and provided long-lasting immunity. Of equal importance for smallpox eradication, however, were the ease of diagnosis of the disease, lack of antigenic variation, and the absence of latently infected human or animal reservoirs. Although the vaccine was immediately successful, eradication of smallpox proved difficult for logistical reasons. In 1967, the World Health Organization implemented a new intensified global ring vaccination strategy that ultimately contained and eliminated variola virus from nature. The last endemic case of smallpox occurred in 1977. Nevertheless, registered stocks of variola virus are preserved in both the United States and Russia, and there is a possibility of unregistered stocks elsewhere. With the eradication of smallpox, the need for vaccination was eliminated, and the practice largely stopped. Therefore, most people are now susceptible to variola virus, as well as other orthopoxviruses, such as monkeypox virus. However, as a precaution against the reintroduction of variola virus from an unregistered stock, a new tissue culture–derived vaccinia virus vaccine ACAM2000 has been developed and licensed in the United States (3). In addition, moderately and highly attenuated strains of vaccinia virus including LC16M8 (4) and modified vaccinia virus Ankara (MVA) (5) are being evaluated as safer alternatives to the conventional vaccine.

Soon after the eradication of smallpox and the cessation of general vaccination, the ability to produce recombinant vaccinia viruses that express genes of other microorganisms was developed (6,7). Such genetically engineered viruses have been employed extensively as research tools to establish the targets of humoral and cell-mediated immunity and are being evaluated as live recombinant vaccines. Similar approaches were used to generate immunogenic avipoxvirus (8,9), capripoxvirus (10), and members of other poxvirus genera.

CONSTRUCTION OF POXVIRUS EXPRESSION VECTORS

Insertion of Foreign DNA into the Poxvirus Genome

The development of expression vectors depended on an understanding of the molecular biology of poxviruses, a subject which is reviewed in detail elsewhere (11). The distinctive characteristics of members of the poxvirus family include: a large complex enveloped virion containing enzymes for mRNA synthesis; a genome composed of a linear double-stranded DNA molecule of about 200,000 base pairs; and the ability to replicate within the cytoplasm of infected cells. Detailed protocols for preparing and characterizing recombinant vaccinia viruses are available (12–15), and only general concepts are dealt with here.

The large size of the vaccinia virus genome posed an initial hurdle to the incorporation of foreign genetic material. In addition, the viral DNA is not infectious because enzymes contained within the virion are essential for gene expression. However, it was known that recombination occurs between homologous DNA sequences of poxviruses (16). Furthermore, recombination was shown to occur between virus-derived genomic DNA and either subgenomic DNA fragments (17,18) or recombinant plasmids (19) that had been transfected into the cell. The latter finding provided a way of inserting foreign DNA into the vaccinia virus genome: a plasmid containing a foreign gene flanked with vaccinia DNA is transfected into an infected cell allowing homologous recombination to occur during viral DNA replication. Of course, for the recombinant vaccinia virus to remain infectious, the foreign DNA must not interrupt any vital viral function. This was not an obstacle, however, because there are many nonessential vaccinia virus genes, and it is also possible to insert DNA between genes. In this manner, foreign DNA segments as large as 25,000 base pairs were recombined into the vaccinia virus genome (20). A variety of methods are available to select recombinant viruses or distinguish the plaques from the parental virus. Similar methods have been used to generate recombinant avian and other poxviruses.

There are additional but less commonly used methods of forming recombinant vaccinia viruses. Two of these methods depend on the in vitro cleavage of the vaccinia virus genome at a unique restriction endonuclease site. In one procedure, a DNA fragment containing the foreign DNA is ligated to the cleaved segments, and then the full-length genome is transfected into cells infected with a helper virus—either a