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Recombinant Vaccinia Viruses as Live Vaccines

G. L. Smith [1]

A. Summary

Recombinant vaccinia viruses can be constructed that express foreign antigens. These viruses retain their infectivity and synthesise the foreign gene product in tissue culture and in vaccinated animals. Following vaccination, specific antibody and cell-mediated immune responses are generated against the foreign protein and in several cases these have protected the animal against subsequent challenge with the corresponding pathogen. The potential use of recombinant vaccinia viruses as medical or veterinary vaccines is discussed.

B. Introduction

Vaccinia virus is the world's oldest vaccine and was successfully used to immunise against and eradicate smallpox. This immunisation campaign remains the most successful ever conducted, and its success derives from a number of factors. The vaccine was cheap, stable and plentiful and the disease was acute, easily identifiable and had no animal reservoir. In addition, the World Health Organisation (WHO) was fully committed to the eradication campaign and vigorously pursued this goal. The last naturally occurring case of smallpox was in Somalia in 1977.

Since that time, interest in vaccinia virus has diminished, but it has remained a subject of active research due to its possession of a number of interesting biological properties (Moss 1985). The virus has a large, complex virion structure and a double-stranded DNA genome of 185000 base pairs. Unlike most DNA viruses, which replicate in the nucleus of infected cells, vaccinia virus (the prototype orthopoxvirus) replicates in the cytoplasm. To enable the virus to do this, it possesses a complete transcriptional enzyme system that is able to transcribe the virus genome into functional mRNAs. The virus-coded RNA polymerase does not transcribe genes normally recognised by the host RNA polymerase II, and the promoters recognised by the vaccinia RNA polymerase are structurally and functionally distinct from those of the host cell and other viruses.

An increased understanding of the molecular biology of vaccinia virus together with the advent of recombinant DNA technology enabled vaccinia virus to be used as a cloning and expression vector. Following the expression of the first foreign genes in vaccinia in 1982, there has been an explosion of interest in recombinant vaccinia viruses. The construction of recombinant vaccinia viruses has recently been reviewed (Mackett and Smith 1986) and so it will only be briefly described here. The unique nature of the virus promoters and presence of the virus-coded RNA polymerase required that vaccinia promoters be used to drive expression of foreign genes in vaccinia virus. So the first step in construction of a recombinant virus is to link the foreign protein-coding sequences to a vaccinia promoter. This gene is

[1] Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP, UK

then inserted into the vaccinia genome by homologous recombination in cells infected with wild type (WT) vaccinia virus and transfected with a plasmid containing the foreign gene. To facilitate this process, the plasmid also contains vaccinia virus DNA, flanking the foreign gene, that is taken from a nonessential locus of the virus genome. Within the transfected cells, homologous recombination results in insertion of the foreign gene into the position of the virus genome specified by the flanking vaccinia DNA. Several such loci have been identified (Panicali and Paoletti 1982) but the most widely used one is the vaccinia thymidine kinase (TK) gene, since this permits genetic selection of the recombinant viruses as TK^- mutants (Mackett et al. 1982, 1984). Other methods of selecting the recombinant viruses have been used such as DNA:DNA hybridisation (Panicali and Paoletti 1982), expression of selectable genetic markers (Mackett et al. 1982; Franke et al. 1985), or expression of enzymes which permit visual detection of recombinants due to conversion of chromogenic substrate (Chakrabarti et al. 1985). A variety of plasmid vectors have been constructed for the cloning of foreign genes into vaccinia (Mackett et al. 1984; Boyle et al. 1985; Chakrabarti et al. 1985).

C. Expression of Foreign Antigens

Many foreign genes have been expressed in recombinant vaccinia viruses and so far none have proved toxic to the virus replication. Since vaccinia replicates in the cytoplasm and does not splice its mRNAs, only cDNAs or genes without introns may be expressed. Correct DNA engineering, to ensure utilisation of the translational start and stop codons of the foreign gene, results in the foreign protein being of the predicted size. Additionally, posttranslational modifications such as glycosylation, proteolytic cleavage and carboxylation also occur within the vaccinia-infected cells. The foreign gene products have been indistinguishable antigenically from the authentic antigen.

The time and level of expression depend upon the type of vaccinia promoter chosen. These are either early (before virus DNA replication commences), late (after DNA replication commences) or early and late. Promoters from genes of all three types have been used for expression of foreign genes. A commonly used promoter is one taken from a gene that maps within the inverted terminal repetition of the virus genome, which is expressed throughout the virus replicative cycle and which codes for a protein of 7500 daltons (Mackett et al. 1984).

D. Immunisation of Animals

Recombinant vaccinia viruses expressing a variety of foreign antigens, particularly eukaryotic viral glycoproteins, have been used to immunise experimental animals. Following dermal inoculation a local vaccinial lesion appears, which heals in 2–3 weeks. During this time no viraemia has been found (Smith et al. 1983a) and the virus does not establish latent or persistent infections. Both antibody and cell-mediated immune responses against the foreign antigen have been subsequently detected. For instance, a recombinant expressing the influenza virus haemagglutinin (HA) produced antibody in vaccinated rabbits that was able to neutralise influenza virus infectivity in vitro (Panicali et al. 1983; Smith et al. 1983b). This recombinant virus also induced murine cytotoxic T lymphocytes (CTL) that recognised the influenza HA in a major histocompatibility complex (MHC) class I restricted manner (Bennink et al. 1984, 1986).

The ability of the recombinant viruses to stimulate specific immune responses is obviously important for the potential use of these viruses as live vaccines. However, there are also other scientific applications. For instance, the virus may be used to raise specific antisera against the foreign gene product. Such antisera may be useful in characterising the corresponding antigen within its normal environment, and for determining if the antigen has potential use in future vaccines (Cranage et al. 1986). The recombinant viruses are also useful for the study of antigen recognition by CTL. This is possible because (a) the recombinant virus can be used to make target cells against which effector CTL can be tested in cytotoxicity assays and (b) the recombinant virus can itself

prime animals for a CTL response against the foreign gene product. Experiments of these types have demonstrated that several influenza virus antigens, previously considered to be intracellular, can be recognised by class I MHC antigen-restricted CTL in a manner cross-reactive among different influenza A virus subtypes (Yewdell et al. 1985).

E. Protection of Vaccinated Animals Against Challenge with Pathogens

Experimental animals vaccinated with recombinant vaccinia viruses have been protected against several pathogenic eukaryotic viruses including influenza, hepatitis B, rabies, herpes simplex, vesicular stomatitis and respiratory syncytial virus. As a specific example, experiments involving the hepatitis B virus surface antigen are described in more detail.

Hepatitis B virus (HBV) remains a serious global health problem, with approximately 200 million chronic carriers of the disease. The virus infection has been associated with several pathological conditions including liver cirrhosis, fulminant hepatitis and primary hepatocellular carcinoma, one of the most common male cancers. A subunit vaccine against HBV has been developed and licensed. The vaccine is composed of the surface antigen of the virus (HBsAg) and is purified from the plasma of chronically infected patients, where it can circulate at high concentrations. Although effective, this vaccine is expensive and available in insufficient quantities to meet the enormous global demand. Cloning and sequencing of the HBV genome identified the gene coding for HBsAg and permitted its expression in a variety of vector systems. This is providing an alternative and potentially greater source of the antigen, which may lead to a cheaper and more universally available vaccine.

As another approach to the development of new HBV vaccines, the HBsAg gene was expressed in recombinant vaccinia virus (Smith et al. 1983a; Paoletti et al. 1984). Since the recombinant virus would simultaneously synthesise the HBsAg and present it to the immune system of the vaccinated host, no expensive protein purification would be necessary. Initial experiments demonstrated that the HBsAg was excreted from cells infected with the recombinant virus and that it had biochemical and immunological properties indistinguishable from authentic HBsAg. Rabbits vaccinated with the recombinant virus produced antibodies against HBsAg (anti-HBs) at levels far greater than those necessary to confer protection against HBV in humans. However, due to the restricted tropism of HBV, protection experiments can only be done in primates (usually chimpanzees). Accordingly, two chimpanzees were vaccinated with the recombinant virus and another animal immunised with WT vaccinia. Disappointingly, the animals failed to produce anti-HBs. Nonetheless, when challenged with HBV by intravenous injection 14 weeks later, there was a dramatic difference between the WT- and recombinant-vaccinated animals (Moss et al. 1984). The control animal developed a typical acute HBV infection with antigenaemia, biochemical evidence of liver disease and subsequently anti-HBs and antibodies against the virus core antigen (anti-c). In contrast, the two animals vaccinated with the recombinant vaccinia virus had no antigenaemia or liver disease but rapidly produced high levels of anti-HBs. Subsequently they both also produced low levels of anti-c, indicating that a HBV infection had been initiated. The animals had been antigenically primed following the original vaccination and had produced anti-HBs following re-exposure to the antigen when challenged with HBV.

Although the animals were not completely protected from HBV infection, the single vaccination had protected them against liver disease following severe challenge with HBV. The vaccine might be improved to provide complete protection in a number of ways. First, by using a stronger vaccinia virus promoter, larger amounts of HBsAg would be produced that might induce a strong primary antibody response. Secondly, HBsAg is a mixture of three polypeptides called S, MS and LS, and the first recombinant vaccinia virus expressed only the S form. All share the same 226 carboxyl terminal amino acids, but MS and LS have an additional 55 and 174 amino terminal amino acids, respectively. Recently an epitope on the LS molecule has been identified

which acts as a receptor binding site for hepatocytes. Antibody to this epitope might be expected to block virus attachment to the target organ in vivo; accordingly, a vaccinia recombinant that expresses the LS protein was constructed. In vaccinated rabbits, antibodies are produced which recognise a synthetic peptide specific for LS (Cheng et al. 1986). The ability of such a recombinant virus to protect chimpanzees is under evaluation.

F. Discussion

These experiments demonstrate the potential use of recombinant vaccinia viruses as new vaccines against HBV. As mentioned already, vaccinia recombinants have also protected animals against several other pathogenic viruses, and specific immune responses against AIDS virus antigens and malarial antigens have been demonstrated. Although there are numerous advantages to using vaccinia recombinants as a means of delivering vaccine antigens to the immune system (see below), there are also potential problems. First, vaccination against smallpox with vaccinia carried with it a small but finite risk of postvaccinial complications. Although these were acceptable in the face of life-threatening smallpox, they became of greater concern as smallpox disappeared, and the risk from vaccination eventually outweighed any possible benefit. If recombinant vaccinia viruses are to be reintroduced as a vaccinating agent, safer, more attenuated strains of the virus are desirable. Fortunately, recombinant DNA technology permits the deletion of vaccinia genes as well as insertion of foreign genes. As the genes responsible for vaccinia pathogenicity are identified, these may be specifically deleted to construct safer vaccine strains; already some success has been achieved towards this goal (Buller et al. 1985). A second potential problem with reuse of vaccinia as a vaccine is the existing immunity to vaccinia in a large proportion of the world's population. However, since smallpox vaccination has been discontinued for 10 years or more, an ever growing population of nonimmune children exists. Vaccination against most infectious diseases is carried out during childhood. Revaccination can also be successful, as demonstrated by the accidental vaccination of a human with a recombinant vaccinia virus. Despite the vaccinee having previously had a smallpox vaccination, a good antibody response to the foreign gene was evoked.

The advantages of live recombinant vaccinia virus vaccines include (1) the ability to stimulate both antibody and cell-mediated immune responses, (2) the capacity for large amounts of foreign DNA encoding multiple foreign antigens (Smith and Moss 1983; Perkus et al. 1985), (3) the low cost of the vaccine to manufacture and administer, (4) the vaccine stability without refrigeration, and (5) the wide host range permitting application in veterinary and human medicine.

If vaccinia is to be reused in humans, efficacy will need to be demonstrated initially in limited clinical trials. Probably these would take place in populations where the advantages from successful vaccination against a particular disease far outweigh the worst conceivable rate of vaccine-associated complications. Life-threatening diseases such as AIDS, malaria or hepatitis B would seem to provide such a scenario in certain populations.

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