Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Viral and bacterial vectors of immunogenes

David Cavanagh

A recent development in the production of experimental vaccines has been the use of the smallpox vaccine virus (vaccinia virus) as a carrier (vector) of the genes (immunogenes) which code for the protection-inducing proteins (immunogens) of unrelated viruses. The potential of these vector vaccines lies in the hope that such a vaccine would be cheaper, safer and/or more effective than existing vaccines to some pathogens. Vaccinia virus as a vector has attracted most attention to date because: (a) several immunogenes can be inserted into its genome without destroying its infectivity; (b) the immunogens appear to be produced normally; (c) vaccinia virus has been used highly successfully to eradicate smallpox; and (d) it has a wide host-range and thus might find veterinary as well as human medical application. Experimental vaccines, successfully tested in animals, have been prepared using immunogenes from influenza virus, hepatitis B virus and herpes simplex virus. Apathogenic enteric bacteria have some potential as vectors, most probably against enteric pathogens, although the potential of viral vectors is likely to be realized first. Parasitic worms and protozoa devastate millions of people. When the immunogens of these organisms have been identified there will be added impetus to investigate the potential of vector vaccines against these pathogens.

Keywords: Vectors; bacterial vectors; viral vectors; vaccinia virus; immunogens; immunogenes

For many years the term vector, the Latin word for a carrier or bearer, has meant for the biologist an agent transferring a parasite to a host. Invertebrate vectors come most readily to mind, for example, mosquitoes and tsetse flies transmitting the protozoans which cause malaria and sleeping-sickness, respectively. More recently the term has been applied to plasmids which have been manipulated by biologists to transfer selected genes for expression of bacteria and yeast cells. The papovavirus simian virus 40 (SV40) and bovine papilloma virus have been used as vectors in conjunction with eukaryotic cells. These vectors were used to transfer genes from other biological entities (foreign genes) to cells *in vitro*, either for fundamental research purposes or for the production of proteins which would subsequently be purified for prototype vaccines e.g. hepatitis B virus surface antigen (HBsAg) from yeast[1-3], and foot and mouth disease virus VP1 protein from *Escherichia coli*[4,5].

It was a logical step to produce vectors which could be used to immunize animals directly, so that the foreign gene protein product, the immunogen, was produced *in vivo*. The potential advantages of this approach are discussed later in this paper. For convenience I shall use the word 'immunogene' to denote a gene which specifies the production of a protein which, in the course of natural infection with a pathogen, induces a protective immune response.

The focus of attention at the moment is the vector potential of vaccinia virus, the vaccine virus which has eradicated smallpox. By viral standards the vaccinia virus DNA genome is enormous, comprising 180 000 base pairs. Some of the vaccinia virus genes are not essential for its replication. This fact raised the possibility that immunogenes, from other viruses, might be inserted into the vaccinia DNA at one of the non-essential regions. If done appropriately transcription and translation of the immunogene might occur following infection of cells susceptible to vaccinia virus. Moreover, it would be expected that the resultant protein would induce immune responses following infection of an animal which was susceptible to vaccinia virus. The recent staggering advances in molecular biological techniques combined with the results of much fundamental research with poxviruses and other viruses have turned this possibility into reality.

Among the requirements for the insertion and translation of an immunogene in vaccinia virus are: (1) the incorporation of the gene into a cloning vehicle e.g. a plasmid, so that sufficient quantities of DNA are available for transfection of vaccinia virus infected cells; (2) the presence in the plasmid, at both ends of the immunogene, of DNA sequences which are homologous to sequences in vaccinia virus, thus permitting recombination; (3) recombination should take place in a non-essential region of the vaccinia virus genome; (4) the immunogene must be adjacent to regulatory sequences recognized by vaccinia virus transcription machinery, for expression to occur.

Volumes 79, 80 and 81 of the Proceedings of the National Academy of Sciences of the USA contain papers which are milestones on the road to the production of vaccines based on eukaryotic viruses as vectors of immunogenes. The scene was set in 1982 with publication of four papers, two from the group of Bernard Moss of the National Institutes of Health, Bethesda, and two from Enzo Paoletti and colleagues at the New York State Department of Health, Albany. Weir *et al.*[6] showed that vaccinia virus which lacked the thymidine kinase (TK) gene (TK^- virus) could be given a TK gene as a consequence of recombination with a plasmid containing the TK gene. The absence from the virus of the TK gene is a readily detectable mutation i.e. a genetic 'marker'; the recovery of the TK marker by the previously TK^- virus is termed 'marker rescue'. Simultaneously Nakano *et al.*[7]

Department of Microbiology, Houghton Poultry Research Station, Houghton, Huntingdon, Cambridgeshire PE17 2DA, UK

This paper was presented at the 'New Routes to Vaccine Prophylaxis' Symposium organized by the Biotechnology Group of the Society of Chemical Industry on 26 June 1984

0264-410X/85/010045-04 $03.00

also demonstrated marker rescue using another deletion mutant of vaccinia virus. Panicali and Paoletti[8] then showed that the TK gene from the unrelated herpes simplex virus (HSV) could be inserted into the genome of infectious vaccinia virus and that a functional TK enzyme was produced. Very soon afterwards Mackett *et al.*[9] presented similar findings, again exploiting the TK genes of vaccinia virus and HSV. Thus it had been shown that vaccinia virus was a very useful selectable cloning and expression vector. The potential of this vector for vaccine purposes was not lost on either group.

In the subsequent two years several papers have been published which further illustrate this potential. A recombinant vaccinia virus has been formed containing the HBsAg gene. Not only was this gene expressed *in vitro* but rabbits vaccinated intradermally with the live recombinant produced antibody against HBsAg[10,11]. The haemagglutinin (HA) immunogene of influenza virus has been inserted in the vaccinia virus genome. Tissue culture cells infected by the recombinant produced glycosylated HA and transported it to the cell surface[12,13]. Both rabbits and hamsters which had been inoculated with the recombinant produced serum antibody that neutralized influenza virus. Moreover, hamsters which had been inoculated intradermally with the recombinant were protected against respiratory infection by live influenza virus applied intranasally[13]. Mice which had been inoculated intraperitoneally with a vaccinia virus recombinant containing the gene for glycoprotein D of HSV resisted a lethal dose of live HSV[11].

Most recently vaccinia virus recombinants containing the gene coding for the circumsporozoite antigen of the malaria parasite *Plasmodium knowlesi* have been made[14]. Rabbits inoculated intradermally with these recombinants produced antibody which reacted with extracts of *P. knowlesi* sporozoites. The authors have suggested that it should be possible to express several antigens from different life cycle stages of the parasite to make a more potent vaccine.

Is vaccinia virus the only virus that can be used for vector vaccines? Several other eukaryotic viruses have established or potential use as vectors e.g. SV40, adenovirus, bovine papilloma virus, herpes virus and retroviruses. However, it has been pointed out that these viruses can be oncogenic while vaccinia virus is not[8]. Another advantage of vaccinia virus is that it has been used highly effectively in its own right as a vaccine to eradicate smallpox. Its large genome permits the inclusion of large amounts of DNA. The construction of stable and infectious recombinants containing more than 20 000 bases of foreign DNA have been claimed by both American groups. Thus there is the potential for constructing polyvalent vaccines.

Vaccinia virus has a large host range which may make it suitable for the production of vaccines for some animals. Alternatively other pox viruses may be used. The poultry industry would seem to be a suitable one in which to use vectors. Chickens are kept at very high stocking densities and this is conducive to the spread of infectious diseases. Vaccines and drugs are essential for intensive poultry keeping. However, the UK poultry industry spends only about 0.1% of its turnover on vaccines. Profit margins are very slim. Indeed, for much of 1983 the UK broiler growers sold at a loss. Thus vaccines must be cheap to buy. They should also be cheap to apply. Handling birds for individual vaccination is costly but for some vaccines this is currently unavoidable. Some live vaccines can be applied inexpensively in drinking water or by spray. They generally induce good immunity but can cause problems. Thus vaccine strains of avian infectious bronchitis virus (IBV, a coronavirus) can cause damage to the oviduct and kidneys of chickens. IBV can also predispose young chickens to secondary infection by some strains of E. coli, causing colisepticaemia. On multi-age sites the vaccine virus may spread to very young chickens with deleterious effects. Inactivated virus vaccines are not associated with these problems i.e. they are safer, but they are costly to buy and apply. In the case of IBV the inactivated virus alone does not induce good protection.

For coccidiosis of chickens, caused by several species of the protozoan Eimeria, no effective vaccines exist. Inactivated vaccines have been prepared but they did not induce protection. The vector approach may be the solution to these problems with some pathogens. In many parts of the world fowl pox is a problem and a live pox virus vaccine is used for its control. A pox virus recombinant carrying the immunogene of, say, IBV would not be associated with the problems of current live IBV vaccines i.e. it would be safer and would still be inexpensive. The successful protection of hamsters against another respiratory virus, influenza virus, by a vaccinia virus recombinant carrying the influenza virus HA gene, holds the promise that a similarly constructed avian pox vaccine would protect chickens against IBV.

Bacteria have been suggested as potential vectors of immunogenes in connection with vaccines for poultry[15-17] and human rotaviruses[18]. The bacterium chosen, probably an *E. coli* strain, would have to be non-pathogenic but capable of colonizing the gastrointestinal tract for a time sufficient to permit a protective immune response to be mounted against the immunogen. Expression of the immunogen at the bacterial cell surface would probably induce a better immune response than if the immunogen was exposed only after the death of the bacterium. The requirements for the transport of proteins to the surface of bacteria is under study[19]. It would be expected that the use of an enteric bacterial vector would be most effective against pathogens of the gastrointestinal tract. Protection at other mucosal surfaces might arise if the common mucosal immune system was effective[20].

Apart from the isolation of suitable bacteria, selection of plasmids for transfer of the immunogen and obtaining expression at the bacterial surface, other potential problems to be overcome include (a) the tendency of some foreign proteins synthesized in bacteria to be hydrolysed rapidly or to be toxic for the bacterium, (b) the lack of glycosylation which may be structurally important, and (c) the possible failure of the polypeptides of normally oligomeric proteins to be assembled, which may result in decreased antigenicity. However, proteins of several viruses have been produced in *E. coli*. In the case of FMDV, when the resultant protein was inoculated into cattle and swine, they were protected against challenge with FMDV[5]. Murray *et al.*[3] have reported that preparations of hepatitis B core antigen produced in *E. coli* were protective or reduced the severity of infection of chimpanzees with hepatitis B virus. Bacteria as vectors have potential advantages which warrant continued consideration. Innocuous *E. coli* strains occur naturally in many animals. Technology for the large-scale production of bacteria is well developed. The bacteria would be expected to be more stable than many live virus vaccines, stability being an important attribute for any vaccine. Administration would be very simple and inexpensive; for animals the bacterium could be added to food and water.

When might a vector be chosen as the basis for a

vaccine? First it must be asked whether, when a vaccine against a pathogen exists, a new type of vaccine is warranted. Recently Broomby[21] has listed a number of advantages that a product of new biological techniques might have: simplified production; increased safety; greater stability; improved potency; reduced allergenicity; patentability. A new product, probably developed at high cost, will have to have strong advantages in order to compete with an established product and recover its costs. Vaccines which are totally new may be commercially more attractive e.g. against coccidiosis in poultry, endo- and ecto-parasites, herpes simplex virus. A vector-based vaccine may be more cheaply produced or safer than current vaccines against, for example, hepatitis B virus and IBV, respectively. Other new approaches to vaccine production are currently being investigated: synthesis of proteins or peptides in bacteria, yeast or eukaryotic cells; chemical synthesis of peptides; rational manipulation of virus genomes; and development of more suitable adjuvants. Although it is most unlikely that the vector approach will be suitable for all diseases it does have several major potential advantages, some of which stem from experimental observations; immunogenes have been transferred to vaccinia virus; the genes were expressed; the immunogen had properties very similar to the immunogen derived from the 'parent virus'; inoculated animals did respond with protective immune responses.

Especially exciting was the protection of the lower respiratory tract of hamsters against infection by influenza virus following intradermal inoculation of a vaccinia virus recombinant carrying the influenza virus HA gene. Production of immunogens in infected cells, following the use of the live poxvirus vector, might stimulate a much more comprehensive array of immune responses than would arise following the parenteral administration of an inactivated virus or subunit vaccine. The transport of immunogens to the surface of a cell may, in some cases, be necessary for the induction of an efficient protective immune response.

Other advantages of vaccinia virus are that it has been used as a vaccine for many years, experience of it in the medical community is worldwide, little expertise is required for its administration and its success in eliminating smallpox is beyond dispute. However, some misgivings have been expressed because a number of people who had been vaccinated with vaccinia virus had serious complications, some of which led to death. Surveys undertaken during the 1960s in the USA indicated that the risk of death from all vaccination complications was, for primary vaccinees, (a) one per million vaccinees of all ages, (b) five per million vaccinees under 12 months of age and (c) 0.5 per million vaccinees 1–19 years old[22]. In addition the combined rate of post-vaccinial encephalitis and vaccinia necrosum was 6.5 per million for infants and three per million for those aged 1–19 years[22].

Figures such as these must be taken into account when consideration is given to the use of vaccinia virus as a vector. However, pathogens afflict mankind on a much greater scale. Parasitic worms (helminths) infect some 3000 million people[23]. On the African continent alone some 160–200 million people are affected by malaria, causing about one million deaths per year[24]. An estimated 200 million people are affected by hepatitis B virus[25]. These terrible figures demand that vaccines be developed. Apart from being safe and efficacious they must be cheap to produce and to administer. A vector vaccine, based on vaccinia virus, would seem to be a very suitable approach to this challenge, especially for developing countries where the disease problems are greatest and the ability to pay is least.

It is not being suggested that the vector approach in general, or the use of pox viruses in particular, will be a panacea for our outstanding vaccine problems. Nevertheless, this approach has great potential and warrants investigation with immunogens of many pathogens. If just one human and one veterinary vaccine were to be successfully based on vectors, the gain to mankind could be enormous.

References

1 Valenzuela, P., Medina, A., Rutter, W.J., Ammerer, G. and Hall, B.D. Synthesis and assembly of hepatitis B virus surface antigen particles in yeast. *Nature* 1982, **298**, 347

2 McAleer, W.J., Buynak, E.B., Maigetter, R.Z., Wampler, D.E., Miller, W.J. and Hilleman, M.R. Human hepatitis B vaccine from recombinant yeast. *Nature* 1984, **307**, 178

3 Murray, K., Bruce, S.A., Hinnen, A., Wingfield, P., van Erd, P.M.C.A., de Reus, A. and Schellekens, H. Hepatitis B virus antigens made in microbial cells immunise against viral infection. *EMBO J.* 1984, **3**, 645

4 Kupper, H., Keller, W., Kurz, Ch., Forss, S., Schaller, H., Franze, R., Strohmaier, K. *et al.* Cloning of cDNA of the major antigen of foot-and-mouth disease virus and expression in *E. coli*. *Nature* 1981, **289**, 555

5 Kleid, D.G., Yansura, D., Small, B., Dowbenko, D., Moore, D.M., Grubman, M.J., McKercher, P.D., Morgan, D.O., Robertson, B.H. and Bachrach, H.L. Cloned viral protein vaccine for foot and mouth disease: responses in cattle and swine. *Science* 1981, **214**, 1125

6 Weir, J.P., Bajszar. G. and Moss, B. Mapping of the vaccinia virus thymidine kinase genes by marker rescue and by cell-free translation of selected mRNA. *Proc. Natl Acad. Sci. USA* 1982, **79**, 1210

7 Nakano, E., Panicali, D. and Paoletti, E. Molecular genetics of vaccinia virus: demonstration of marker rescue. *ibid*, 1593

8 Panicali, D. and Paoletti, E. Construction of poxviruses as cloning vectors: insertion of the thymidine kinase gene from herpes simplex virus into the DNA of infectious vaccinia virus. *ibid*, 4927

9 Mackett, M., Smith, G.L. and Moss, B. Vaccinia virus: a selectable eukaryotic cloning and expression vector. *ibid*, 7415

10 Smith, G.L. Mackett, M. and Moss, B. Infectious vaccinia virus recombinants that express hepatitis B virus surface antigen. *Nature* 1983, **302**, 490

11 Paoletti, E., Lipinskas, B.R., Samsonoff, C., Mercer, S. and Panicali, D. Construction of live vaccines using genetically engineered poxviruses; biological activity of vaccinia virus recombinants expressing the hepatitis B virus surface antigen and the herpes simplex virus glycoprotein D. *Proc. Natl Acad. Sci. USA* 1984, **81**, 193

12 Panicali, D., Davis, S.W., Weinberg, R.L. and Paoletti, E. Construction of live vaccines by using genetically engineered poxviruses: biological activity of recombinant vaccinia virus expressing influenza virus hemagglutinin. *Proc. Natl Acad. Sci. USA* 1983, **80**, 5364

13 Smith, G.L., Murphy, B.R. and Moss, B. Construction and characterization of an infectious vaccinia virus recombinant that expresses the influenza hemagglutinin gene and induces resistance to influenza virus infection in hamsters. *ibid*, 7155

14 Smith, G.L., Godson, G.N., Nussenzwieg, V., Nussenzweig, R.S., Barnwell, J. and Moss, B. *Plasmodium knowlesi* sporozoite antigen: expression by infectious recombinant vaccinia virus. *Science* 1984, **224**, 397

15 Cavanagh, D. New Biotechnology for Animal Health and Production (Ed. Ross, D.B. and Sellers, K.) Association of Veterinarians in Industry, London, 1984, pp. 67–78

16 Brown, T.D.K. 'Engineering' offers better vaccines. *Poultry World*. 1984, **138**(6) 16

17 Cavanagh, D., Binns, M.M., Boursnell, M.E.G. and Brown, T.D.K. Modern Approaches to Vaccines (Ed. Chanock, R.M. and Lerner, R.A.) Cold Spring Harbor Laboratory, 1984, pp. 215–218

18 Flores, J., Sereno, M., Kalica, A., Keith, J., Kapikian, L. and

Chanock, R.M. *ibid,* pp. 159–164
19 Bouges-Bocquet, B., Guesdon, J-L., Marchal, C. and Hofnung, M. *ibid,* pp. 225–231
20 Bienstock, J., Befus, D., McDermott, M., Mirski, S. and Rosenthal, K. Regulation of lymphoblast traffic and localization in mucosal tissues, with emphasis on IgA. *Fed. Proc.* 1983, **42**, 3213
21 Broomby, M.R. See ref. 15. p. 71–75
22 Koplan, J.P. and Axnick, N.W. Benefits, risks and costs of viral vaccines. *Prog. Med. Virol.* 1982, **28**, 180
23 Ash, C., Crompton, D. and Keymer, A. Nature's unfair food tax. *New Scientist* 1984, **101**(1400), 17
24 Bruce-Chwatt, L. Malaria: from eradication to control. New Scientist 1984, **102**(1406), 17
25 Tiollais, P, Charnay, P. and Vyas, G.N. Biology of hepatitis B virus. *Science* 1981, **213**, 406