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THE RELEASE INTO THE ENVIRONMENT OF GENETICALLY ENGINEERED VIRUSES, VACCINES AND VIRAL PESTICIDES

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Vaccination of man and domestic animals against a virus disease can be accomplished using either an appropriate live virus (including, in the future, genetically engineered viruses), or a killed virus (or subunits of a virus) either extracted from infectious material, or made in a genetically engineered vector. The use of a killed virus or subunit vaccine does not constitute an environmental release, provided there is no residual infectivity. Live virus vaccines, including genetically engineered viruses, may result in a release into the environment if infectious virus escapes from a vaccinated individual. Likewise, the use of infectious viruses that express foreign genes (viral, bacterial, mammalian, etc.) may, depending on the replication characteristics of the vectors, also result in releases.

Live attenuated virus vaccines are effective immunogens for several virus diseases, including smallpox, yellow fever, polio, mumps, rubella and measles: they afford protection against disease to most vaccinated hosts. The classic example is smallpox, a disease which was eliminated by an international programme of vaccination using live vaccinia virus. (Vaccinia virus is a member of the Poxviridae and is immunologically related to smallpox virus.) Although for some people (such as immunosuppressed individuals) vaccination with vaccinia virus is not advisable, and although virus can be transferred from a vaccinee to others, the smallpox eradication programme was a milestone in human medicine that probably would not have been achieved as cheaply or as proficiently if a killed, or subunit, vaccine had been used. The eradication programme undoubtedly also owed its success to the fact that smallpox infection is limited to man. There is no other natural vertebrate or invertebrate reservoir of the virus. Other virus diseases exclusive to man (e.g. polio, measles and mumps) could, theoretically, also be eliminated with appropriate vaccination programmes. By contrast, it is unlikely that human viral diseases that also involve wildlife or arthropod hosts could be eradicated. Preventive vaccination is the only possibility to limit their effects on man.

As with vaccinia virus, the use in man of other available live virus vaccines (even the highly effective yellow fever vaccine) is not recommended in certain circumstances. Where live viruses are shed from an immunized host, as in live polio virus immunization, use of the vaccine may be advantageous since it may confer infection and immunity to non-vaccinated associates of the primary vaccinees. However, it may also be disadvantageous, producing unwanted infections in those associates, for example.

Genetically engineered live virus vaccines

Genetic engineering procedures may improve live vaccines; for instance, by enhancing their immunogenicity, by further reducing their virulence, or by reducing the possibility of transfer from the primary host. Genetic engineering is most easily accomplished in viruses with a DNA genome. Human diseases caused by DNA viruses include cold sores (herpes simplex virus), chickenpox (varicella-zoster), cytomegalovirus infections, glandular fever (Epstein-Barr virus), certain respiratory tract infections (human adenoviruses), hepatitis (hepatitis B virus), warts and, probably, certain cervical carcinomas (papilloma viruses). Many animal diseases also have aetiology involving a DNA virus (e.g. pseudorabies and African swine fever). There are compelling reasons to develop new or improved virus vaccines for all of these diseases. As with other agents of infection, DNA viruses are transferred from an infected individual by a variety of routes, e.g. respiratory secretions, saliva, faecal excretions, sexual activity, transdermal abrasions, transfer in utero or at parturition.

Some RNA viruses have an infectious DNA form in their replication cycle, for example retroviruses such as the human immunodeficiency viruses that cause AIDS. Others can be copied in vitro into infectious DNA using reverse transcriptase. These include the positive-stranded viruses such as the picornaviruses (e.g. polio and certain common cold viruses), alphaviruses, coronaviruses and flaviviruses (e.g. dengue and yellow fever virus). Since DNA can be manipulated and RNA cannot (at present), sequence changes can be introduced

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Fig. 1. Construction of vaccinia virus recombinants that express a foreign gene.
into these RNA viruses through the DNA intermediate.

The opportunities to manipulate directly RNA viruses that have a duplex RNA genome (e.g. human rotavirus that are responsible for certain forms of viral diarrhoea), or a single-stranded, negative-sense RNA genome (e.g. measles, mumps, influenza, rabies) are limited. As an alternative, their genes may be incorporated into a DNA virus vector such as vaccinia virus (Fig. 1) and immunity raised via the replication and expression capabilities of the vector. Since vaccinia virus was used to eliminate smallpox disease, and since it has been widely studied at the molecular and genetic level, there are good reasons to consider it as a vector of immunogens in man. Although other human DNA viruses are potential vectors (e.g. herpes viruses, adenoviruses), most research has focused on vaccinia.

Before genetically engineered viruses are widely used in human or animal medicine, risks and benefits will have to be assessed. Crucial to such analyses will be the genetic stability of the viruses and their ability to promote gene exchange. It needs to be determined whether the vector’s host range will provide opportunities for the introduction of the foreign gene into a host that otherwise would not be exposed to it. The environmental consequences of the release of genetically engineered virus from a vaccinated individual have to be considered in relation to the infection and transmission cycles of the virus and any changes in these cycles that result from the manipulations.

Vaccination of animals with recombinant vaccinia viruses

Vaccinia virus recombinants expressing appropriate antigens have been investigated as potential agents for vaccinating animals1-3. Animals have been vaccinated with live vaccinia recombinants that express a variety of immunogens: for example, influenza haemagglutinin and nucleoprotein, vesicular stomatitis virus (VSV) G protein, rabies G protein, herpes simplex virus glycoprotein, hepatitis B surface antigen, Rift Valley fever virus glycoproteins, Lassa arenavirus glycoprotein, and plasmodial circumsporozoite antigen. In each case antibodies were produced that reacted with or neutralized the corresponding virus or protozoan. Mice inoculated with vaccinia recombinants expressing influenza haemagglutinin or nucleoprotein became primed to produce cytotoxic T cells that recognize influenza virus-infected mouse cells4. Likewise, infected cells expressing the same antigens during recombinant vaccinia virus infections were targets for cytotoxic T cells produced in influenza virus-infected mice5. In other words, both humoral and cell mediated responses are induced against the foreign antigens expressed from the recombinant vaccinia viruses. Coexpression of immunogens with interleukin raises the opportunity for enhanced immunological responses5-7.

Vaccination with the appropriate recombinant vaccinia viruses (Ref. 3) protects, at least partially, the following species:

- hamsters against infection with influenza virus;
- mice against lethal intraperitoneal inoculation with herpes simplex viruses (serotypes 1 or 2) and against the development of a latent trigeminal HSV-1 infection, or against lethal intracranial infection with rabies virus, or against lethal intravenous infection with VSV;
- cattle against lingual infection with VSV;
- chimpanzees against hepatitis following intravenous infection with hepatitis B virus;
- sheep against intradermal infection with Rift Valley fever virus;
- primates and guinea-pigs against Lassa virus.

The question of the shedding of recombinant vaccinia viruses from vaccinated hosts and the consequential environmental risks is under study. Opportunities exist to restrict spread via genetic constructions that limit the infectivity and pathogenesis of vaccinia, and they are being explored. These issues are particularly important in the proposed use of vaccinia recombinants to protect wildlife against rabies infection.

Virus insecticides

Among the viruses that are pathogenic for arthropods are members of the Baculoviridae. The Baculoviridae are a family of lipid-enveloped viruses with circular, double-stranded, DNA genomes of 80-150 kilobase-pairs, depending on the virus6,7. Unlike vaccinia virus, the virions neither possess nor require virus-encoded DNA-directed RNA polymerase activities. Naked viral DNA is infectious per se. Baculoviruses are restricted in their host ranges. They do not infect vertebrates, non-arthropod invertebrates, microorganisms, or plants. Indeed, baculoviruses infect only a few arthropod species. Many baculoviruses are pathogenic for these arthropods and a dozen or more are being used commercially as alternatives to chemical insecticides. Their use, which dates back to the 19th century and the induction of 'caterpillar wilt', has been successful and environmentally acceptable. But the commercial use of virus insecticides has been limited,
among other things, by their high specificity (restricted host range) and slowness.

Although specificity is an ecologically desirable feature, a single specific viral insecticide is insufficient to treat crops infected with several unrelated pests. Also, unlike chemicals, viruses may take several days or weeks to kill an insect pest. During that time the pest may cause an unacceptable level of damage to the crop. Chemical insecticides have a broader specificity and kill quickly. However, chemicals that affect beneficial insects as well as the pest species, or preparations that leave residues in the environment that affect other organisms (birds, fish, man), are also not desirable.

Among the Baculoviridae are viruses that are encapsidated by a crystalline ‘arrangement of protein’ (polyhedrin, Fig. 2). In an infected cell or caterpillar this protein is a late viral gene product. The occlusion body derived from the protein protects the infectious virions in the environment and permits their passive dispersal, for example by insectivorous animals. Some, but not all, baculoviruses make occlusion bodies that contain a single infectious virion; others occlude many virions within the particle. In the infection cycle of an occluded baculovirus such as the multiply enveloped nuclear polyhedrosis virus of the alfalfa looper moth Autographa californica (AcMNPV or AcNPV), polyhedra are released from dead caterpillars, passively dispersed in the environment and subsequently ingested by other caterpillars (Fig. 3).

Like vaccinia virus, baculoviruses can be genetically engineered to include foreign genes. The most frequently used system is one in which the foreign gene replaces the polyhedrin gene, allowing selection of non-occluded recombinants. Chimeric genes involving the foreign gene tagged on to part of the polyhedrin protein have also been described. Since in either case polyhedrin protein is not made, recombinants produce polyhedrin-negative plaques in tissue culture, in contrast to wild-type virus which produces plaques that contain cells with visible occlusion bodies. Alternative promoters have also been used, and selection systems similar to those used to recover vaccinia recombinants have been studied. In addition, duplicated promoters have been described, which allow both the native gene (e.g., the polyhedrin gene) and the foreign gene to be expressed. The levels of expression of foreign genes can represent up to 50% of the total protein recovered from infected tissue culture cells, or from infected caterpillars.

Virus insecticides are environmentally acceptable alternatives to chemical insecticides. By genetic engineering procedures it might be possible to improve the efficacy (e.g., speed of action) of such insecticides and induce other changes in phenotype. For example, a bacterial toxin gene or a caterpillar regulatory gene (e.g., one coding for a hormone) could be incorporated into the virus genome.

At the NERC Institute of Virology in Oxford, a cautious and systematic programme of research and development into the genetic engineering of baculovirus insecticides [AcNPV] began with the release, in 1986, in the UK of a genetically marked virus into a cabbage-patch ecosystem to follow its fate and persistence in the environment. As expected for an occluded virus, persistence was demonstrated throughout the six months of study both in soil and on foliage. In 1987, a second genetically manipulated virus insecticide was developed and tested in a field study. In this virus, the gene coding for the protective viral coat (the polyhedrin) was removed. The data from this study indicated that such a genetically crippled virus was still an effective pathogen. The study also demonstrated that the virus could not persist in the environment post infection, thus limiting its possible dispersal or interaction with other baculoviruses. Such a virus is planned to be a substrate for the next phase of the programme — the incorporation of genes designed to alter the virus phenotype.

Research into genetically engineered virus insecticides has as its eventual objective the development of
environmentally acceptable alternatives to other procedures of pest control. As with engineered vaccines that might be introduced into the environment, the initial goal of research in this field is risk assessment. We need to know whether there are any risks associated with the use of these agents. The answer will determine which agents can be deployed in the future.

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GENETIC ENGINEERING, INTEGRATED PEST MANAGEMENT AND THE EVOLUTION OF PESTS

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Let us assume that genes placed in plants and microbes will always stay put and will only have the effect they were designed to have. Will the need for ecologists and evolutionary biologists in biotechnology disappear? I will use a specific case regarding pest control to illustrate how active collaboration between genetic engineers and evolutionary ecologists in the design of genetically engineered organisms could lead to gains for society.

**Insecticidal plants**

The use of synthetic pesticides has sometimes caused serious environmental problems. Genetic engineering has the potential to eliminate some of these problems. By moving genes into crop plants that code for synthesis of environmentally safe compounds, protection from insects and pathogens could be achieved. To this end, scientists have moved a gene from *Bacillus thuringiensis* (the gene codes for *B.t.* δ-endotoxin) into tobacco and tomato. Industry scientists intend to move this gene into major crops, such as cotton, where insecticides are currently applied more than ten times a season in some areas.

Genetic engineers feel challenged to develop plants that kill insects as effectively as conventional insecticides. Such plants would probably be welcomed by farmers, because they would cut costs and reduce hazards to workers. From an environmental perspective, the small amount of biodegradable δ-endotoxin would be a welcome replacement for insecticides.

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**It is well known that pest populations can evolve resistance to pesticides. The bollworm complex (*Heliothis spp*.), which attacks cotton plants, is a particularly good example of a highly adaptable pest. To achieve long-term success, genetic engineering projects for the production of insecticidal crop plants, including cotton, need to take account of the adaptive potential of pests. Using the bollworm complex as a model, it is possible to show how an integrated approach to pest management, involving genetic engineers, evolutionary biologists and ecologists, could lead to the development of safe and effective transgenic cultivars.**

that cause on-site and off-site pollution. While such an approach is certainly an improvement over the prophylactic use of synthetic pesticides, a closer look from an ecological and evolutionary perspective indicates that some changes in this approach could lead to long term benefits.

The current approach for engineering *B.t.* δ-endotoxin genes into crops involves linking the DNA sequence that codes for the toxin with a constitutive promoter sequence. When such a construct is spliced into a plant's DNA, the toxin-encoding sequence should theoretically be expressed in all of the plant's tissues. In practice, there is usually some quantitative variation among tissues in expression of these genes. Nevertheless, the goal of producing a plant in which all structures are highly toxic to insects is in sight.

**The bollworm complex**

Larvae of *Heliothis* *zea* and *H. virescens*, which are generally referred to as the bollworm complex, can cause severe damage to cotton plants. Currently, when damaging densities of these larvae are found in cotton, they are controlled with insecticides. When first released commercially, transgenic cotton plants with a high constitutive level of *B.t.* δ-endotoxin production would be protected from damage by this bollworm complex and would not require insecticide use. Unfortunately, the long term efficacy of such cultivars may decrease because of evolution of the target pest population. Experience with crop pests indicates that whenever a single environmental stress drastically decreases the fitness of a pest, adaptation by the pest to that stress should be anticipated. Repeatedly, pathogens and insect pests have adapted to synthetic pesticides and resistant crop cultivars. The bollworm complex is notorious for the rapidity with which it has adapted to a variety of insecticides by changes in metabolic enzymes or the target site of the pesticide. Thus, if industry succeeds in commercializing cotton that constitutively produces concentrations of endotoxin that kill over 90% of the bollworm population, genetic adaptation of the pest to the toxin is likely.

There are three ways of facing this problem. One is to deny its existence. The second is to search for new toxin-encoding genes that could replace genes that become evolutionarily