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USE OF GENETICALLY MODIFIED VIRUSES AND GENETICALLY ENGINEERED VIRUS-VECTOR VACCINES: ENVIRONMENTAL EFFECTS

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Despite major therapeutic advances, infectious diseases remain highly problematic. Recent advancements in technology in producing DNA-based vaccines, together with the growing knowledge of the immune system, have provided new insights into the identification of the epitopes needed to target the development of highly targeted vaccines. Genetically modified (GM) viruses and genetically engineered virus-vector vaccines possess significant unpredictability and a number of inherent harmful potential hazards. For all these vaccines, safety assessment concerning unintended and unwanted side effects with regard to targeted vaccinees has always been the main focus. Important questions concerning effects on nontargeted individuals within the same species or other species remain unknown. Horizontal transfer of genes, though lacking supportive experimental or epidemiological investigations, is well established. New hybrid virus progenies resulting from genetic recombination between genetically engineered vaccine viruses and their naturally occurring relatives may possess totally unpredictable characteristics with regard to host preferences and disease-causing potentials. Furthermore, when genetically modified or engineered virus particles break down in the environment, their nuclei acids are released. Appropriate risk management is the key to minimizing any potential risks to humans and environment resulting from the use of these GM vaccines. There is inadequate knowledge to define either the probability of unintended events or the consequences of genetic modifications. The objective of this article is to highlight the limitations in environmental risk assessment and raise awareness of the potential risks involving the use of genetically modified viruses and genetically engineered virus-vector vaccines.

Vaccination has been one of the most successful and cost-effective public health interventions ever employed. Following the Second World War and the establishment of the World Health Organization (WHO), many vaccines were developed based on conventional processes. Conventional processes in vaccine development usually depend on inactivation/killing of virulent organisms, purification of immunogens, or attenuation of virulent organisms (Maybury

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Okonek & Peters, 2004; Stephenson, 2001). Despite having considerable successes in eradicating or preventing diseases, these vaccines were developed without an extensive knowledge of the pathogenic mechanisms exerted by these agents, with manufacturing processes either expensive or based on chance appearance. With the recent advancement in genetic engineering technology and growing knowledge of the immune system, the trend is now moving toward development of highly targeted vaccines by design, based on knowledge of both the genomic sequence of pathogen and the mammalian immune response.

GROWTH IN GENETICALLY MODIFIED VIRUSES AND GENETICALLY ENGINEERED VIRUS-VECTOR VACCINES

Genetically modified (GM) viruses and genetically engineered (GE) virus-vector vaccines are made of "live" viruses or virus vectors that are specifically designed to become harmless, nonpathogenic but with their infectivity unaffected (Stephenson, 2001). These vaccines are therefore considered to have advantage over convectional vaccines in term of safety and immunological responses. Theoretically, GM vaccines do not carry the danger of virulence reversion like attenuated vaccines, and they provide more effective and longer lasting immune responses than killed, acellular, or subunit vaccines.

Industry in the development of genetically engineered vaccines is growing quickly. Despite the success of current conventional vaccines, parasitic diseases, chronic diseases of the central nervous system (CNS) (such as Alzheimer's disease and Creutzfeldt–Jakob disease), and infectious diseases (such as malaria, AIDS, herpes, and dengue fever) still remain refractory to those approaches. Additionally, pathogens once thought to be controlled now increasingly gain drug resistance, new diseases emerge, and established diseases emerge in new and virulent forms (Stephenson, 2001). These new problems require new therapies or vaccines. Examples of viruses or virus vectors used for GM vaccine development include avian influenza (H5N1 Hong Kong 2003 strain) vaccine virus for human (NIBSC, 2005) and recombinant vaccinia–rabies glycoprotein virus for foxes and raccoons (Arizona State University, 2004; Pastoret, 2004).

GENETICALLY MODIFIED VIRUSES AND GENETICALLY ENGINEERED VIRUS-VECTOR VACCINES AND THEIR POTENTIAL RISKS

GM viruses and GE virus-vector vaccines intend to provide better immunological protection and a better safety profile than conventional vaccines. However, the real world is too complex and interdependent for a flawless vaccine. These genetically engineered vaccines also carry significant unpredictability and a number of inherent harmful potential hazards. Unintended and unwanted side effects with regard to the targeted or nontargeted individuals were found to occur within the same as well as other species.

GM viruses and GE virus vector vaccines, in particular those for human use, are under tight regulatory scrutiny for unwanted side effects within the targeted individual. Potential undesirable immunological effects associated with this new vaccination technology include unexpected immunopathological reaction, autoimmune reaction (related to induction of anti-DNA antibodies), and long-term tolerance (related to persistent infection or latent infections) (European Agency for the Evaluation of Medical Products [EMEA], 2001; MHRA, 2004; Traavik, 2005). Vaccines also inherit the potential to undergo chromosomal integration or insertional mutagenesis, leading to random insertions of vaccine constructs into host cellular genomes, resulting in alterations of gene expression or activation of cellular oncogenes. Concern is thus raised with respect to potential to induce tumors (EMEA, 2001; MHRA, 2004).

At present, safety concerns related to the use of GM viruses and GE virus vectors on nontargeted individuals within the same or other species remain unresolved. Unintended side effects occur in nontargeted individuals of the same species and constitute acute symptoms. Depending on the genetic differences between virus strains and geographical variants of the same species, these unintended side effects may have relative influence on infectivity of GM vaccines. Over time, new or spontaneous genetic changes may modulate the interplay with host species in new and unpredictable ways (Traavik, 2004b). Another concern raised is the potential to transfer or recombine genetic material from GM viruses or GE virus-vector vaccines to the targeted individual germ line cells (EMEA, 2001; MHRA, 2004). The fact that vaccine strains may persist in the vaccinated recipients also raises concern that if the target species is a food-producing animal, the virus may cascade down the food chain (Pastoret, 2004).

HORIZONTAL GENE TRANSFER AND ITS CONSEQUENCES

Horizontal gene transfer is believed to be the critical process leading to nontarget adverse effects and unintended spread of GM materials into ecosystem. Horizontal gene transfer (1) is defined as nonsexual transfer of genetic information between genomes or between different organs in the same or different species, (2) is distinct from the usual form of gene transfer that takes place vertically from parent to offspring (vertical gene transfer), and (3) involves not only the movement of a new gene into a cell but also long-term maintenance by the recipient cell. The dangerous part of this event is that it is random and unpredictable.

Although horizontal gene transfer is obviously not a general phenomenon, there is now reliable evidence that horizontal transfer really takes place for both genomic (usually nonmobile) sequences and sequences derived from transposable genetic elements or mobile introns (Traavik, 2004b): Field studies showed that GM genes may have transferred from GM pollen to bacteria and yeast in the gut of baby bees (Ho, 2004). Transgenic DNA in food (antibiotic

resistance marker genes) was found to be taken up by bacteria in human gut (ISIS, 2002) or pathogenic bacteria, making infections difficult to treat (ISIS, 2001). There is also experimental evidence that transgenic DNA from plants was taken up by bacteria in soil (ISIS report, 2001).

Genetic recombination between genetically engineered vaccine viruses and naturally occurring relatives is a possibility. It was demonstrated that minor genetic changes in, or differences between, viruses result in dramatic changes in transmission abilities, host preferences, and virulence. The new, hybrid virus progenies resulting from such events may have completely unpredictable characteristics (Traavik, 2004a; Traavik & Smith, 2004).

An example of virulence reversion was documented with the use of recombinant vaccinia–rabies glycoprotein virus vaccine prepared for wild raccoons and foxes. A 28-yr-old pregnant woman was infected with this live, recombinant rabies–vaccinia virus when it contacted her open wound (PHAC, 2001).

The recent outbreak of avian influenza or "bird flu" in Asia serves as another example. Whether the occurrence of the highly pathogenic H5N1 2004 strain is a product of natural evolution or horizontal gene transfer brought about by GM organisms is difficult to trace. The fact that there is now an avian flu virus that can cross species barriers and seems to be more efficiently transmissible to humans represents another alarm in the potential consequences following horizontal gene transfer (PAN, 2004). Although there is no concrete evidence of human-to-human transmission of avian flu, the fear that the latest H5N1 virus strain was to merge with a human flu virus, resulting in a highly contagious flu strain, remains a serious concern. It is worthwhile to mention that a GM H5N1 2004 strain is being developed and currently under investigation for vaccination use (NIBSC, 2005).

Viruses undergo degradation. When the viral particles of these GM vaccines break down in the environment, their nucleic acids are released. These free nucleic acid, depending on their length and surrounding environment, survive for a long time, and act as pollutants in the environment (Traavik, 2004b). Although the chances for viruses to be released, taken up, and exert biological effects are not known, studies showed uptake of free nucleic acid by the skin-associated lymphoid tissues (Raz et al., 1994).

It has been suggested that GM materials and free DNA interact with environmental pollutants (Miller & Bach, 1968; Environmental Health, 1999): Environmental pollutants like dioxins and heavy metals affect cell membrane and/or intracellular functions (Environmental Health, 1999; Life Extension Foundation, 2005) and thus will have potential to influence the ability of cells to take up and horizontally transfer free DNA (Neumann & Kakorin, 2002). In addition, some "mutagenic" xenobiotics, such as radioactive substances that are accidentally release into environment, industrial chemicals (like carbotetrachloride) and plant protectants (like Camphechlor), induce sequence changing of free DNA (Weizmann Institute of Science, 2005; IPCS, 1990) and thus will have the potential to affect uptake, transfer, and long-term establishment in ecosystems (Traavik, 2004b).

CONCLUSIONS

The horizontal transfer of gene to other species is the prime ecological impact with application for GM vaccines. It is not known how often gene transfer takes place, because of the lack of pertinent knowledge. The scenario has changed recently, with the arrival of modern sequencing methods and polymerase chain reaction (PCR). Gene sequences from many different organisms have been identified, and it may be possible to predict the determinates of host specificities and pathogenic potentials of a virus. Together with better understanding of the mechanisms underlying horizontal gene transfer and ecosystem interconnections, more reliable estimation on the frequency of horizontal transfer of genes may be established (Kidwell, 1993; Traavik, 2004b) and consequently allow us to decide whether the described environmental effect produced by GM viruses is significant.

While more information is being sought, increasing numbers of vaccines are being developed using genetically modified viruses and genetically engineered virus vectors. As mentioned earlier, the lack of knowledge has made scientifically based risk assessments on the environmental impact by these GM viruses impossible. Based on guidelines drawn by international biosafety committees, whenever there are GM viruses involved in the developmental process, "precautionary measures" should be performed. Viruses that are relatively stable and nonpathogenic need to be used, and for those cases where pathogenic viruses are necessarily employed in the manufacturing of vaccines, the manufacturer must provide appropriate methods of detection and containment (either by "biological containment" or by "physical containment") or complete removal of those organisms from the final product (Dorsch-Hasler & Spycher, 2003; Gene Technology Technical Advisory Committee, 2004; OECD, 2004). In addition, it was suggested that continual long-term postmarketing monitoring on the use of GM vaccine needs to be performed to provide valuable epidemiology information on the significance of ecological impact of these vaccines. In conclusion, the main purpose of this article is to raise awareness of the potential adverse effects in the use of GM viruses and GE virus-vector vaccines in environment. It is hoped that with appropriate risk management and prudent precautionary measures during the developmental process of vaccines, risks and hazards can be kept to a manageable level before we gain sufficient knowledge to determine the significance of the unintended and unwanted side effects of these GM viruses or virus vectors in the environment.

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