Advancing the Understanding of Biosafety

Latest scientific findings,

policy responses and public participation

Lecture

Genetically Engineered Viruses – Environmental Challenges

Terje Traavik

Session

Ecological Risk Research

From Organisms to Landscapes

Nagoya

7. - 9. October 2010

Prof. Terje Traavik
GenØk - Center for Biosafety, Norway

Introduction

Genetically engineered or modified viruses (GMVs), from a number of taxons, are being increasingly used as live vaccine vectors. There are four broad genetically engineered virus application areas that may have environmental implications: i) Immunization against infectious disease in livestock species; ii) Immunization of wildlife species which are reservoirs of infectious agents causing disease in humans and livestock species; iii) Control of pest animal population densities by either direct lethal control operations or immuno-contraception; and iv) Human vaccination programs with GMVs that are able to jump species barriers directly, or following recombination with naturally occurring viruses.

All these applications may, to varying extents, represent release of GMVs. The different application areas call for different considerations and options with regard to choice of virus vectors and genetic engineering strategies. Generally speaking, there are two strategies: The first is represented by gene-deleted viruses to be used for homologous vaccination, i.e. to achieve protective immunity against the GMV itself. The induced deletions most commonly concern genes that are necessary for the virus to carry out a full multiplication cycle, or are implied in viral virulence. Furthermore, non-essential genes may be deleted in order to obtain markers for monitoring unintended vaccine virus spread.

Recombinant virus vectors obtained by transgenesis represent the second strategy. Such viruses are created in cell cultures by simultaneous transfection with a plasmid carrying a gene from the virus/microbe that is to be targeted, and infection with the virus vector of choice. The plasmid construct is such that the transgene contains DNA sequences homologous to a viral gene at each end. Hence the transgene is transferred and integrated to a predetermined site in the virus vector genome by homologous recombination. The most commonly used vector viruses are members of the DNA virus families Poxviridae and Adenoviridae.

Cross-species transfer of viruses

The opportunities for cross-species transfer of mammalian viruses have increased in recent years due to increased contact between humans and animal reservoirs. It is, however, difficult to predict when such events will take place since the viral adaptations that are needed are multifactorial and stochastic. Recent examples of viruses that have crossed species barriers are HIV, hantaviruses, haemorrhagic fever viruses, arboviruses, avian influenza virus, SARS-associated coronavirus, Nipah and Hendra viruses, and monkeypox virus. The emergence of HIV exemplifies how multiple independent cross-species transmissions of simian viruses that are not associated with disease in their natural hosts eventually resulted in the establishment of two types of HIV in the human population. While adapting to its new host the virus underwent a myriad of molecular changes. Changes in social behaviour of humans may well have offered opportunities for newly evolved HIV strains to become pandemic. Crossing the species barrier from one animal species to another is most readily noticed when it is associated with overt pathology. In the past, such events may have been overlooked as the underlying cause of the emergence of a new disease. When a virus transmitted from non-human host reservoirs to humans causes such a disease, it is called a zoonosis.

The emergence of new viral infections often follows environmental, ecological and technological changes caused by human activities (Louz et al., 2005). Such activities may lead to an

increased contact between humans and livestock on one hand, and animal hosts acting as reservoirs of zoonotic viruses on the other hand. Agricultural development, an increased exploitation of environmental resources, growth and increase in the mobility of the human population and trade and transportation of food and livestock, have been identified as important factors contributing to the introduction and spread of a number of new viruses in the human population. Against this background, the intensified use of viruses and their genetically modified variants as viral gene transfer vectors for biomedical research, experimental gene therapy and as live-vector vaccines is a cause for concern (reviewed by Louz et al., 2005).

Relevant risk assessment questions for GMVs

The different virus families have their specific life cycles and host-preferences. Hence it is impossible to make risk assessment schemes that are valid for all potential virus vectors. Risk assessment must be performed on a case-by-case, step-by-step basis, taking into account the characteristics of the ecosystem into which the virus will be released, and the ability of the virus to engage in transboundary movements (Traavik, 1999; McFadden, 2005). The most evident risk issues related to the release of GMVs or unmodified viruses are the known and unknown unknowns related to (i) infection of non-target species, (ii) recombinations with naturally occurring relatives and (iii) integration of GMV DNA into host cell chromosomes. Ideally, before any GMV, or unmodified virus intended for release, becomes implanted into a new location/ecosystem, a number of crucial questions should be answered, for example:

- Can the released virus engage in genetic recombination, or by other means achieve new genetic material? If so, will the hybrid offspring have changed their host preferences and virulence characteristics?
- Can the released virus or any hybrid or mutated offspring infect unintended species?
- Can the released virus or any hybrid or mutated offspring integrate into the genomes of host cells?
- Can other viruses that are present within the ecosystem influence the infection with the released virus or its offspring?
- Can insects or migrating birds or animals function as vectors for the released virus or its offspring, to disseminate viruses out of their intended release areas?
- For how long can the virus and its offspring survive outside host organisms under realistic environmental and climatic conditions?
- Are the virus and its offspring genetically stable over time?
- Can the virus or its offspring establish long-lasting, clinically mute, persistent or latent infections in naturally accessible host organisms?
- Can the virus or its offspring activate or aggravate naturally occurring latent or persistent virus infections?

Some of these questions deal with the biological and phenotypical characteristics of a supposed genetically stable GMV. But the situation becomes even more complex and unpredictable if the GMV parental strain under certain conditions or circumstances is genetically unstable, giving rise to viral strains with altered characteristics (Traavik, 1999).

Gaps in information necessary to perform environmental risk assessments (ERAs) for released or escaped GMVs

So far no GMVs have been thoroughly risk assessed from an environmental point of view. Risk assessments have focused on unintended effects of the vaccine arising in the vaccinated individuals, or in individuals of the same species that are infected by viruses shed from vaccinated individuals. The main areas of information gaps related to GMVs are:

- Lack of knowledge about naturally occurring relatives in the actual ecosystem. Such information is necessary to assess the possibilities of new viruses through recombination.
- Lack of knowledge concerning recombination events and their consequences.
- Lack of knowledge concerning non-target effects and transboundary spread of the GMV or its offspring.
- Lack of knowledge concerning integration of GMV DNA, or fragments of it, into host cell chromosomes.
- Lack of knowledge concerning the genetic stability of the GMV and its offspring. If the transgene is deleted over time, monitoring of GMV spread and changed phenotypic traits may become difficult/impossible.

Current research on risk assessment of GMVs

There is little information available that relates to ERA of virus releases. To our knowledge research related to environmental effects is only being performed for alphaherpesviruses (Thiry et al., 2006) and poxviruses (orthopox and avipoxviruses). Such environmental biosafety-related research has been performed for a number of years in Norway, but we have no present knowledge of other research groups with a similar focus. We have focused on biosafety issues of the orthopoxvirus strain MVA (Modified Vaccinia Ankara), considered to be a very safe vaccine vector because of high gene expression capacity and lack of viral replication in mammalian cells (Drexler et al., 2004).

The most relevant conclusions from our studies may be summed up as follows:

Orthopoxviruses, and hence potential recombination partners for orthopoxvirus vectored vaccines, are common in different small rodent species populations all over the country, and small rodent predator species have antibodies to such viruses (Sandvik et al., 1998; Tryland et al., 1998).

Recombination between an influenza-transgenic MVA and a naturally occurring orthopoxvirus is readily demonstrated in cell cultures. The recombinants may have phenotypic characteristics different from the parental viruses. Recombinants may be genetically unstable and “throw out” the influenza transgene. This will eliminate the most logical tag for vaccine monitoring (Hansen et al., 2004).

The absolute and relative permissivities for MVA multiplication and viral shedding have not been thoroughly studied. GM and unmodified MVA may, contrary to the general dogma, perform fully productive infections in highly relevant mammalian cell types, and other mammalian cell cultures are semi-permissive to infection (Okeke et al., 2006).

DNA sequencing revealed that orthopoxviruses can be clearly separated into geographically distinct strains, and it was inferred that these strains have distinct evolutionary histories in different rodent lineages (Hansen et al., 2009). Upon sequencing of an orthopoxvirus isolated from a human clinical case, it was established that this strain was a naturally occurring hybrid

between two distinct orthopoxvirus species. This is the first proof of concept for orthopoxvirus recombinations taking place under authentic ecological circumstances (Hansen et al, 2010).

Homologous recombination between orthopoxvirus-vectored vaccines and naturally circulating orthopoxviruses, genetic instability of the transgene, accumulation of non-transgene expressing vectors or hybrid virus progeny, as well as cell line/type specific selection against the transgene are potential complications that may result if poxvirus vectored vaccines are extensively used in animals and humans (Okeke et al 2009a). Phenotypic characteristics of recombinants between genetically modified and naturally occurring orthopoxviruses may be unpredictably different from any of the parental viruses (Okeke et al 2009b). Contrary to common assumptions, some avipoxviruses may carry out productive infections in mammalian cells, and avipoxviruses within a restricted geographical area may be more genetically diverse than realized so far (Weli et al., 2004 and 2005).

The implications of these studies for ERA (Environmental Risk Assessment) of transgenic viruses, and the lack of GMV biosafety relevant research will be further discussed.

References

Drexler, I., Staib, C. & Sutter G. 2004. Modified vaccinia virus Ankara as antigen delivery system: how can we best use its potential? Curr Opin Biotech 15: 506-512. http://tinyurl.com/399v2wx

Hansen, H., Okeke, M. I., Nilssen, Ø. & Traavik, T.. 2004. Recombinant viruses obtained from co-infection in vitro with a live vaccinia-vectored influenza vaccine and a naturally occurring cowpox virus display different plaque phenotypes and loss of the transgene. Vaccine 23: 499-506.

Hansen, H., Okeke, M.I., Nilssen, Ø. & Traavik, T. 2009. Comparison and phylogenetic analyses of orthopoxviruses isolated from cats and humans in Fennoscandia. Archives of Virology 154: 1293-1302

Hansen, H., Okeke, M.I. & Traavik, T. 2010. A cowpox virus with an ectromelia virus A-type inclusion protein. Manuscript in preparation

Louz, D., Bergmans, H.E., Loos, B.P. & Hoeben R.C. 2005. Cross-species transfer of viruses: implications for the use of viral vectors in biomedical research, gene therapy and as live-virus vaccines. The Journal of Gene Medicine 7: 1263-1274. http://onlinelibrary.wiley.com/doi/10.1002/jgm.794/abstract

McFadden, G. 2005. Poxvirus tropism. Nature Reviews Microbiology 3: 201-2013. http://www.nature.com/nrmicro/journal/v3/n3/execsumm/nrmicro1099.html

Okeke, M.I., Nilssen, Ø & Traavik, T. 2006. Modified Vaccinia virus Ankara multiplies in rat IEC-6 cells and limited production of mature virus occurs in other mammalian cell lines. J Gen Virol 87: 21-27. http://vir.sgmjournals.org/cgi/content/abstract/87/1/21

Okeke, M.I., Olayiwola, A.A., Moens, U., Tryland, M., Traavik, T. & Nilssen Ø. 2009. Comparative sequence analysis of A-type inclusion (ATI) and P4c proteins of orthopoxviruses that produce typical and atypical ATI phenotypes. Virus Genes 39: 200-209. http://www.springerlink.com/content/r2v006710mq20617/

Okeke, M.I., Nilssen, Ø., Moens, U., Tryland, M. & Traavik, T. 2009. In vitro host range, multiplication and virion forms of recombinant viruses obtained from co-infection in vitro with a vaccinia-vectored influenza vaccine and a naturally occurring cowpox virus. Virology J 6: 55. http://www.virologyj.com/content/6/1/55/abstract

Sandvik, T., Tryland, M., Hansen, H., Mehl, R., Moens, U., Olsvik, O. & Traavik, T. 1998. Naturally occurring orthopoxviruses: Potential for recombination with vaccine vectors. J Clin Microbiol. 36(9): 2542-2547. http://jcm.asm.org/cgi/content/full/36/9/2542

Thiry, E., Muylkens, B., Meurens, F., Gogev, S., Thiry, J., Vanderplasschen, A. & Schynts, F. 2006. Recombination in the alphaherpesvirus bovine herpesvirus 1. Veterinary Microbiology 113: 171-177. http://tinyurl.com/2vhxync

Traavik, T. 1999. An orphan in science: Environmental risks of genetically engineered vaccines. Research Report for DN 199-6 (92 pages). Directorate for Nature Management, Trondheim, Norway. (ISBN 82-7072-351-7). http://www.biosafety-info.net/article.php?aid=515

Tryland, M., Sandvik, T., Arnemo, J. M., Stuve, G., Olsvik, O. & Traavik, T.. 1998. Antibodies against orthopoxviruses in wild carnivores from Fennoscandia. J Wildl Dis. 34(3): 443-450. http://www.jwildlifedis.org/cgi/reprint/34/3/443.pdf

Weli, S.C., Nilssen, Ø . & Traavik, T. 2005. Avipoxvirus multiplication in a mammalian cell line. Virus Res 109: 39-49. http://tinyurl.com/39b373z

Weli, S.C., Okeke, M.I., Tryland, M., Nilssen, Ø & Traavik, T. 2004. Characterization of avipoxviruses from wild birds in Norway. Can J Vet Res 68: 140-145