Recombinant and chimeric viruses:

Evaluation of risks associated with changes in tropism

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RECOMBINANT AND CHIMERIC VIRUSES: EVALUATION OF RISKS ASSOCIATED WITH CHANGES IN TROPISM

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EXECUTIVE SUMMARY

With molecular biological techniques the genetic make-up of virtually any (micro-)organism can be modified. This process is know as 'genetic modification'. The availability of cloned copies of viral genomes facilitates the generation of genetically-modified recombinant and chimeric viruses. A chimeric virus is a recombinant virus that contains at least one functional gene from another virus. Such viruses become increasingly important for vaccine development, experimental gene therapy and for scientific research purposes.

Virus particles bind and enter living cells via specific receptors at the cell surface. These and other host factors determine whether the host is permissive for this virus. Rare spontaneous mutations in the viral genome can alter the cell-, tissue-, and host-specificity of a virus. Hardly ever this leads to an extension of the host range of a virus. However, in rare cases this may lead to new viral diseases. HIV, influenza virus, and SARS-associated coronavirus are notorious examples of viruses that in recent history extended their host specificity from animals to humans.

The generation of recombinant and chimeric viruses by means of targeted genetic modification, may – either intentionally or unintentionally – result in the generation of viruses with a modified host specificity. Due to the infectious nature of viruses, and the fact that they can cause disease, applications involving such viruses are not completely without risk. A desk study was performed to gain a better understanding of the risks associated with changes in the tropism of recombinant or chimeric viruses. Here a state-of-the-art review is provided that covers the recent developments and uses of such viruses. It describes the viral and cellular factors that can affect the tropism of viruses, and identifies the potential risk factors associated with the use of recombinant and chimeric viruses.

Four classes of risk factors are distinguished: *i.e.*, (1) those related to biological and physical properties of the vector virus, (2) those related to changes in virus - receptor interactions, (3) those related to changes in the virus that affect the host's antiviral responses, and (4) those related to the health status of the host. Although it cannot always be precisely predicted whether a change in tropism will result in (increased) pathogenicity, criteria are identified that allow for educated estimates of such events and their associated risks.

It is concluded that our knowledge of the relationship between tropism and pathogenicity of viruses is still incomplete. Therefore developments in this area must be closely monitored and, where pertinent, dedicated research should be stimulated. This will allow scientifically supported assessment of the risks and risk factors associated with the use of recombinant or chimeric viruses for research and biomedical applications.

INTRODUCTION

Genetic engineering can be used for the production of recombinant and chimeric viruses of which the genome consists of a combination of the genetic information from two different viruses. By combining the sequences from two different viruses, the biological properties of recombinant and chimeric viruses may differ from those of the original parent strains. These changes in biological properties may be intentional - for instance to achieve changes in tropism - or unintentional. Due to our limited knowledge of the intricate interactions between viruses and their hosts, it is not always possible to faithfully predict all consequences of the new genetic constellation of recombinant and chimeric viruses. Therefore, applications involving such viruses are not completely without risk, especially when it concerns viruses which are infectious to humans. In this report different applications of recombinant and chimeric viruses are reviewed in the context of potential risks associated with the use of viruses of which the tropism has been modified either intentionally or unintentionally.
Viruses of which the tropism has been intentionally modified have mainly been developed for gene therapy and cancer gene therapy applications. Many viral gene therapy vectors have a cell- and tissue specificity that is often insufficient for efficacious delivery to specific target cells or tissues. Therefore, retargeting of such vectors has been explored by using pseudotyping of virus particles or modification of viral attachment proteins by the insertion of specific targeting ligands. Recombinant and chimeric viruses have also been developed for vaccination purposes. Since viral attachment proteins belong to the most immunogenic viral proteins, they are often the proteins of choice for inclusion in recombinant and chimeric live vaccines. However, since these proteins are also involved in cell recognition, attachment and entry, incorporation of such proteins may also lead to unintentional changes in viral tropism. Here, we give an account of recent developments in virus retargeting for gene therapy as well as of the development of recombinant chimeric viruses containing heterologous viral surface proteins for vaccination purposes and fundamental studies. Potential risks associated with changes in tropism are discussed in view of our current knowledge of viral and host factors that determine virus tropism and host range.

This report is organized into seven sections. After a short introduction on the genetic modification of viruses and the definition of recombinant and chimeric viruses, a brief overview is given of the different applications for which recombinant and chimeric viruses are used. These sections are followed by detailed descriptions of recent developments in virus retargeting, with an emphasis on new techniques aimed at redirecting viruses and viral vectors to specific cell-types. Section 5 deals with chimeric viruses that express foreign viral surface proteins. This part is focussed on chimeric viruses in which the foreign protein is actually incorporated and displayed at the surface of the virus particle, thereby potentially altering the tropism of such chimeric virions. Section 6 summarizes our current knowledge of the viral factors and host factors that determine host-range and tropism. Without being exhaustive, in this section relevant examples are given to illustrate how such factors may affect virus tropism and host range. Finally, potential risks associated with the use of recombinant and chimeric viruses are discussed in view of our current knowledge of viral and host factors that determine virus tropism and host range.

1. GENETIC MODIFICATION OF VIRUSES

Developments in molecular biology over the past few decades have resulted in techniques that allow the modification of genetic material, a process know as 'genetic manipulation', 'genetic engineering' or 'genetic modification'. Due to their relatively small sizes and simple genetic organization, viruses have been used extensively for genetic modification, thereby serving as model systems to unravel the complex molecular mechanisms of all kinds of biological processes. Furthermore, genetic modification of viruses has been used for the production of recombinant viruses with new properties that may be used as tools for fundamental scientific studies or practical applications such as vaccination and gene therapy.

Recombinant DNA technology makes it possible to specifically modify DNA. Therefore, the first viruses that were successfully modified consisted of viruses which contain a DNA genome. The first recombinant virus, simian virus 40 (SV40), was constructed in the late 1970th (Goff & Berg, 1976) and since then, a variety of DNA viruses and retroviruses have been genetically modified. When transfected into cells, the *in vitro* modified genome of many DNA viruses is infectious and gives rise to new recombinant viruses. Alternatively, recombinant DNA can be introduced into a viral genome by means of homologous recombination between transfected DNA (usually in the form of a 'transfer plasmid') and the genome of a helper virus. The latter process is commonly used for the modification of large DNA viruses such as herpesviruses or poxviruses.

Since genetic modification of RNA is hardly possible, the modification of RNA viruses has somewhat lagged behind that of DNA viruses. However, by conversion of the RNA genome into complementary DNA (cDNA), an intermediate can be generated that is amenable to genetic modification and which can subsequently be converted back – either *in vitro* or *in vivo* - into an RNA genome which is able to yield infectious virus. This process is known as 'reverse genetics'. Two major RNA virus groups - the positive-stranded and negative stranded RNA viruses - can be distinguished based on whether their RNA genomes can be directly used for translation by the host cell machinery or whether they first have to be converted into complementary RNA. In other words, positive-stranded viral RNA genomes can initiate an infectious cycle whereas negative-stranded RNA genomes cannot. For the latter group, the infectious unit is not an RNA molecule but a ribonucleoprotein complex (RNP) consisting of the RNA genome and associated replication proteins. After reconstitution of such an RNP complex the negative-stranded genome is converted into a positive-stranded anti-genome and subgenomic mRNA's, leading to an infectious cycle. Nowadays, genetic modification is possible for members of almost all virus groups containing either a DNA or RNA genome, a positive-stranded or negative-stranded RNA genome, or a non-segmented or segmented genome (Almazan *et al.*, 2000, Boyer & Haenni, 1994, Burton *et al.*, 2002, Conzelmann, 1998, Conzelmann & Meyers, 1996, Efstathiou & Minson, 1995, Enjuanes *et al.*, 2005, Evans, 1999, Fodor *et al.*, 1999, Kuo *et al.*, 2000, Lundstrom, 2000, Lundstrom, 2003b, Masters, 1999, Moss, 1996, Neumann & Kawaoka, 2001, Neumann & Kawaoka, 2004, Palese *et al.*, 1996, Rice *et al.*, 1989, Sola *et al.*, 2003, Yun *et al.*, 2003).

2. RECOMBINANT AND CHIMERIC VIRUSES

The ability to manipulate viral genomes has resulted in the generation of recombinant viruses and chimeric viruses. The term ‘recombinant virus’ is generally used for a genetically modified virus (‘vector’) that carries nucleotide sequences from a viral or non-viral species. An example of a recombinant virus would be a virus that carries and expresses a foreign therapeutic gene or reporter gene. The term ‘chimeric virus’ is generally used for a recombinant virus that consists of a combination of the genomes of two viruses and which may display biological properties characteristic for both parent viruses. An example of a chimeric virus would be a virus that contains the non-structural genes (*i.e.*, genes encoding proteins which are not incorporated in virus particles) from one virus and one or more structural genes (*i.e.*, genes encoding proteins which are incorporated in virus particles) from another virus. However, the distinction between a recombinant virus and a chimeric virus is not always clearly defined. For instance, a recombinant virus may express a hybrid protein encoded by a gene consisting of a fusion between a gene from the vector virus and a gene from a donor virus. Such a virus might be best referred to as a recombinant virus expressing (or carrying) a hybrid or chimeric protein. Other viruses may express a viral gene product that is modified to display only a short immunogenic epitope from a heterologous virus, *e.g.*, for vaccination purposes. In order to clearly distinguish between a chimeric virus and a recombinant virus, we will define a chimeric virus as a recombinant virus (acceptor or vector) that contains the genetic information for at least one functional gene from another virus (donor). The gene(s) from the donor virus can be either an addition or a replacement. The donor virus may be a different species but may also be closely related, *e.g.*, a different serotype but belonging to the same species as the acceptor virus. This definition would include reassortants of viruses with segmented genomes, such as influenza virus. However, since a new genetic constellation is inherent to the term ‘reassortant’, we will also use this term when referring to influenza viruses of which the genome consists only of influenza virus gene segments of one type. If at least one gene segment is derived from, *e.g.*, influenza B, and all other gene segments from influenza A, we will refer to this virus as a chimeric virus.

3. APPLICATIONS OF RECOMBINANT AND CHIMERIC VIRUSES

The technological developments that enabled the manipulation of viral genomes have led to a large number of applications in which viruses are used for the incorporation of heterologous nucleotide sequences or for the generation of chimeric viruses. Incorporation of foreign sequences may result in changes in the biological behaviour of the viral vector. These changes may include the acquisition of new pathogenic properties. Therefore, precautions should be taken when handling recombinant and chimeric viruses in the laboratory or when using them in association with animals or humans. In the following paragraphs, an overview is given of the main activities for which recombinant viruses and chimeric viruses are currently being used. Since this report deals with potential risks as a result of changes in tropism, the emphasis will be on those applications and developments which may lead to an increase in pathogenicity or the acquisition of new pathogenic properties by acquiring a different tissue or host tropism. The expression of immune modulating proteins by recombinant viruses will also be briefly discussed since this may also affect viral tropism.

3.1. Fundamental studies of host-pathogen interactions

In order to understand the complex interactions of viruses with their host, recombinant viruses have been used as tools to unravel these interactions, mainly at the level of virus-cell interaction. Viruses can be divided into two main categories; enveloped viruses, which have a lipid membrane (envelope) that is derived from the host cell; and non-enveloped viruses, which lack a membrane. Both non-enveloped and enveloped viruses share the same main steps and routes of virus entry which begin with attachment to cell-surface receptors and end with the delivery of the viral genome to the cell cytoplasm. After binding to receptors, viruses use two main routes to enter the cell, *i.e.*, the endocytic and non-endocytic routes. The endocytic route is used by most - if not all - non-enveloped viruses and usually involves transport by clathrin-coated vesicles or caveolae. Enveloped viruses can either use the non-endocytic route by directly crossing the plasma membrane by fusion of the viral envelope with the cell membrane at neutral pH, or by the endocytic pathway which involves uptake of virus particle in endosomes followed by acidification of the endosome which leads to fusion of the viral envelope and the endosomal membrane as a results of conformational changes in the viral fusion proteins (Dimitrov, 2004, Sieczkarski & Whittaker, 2002). Viruses from many different virus families are associated with diseases in humans. Therefore, it is important to understand how different viruses are able to enter cells and how this process can be inhibited. Although the use of recombinant and chimeric viruses has added significantly to our understanding of the interactions between viruses and receptors and the subsequent process of entry into cells, only few successful antiviral drugs that target viral entry have been developed. Major problems are the occurrence of virus mutants that are resistant to such drugs, low potency *in vivo* and toxic side effects.

3.2. Gene therapy

Many viruses have evolved to become highly efficient at nucleic acid delivery (‘transduction’) to specific cell-types while simultaneously avoiding immune surveillance by an infected host. These

properties make viruses attractive gene delivery vehicles for gene therapy. The definition of viral gene therapy has become quite broad. It no longer refers strictly to the treatment of an existing genetic disorder by replacement of a defective gene with a functional copy by gene transfer (Mulligan, 1993). The delivery of recombinant viruses to tumors, and prophylactic or therapeutic cancer vaccination by immunization with recombinant viruses are also examples of gene therapy (Lundstrom, 2003b, Robbins & Ghivizzani, 1998). However, we will deal with them separately because potential risks associated with the use of recombinant and chimeric viruses may differ significantly between classical gene therapy studies, in which replication incompetent vectors are generally used, and cancer gene therapy studies, in which replication competent vectors are typically used.

Several types of viruses, including adenovirus, adeno-associated virus (AAV), retrovirus, lentivirus, herpes virus, pox virus and several RNA viruses have been modified for use in gene therapy applications. Because these vector systems have unique advantages and limitations, each has applications for which it is best suited. Retroviral and lentiviral vectors can permanently integrate into the genome of the infected cell. Adenoviral vectors can efficiently deliver genes to a wide variety of dividing and non-dividing cell-types, but immune elimination of infected cells often limits gene expression *in vivo*. Herpes simplex virus can deliver large amounts of exogenous DNA; however, cytotoxicity and maintenance of transgene expression remain as obstacles. AAV also infects many nondividing and dividing cell-types, but has a limited DNA capacity. Alternatively, hybrid viral vector systems that combine advantageous properties of two or more viral systems are also being explored. In general, viral vectors for gene therapy are unable to produce infectious progeny virions after application to target cells or tissues. Replication incompetent vectors can consist of gene-deleted or 'empty' vectors (*e.g.*, adenovirus, AAV and retrovirus/lentivirus), or of highly attenuated strains which contain mutations that do not allow productive replication in the target cells and tissues (*e.g.*, poxvirus, herpesvirus). Whatever their specific advantages and disadvantages may be, no virus is optimally suited for the delivery of genes to each cell-type. Therefore, attempts have been made to change the cell-, tissue- or even host specificity of gene therapy vectors either by broadening or limiting their tropism.

3.3. Cancer gene therapy

The goal of viral cancer gene therapy is to kill cancer cells either directly as an effect of viral replication, by introducing genes that will kill cells by sensitising them to other therapies, or by enhancing an immune response against transfected cancer cells (Bonnet *et al.*, 2000, Kirn *et al.*, 2002). Most classical gene therapy studies use replication defective viruses because of the risk of uncontrolled viral spread of replication competent viruses. However, if it is the goal to eliminate the transfected cells, as is the case in cancer, one may take advantage of the inherent cytotoxic characteristics of viral replication. Infection of cells with replication competent viruses will often lead to cell death. In addition, viral replication often results in lysis of the infected cells allowing further spread of progeny virus particles to other cells. This idea of using replication competent viruses has resulted in the use of such viruses for cancer therapy, know as 'oncolytic virotherapy' (Biederer *et al.*, 2002, Everts & van der Poel, 2005, Lin & Nemunaitis, 2004, Ries & Brandts, 2004). Currently used oncolytic

viruses can be divided in two groups, *i.e.*, (1) natural tumor selective viruses with inherent oncolytic properties such as Newcastle disease virus (NDV), Vesicular stomatitis virus (VSV), Measles virus, Reovirus and autonomous parvovirus, and (2) genetically engineered tumor selective viruses such as adenovirus, herpesvirus, poxvirus, poliovirus and influenza virus. Whereas replication of oncolytic viruses is more or less restricted to cancer cells, the biodistribution of the virus after inoculation of patients is determined by the interaction of the viral envelope or capsid proteins with cellular receptors. Since these receptors are generally not restricted to specific cell-types but are present on a wide range of cells and tissues, specific targeting of oncolytic viruses to cancer cells is highly desirable. Examples of how specific retargeting of several classes of viruses to cancer cells may be achieved are discussed below.

3.4. Vaccination

Vaccination has been one of the most successful and cost-effective means to combat infectious viral diseases. Conventional vaccines have been generated by attenuation of virulent viruses, inactivation of virulent viruses, or the purification of immunogenic molecules from whole viruses. In this way, effective vaccines which give protection against diseases, such as smallpox, polio, rabies, measles, influenza and yellow fever, have been generated. However, in many cases these protocols have failed to produce safe and efficacious vaccines against a number of other important infectious diseases. Consequently, fundamentally new technologies are required to tackle these infections. One of the most promising has been the development of genetically modified viruses. This process normally involves taking a proven safe and efficacious vaccine virus, and modifying its genome to include genes coding for immunogenic proteins from other viruses. In many cases, the most immunogenic proteins of viral pathogens are structural components of the viral particle which are recognized primarily by the humoral immune system. Thus, in order to generate efficacious recombinant vaccines, viral structural proteins are the proteins of choice for the inclusion in such vaccines. In order to display these proteins to the immune system in a similar way as the original pathogen, incorporation of the heterologous protein in recombinant viral particles is often desirable. However, since structural proteins are often involved in cell recognition and entry, incorporation of foreign structural proteins may result in changes in the biological properties, including tropism, of the vector virus.

3.5. Immune modulation

The clinical use of cytokines to modulate *in vivo* immune responses has been well established. However, in many cases the practical use has been problematic due to the high cost and systemic toxicity of purified recombinant cytokines. Furthermore, multiple injections are often required to elicit the desired effect. These problems may be overcome by incorporating genes encoding cytokines in viral vectors which can be delivered topically and which are able to express the cytokine *in vivo* for extended periods of time. In addition, since cytokines may function as potent adjuvants, incorporation of cytokine genes in live recombinant viral vaccines may greatly enhance their efficacy (Hubel *et al.*, 2002). Cytokines are involved in many immunological processes and play a key regulatory role in the differentiation and proliferation of CD4+ T-helper cells into Th1 and Th2 subsets, which is important in

stimulating either humoral or cellular immune responses. Thus, by incorporating Th1 or Th2 specific cytokine genes as adjuvants in vaccines, the immune response may be shifted in the desired direction. However, in view of the complex nature and multiple intricate interactions of the cytokine network, the outcome of the adjunct cytokine treatment on vaccination may not always be predictable and indeed can sometimes result in adverse effects such as uncontrolled virus spread and infection.

4. RECENT DEVELOPMENTS IN VIRUS RETARGETING

With respect to retargeting of recombinant or chimeric viruses, a distinction must be made between conjugate-based retargeting and classical pseudotyping on the one hand and genetic retargeting strategies on the other. Neither classical pseudotyping nor conjugate-based retargeting requires genetic modification of the vector virus. Conjugate-based retargeting is based on the use of bi-specific conjugates or adapter molecules of which one component binds specifically to a viral capsid or envelope protein whereas the other component binds to a specific cellular surface molecule. After pre-incubation of the virus and the conjugate to allow binding of the virus-binding component to the viral surface, the complex is added to cells that are specifically recognized by the other component of the conjugate. In this way the conjugate functions as a retargeting device. Bi-specific conjugates can consist of a protein component that is chemically linked to a synthetic or natural substance. However, in most cases the conjugate consists either of two peptide domains in the form of a fusion between a Fab fragment or single-chain immunoglobulin variable region (scFv) and a peptide ligand that is recognized by a cellular receptor, or a combination of two scFv's (Barnett *et al.*, 2002a, Bartlett *et al.*, 1999, Bian *et al.*, 2005, Curiel, 1999, Kanerva *et al.*, 2002, Ponnazhagan *et al.*, 2002, Volpers & Kochanek, 2004, Volpers *et al.*, 2003). Pseudotyping refers to the process of generating virions that have incorporated a capsid or envelope protein from another virus but which do not carry the genetic information encoding that heterologous protein. Pseudotyping may, for instance, occur when two different viruses infect the same cell ('phenotypic mixing'). However, specific pseudotyping is usually achieved by expressing a single envelope or capsid protein (Sena-Esteves *et al.*, 2004). As a result of the incorporation of the heterologous protein, the tropism of the resulting virions resembles that of the donor virus (Balliet & Bates, 1998, Bruett & Clements, 2001, Engelstadter *et al.*, 2001, Kafri, 2004, Kang *et al.*, 2002, Kobayashi *et al.*, 2003, Ma *et al.*, 1999, Mebatsion & Conzelmann, 1996, Schauber *et al.*, 2004, Schnierle *et al.*, 1997, Spector *et al.*, 1990, Spiegel *et al.*, 1998, Sung & Lai, 2002, Tatsuo *et al.*, 2000). Since their genome was not modified, retargeted virions will produce progeny virions that carry the original capsid or envelope proteins of the vector virus. Although it cannot be excluded that these forms of retargeting may lead to changes in pathogenicity for the individual host, they probably do not represent an enhanced environmental risk over the use of the original viral vector. Therefore, we will only deal with potential risks associated with changes in the tropism of genetically modified viruses.

Another form of virus or vector retargeting concerns the use of so-called 'transcriptional targeting'. In this case, virus replication in different cell-types is not determined by the entry proteins on the viral surface ('transductional targeting') but by transcriptional control elements in the viral genome that are only operative in certain cell-types due to their dependence on specific host cell factors (Glasgow *et al.*, 2004a, Rots *et al.*, 2003).

4.1. Adenovirus

Probably the most popular viral gene therapy vector is adenovirus (Barnett *et al.*, 2002a, Kanerva & Hemminki, 2004, Lundstrom, 2003b, Rots *et al.*, 2003, Volpers & Kochanek, 2004). Several biological properties of adenoviruses make them the vector of choice for human gene therapy because they

efficiently introduce DNA into host cells, can be produced easily to high titres, and are able to transduce a relatively wide range of cell-types (both dividing and quiescent). Moreover, they are not inactivated by complement *in vivo* and are not oncogenic in humans. Limitations of adenoviruses as gene vectors is their immunogenicity (which interferes with multiple serial applications), the suboptimal distribution of adenovirus particles *in vivo*, the lack of specificity of vectors for malignant tissues, and a poor infectivity for several therapeutically relevant cell-types.

Adenovirus is a non-enveloped, icosahedral virus of 60–90 nm in diameter with a linear, double-stranded DNA genome of 30–40 kb. The capsid is composed of 240 hexon capsomers forming the 20 triangular faces of the icosahedron, and 12 penton capsomers with spike-shaped protrusions located at the 12 vertices. The terminal globular domain or 'knob' region of the homotrimeric protruding fibers of the adenovirus capsid is responsible for the primary virus attachment to the cellular receptor, the coxsackie- and adenovirusreceptor (CAR), which is used by human group C adenoviruses such as Ad2 and Ad5, or CD46, which is used by human group B adenoviruses such as Ad3 and Ad35. Following the initial attachment, an arginine-glycine-aspartic acid (RGD) motif exposed in a protruding loop of the penton base protein interacts with a cell surface integrin molecule ($\alpha_V\beta_1$, $\alpha_V\beta_3$, or $\alpha_V\beta_5$) serving as secondary or internalisation receptor that triggers virus uptake by endocytosis. The endosomal uptake of the virus and release into the cytoplasm is accompanied by a stepwise dismantling of the capsid, leading to the transport and delivery of the viral genome to the nucleus. Several therapeutically relevant cell-types express only low levels of CAR or completely lack expression of CAR and, therefore, are almost refractory to adenovirus vector infection. These cell-types include skeletal and smooth muscle cells, endothelial cells, haematopoietic cells, differentiated airway epithelial cells, lymphocytes, fibroblasts, dendritic cells, and many tumor cells. Based on these observations, strategies to modify adenovirus tropism have focused on alterations to the viral capsid to allow CAR-independent infection. The interaction of the adenovirus fiber and CAR only serves to bring the viral particle into close contact with the cellular surface. Since other cellular receptors could fulfil the same function, changes to the fiber to disrupt CAR binding and to redirect binding to other cellular receptors would allow for CAR-independent binding. Two general approaches have been used to achieve this goal. One approach involves the use of adaptor molecules or bispecific proteins which bind to the viral capsid with one arm and to a cellular surface molecule with the other. In the second approach, the viral genome is genetically modified in such a way that it expresses a modified capsid protein which binds to a specific cellular surface molecule (Barnett *et al.*, 2002a, Curiel, 1999, Volpers & Kochanek, 2004). Genetic modification of the adenovirus capsid protein has been achieved in a number of different ways (summarized in Table 1). In one approach, the original capsid is replaced by substituting the fiber gene from the most commonly used group C serotypes Ad2 or Ad5 with genes encoding fiber proteins from other adenovirus serotypes with different tissue tropism. This process of genetic pseudotyping is also know as 'fiber swapping' (Gall *et al.*, 1996, Goossens *et al.*, 2001, Havenga *et al.*, 2001, Havenga *et al.*, 2002, Rea *et al.*, 2001, Zabner *et al.*, 1999). In all cases, substitution of the fiber protein resulted in enhanced gene transfer to the targeted tissue. Replacement of only the knob domain of the fiber can also alter tropism. This has been shown, for instance, by replacement of the Ad5 knob with the Ad3 or Ad35 knob. The resulting chimeric fiber conferred altered

tropism on the vector (Haviv *et al.*, 2002, Kanerva *et al.*, 2002, Kanerva *et al.*, 2003, Krasnykh *et al.*, 1996, Mizuguchi & Hayakawa, 2002, Rea *et al.*, 2001, Shayakhmetov *et al.*, 2000, Stevenson *et al.*, 1997, Von Seggern *et al.*, 2000). Recently, chimeric fibers have been generated by using the knob region from adenoviruses from non-human species such as canine adenovirus type 2 (Glasgow *et al.*, 2004b), and avian adenovirus type 1 or bovine adenovirus type 4 (Renaut *et al.*, 2004). Gene delivery to CAR-deficient cells was increased up to 30-fold by using the canine adenovirus knob, whereas recombinant viruses containing the bovine adenovirus knob demonstrated CAR-independent binding and increased infectivity of cell lines from ovarian origin. Conversely, insertion of a chimeric fiber containing the human Ad5 knob in bovine adenovirus type-3 resulted in expansion of the tropism of the recombinant virus from strictly bovine to non-bovine cells, including human cells (Wu & Tikoo, 2004).

Although pseudotyping and the use of chimeric fibers is a convenient way to manipulate the tropism of adenovirus, this approach suffers from the same disadvantage as a vector with a native fiber, *i.e.*, the tissue distribution of the receptor for the fiber limits the targeting potential. One approach to overcome this limitation is to incorporate cell-specific targeting ligands into the fiber. This was first reported by Michael and co-workers who showed that a peptide ligand could be genetically fused to the fiber protein without disrupting trimerization of the fiber (Michael *et al.*, 1995). Subsequently, it was shown that the incorporation of either an integrin-targeting RGD-peptide or a heparin-binding peptide consisting of a stretch of lysine residues at the C-terminus of the fiber protein resulted in efficient infection of cell lines that were refractory to infection by the unmodified parent virus (Dmitriev *et al.*, 1998, Gonzalez *et al.*, 1999a, Gonzalez *et al.*, 1999b, Staba *et al.*, 2000, Wickham *et al.*, 1997). The availability of the crystal structure of the fiber protein resulted in a refinement of the insertion of targeting ligands. The so-called HI-loop localized on the surface of the knob allowed the insertion of ligands without disrupting the intramolecular interactions (trimerization) which are essential for the stability of the fiber protein. Krasnykh and co-workers showed that the native trimeric configuration of the fiber protein was retained after the insertion of the octapeptide FLAG into the HI loop and that the FLAG peptide was able to bind to an anti-FLAG monoclonal antibody. Moreover, the vector retained the ability to bind and infect CAR-positive cells via a CAR-mediated pathway, indicating that the native tropism was not abolished (Krasnykh *et al.*, 1998). The HI-loop has subsequently been used for the insertion of other cell-type specific ligands, especially for the targeting of different types of CAR-deficient cancer cells (Biermann *et al.*, 2001, Cripe *et al.*, 2001, Fontana *et al.*, 2003, Garcia-Castro *et al.*, 2001, Kasono *et al.*, 1999, Mizuguchi *et al.*, 2001, Vanderkwaak *et al.*, 1999). Work and co-workers inserted linear targeting ligands, which were selected for selectivity for vascular smooth muscle cells by means of the phage display technology, in the HI-Loop. Of two peptides which were tested, only one resulted in a significant improvement in transduction of smooth muscle cells (Work *et al.*, 2004a). This indicates that ligands do not always retain their binding specificities when grafted into a different protein. To overcome these limitations, Fontana and co-workers expressed a functional Ad5 fiber domain on the surface of bacteriophage lambda. This phage display system was used to create a library in which 14 amino acid residues were displayed in the HI-Loop. Panning this library on CAR-negative NIH 3T3 cells resulted in the identification of three clones with increased binding to these

cells. Adenovirus recombinants incorporating these ligands in the fiber gene transduced NIH 3T3 cells 2 to 3 orders of magnitude better than the parental vector. Furthermore, these recombinant viruses transduced mouse and human primary immature dendritic cells with up to 100-fold increased efficiency (Fontana *et al.*, 2003).

Modifications other than in the HI-Loop include the incorporation of the RGD-motif in the hexon monomers (Vigne *et al.*, 1999) and the replacement of the RGD-motif in the penton base by another receptor-specific peptide (Wickham *et al.*, 1995). Zerbini and co-workers substituted 44 amino acids of the Ad2 fiber globular region by the C4 domain of gp120 of human immunodeficiency virus type 1 (HIV-1) (Zerbini *et al.*, 2002). Furthermore, it was shown that also minor capsid protein IX, a minor capsid component that is non-essential for capsid formation, can be used for the incorporation of the heparan-sulfate binding poly-lysine motif (Dmitriev *et al.*, 1998) or the RGD-motif (Vellinga *et al.*, 2004).

Table 1. Strategies for tropism modification and retargeting of adenoviruses and adenovirus vectors			
Genetic fiber pseudotyping	Human serotypes	Ad7a Ad16 Ad17 Ad35	(Gall *et al.*, 1996) (Goossens *et al.*, 2001) (Havenga *et al.*, 2001) (Zabner *et al.*, 1999) (Rea *et al.*, 2001)
	Non-human serotype	Bovine Ad4 Canine Ad2 Avian Ad1	(Renaut *et al.*, 2004) (Glasgow *et al.*, 2004b) (Renaut *et al.*, 2004)
Chimeric fiber proteins		Ad3	(Krasnykh *et al.*, 1996) (Stevenson *et al.*, 1997) (Von Seggern *et al.*, 2000) (Kanerva *et al.*, 2002) (Kanerva *et al.*, 2003) (Haviv *et al.*, 2002)
		Ad35	(Mizuguchi & Hayakawa, 2002) (Rea *et al.*, 2001) (Shayakhmetov *et al.*, 2000)
Incorporation of peptide-ligand	C-terminus	Poly-lysine	(Gonzalez *et al.*, 1999a) (Gonzalez *et al.*, 1999b) (Staba *et al.*, 2000) (Wickham *et al.*, 1997) (Cripe *et al.*, 2001)
		RGD-motif	(Dmitriev *et al.*, 1998) (Cripe *et al.*, 2001)
	HI-loop	RGD-motif	(Biermann *et al.*, 2001) (Garcia-Castro *et al.*, 2001) (Kasono *et al.*, 1999) (Mizuguchi *et al.*, 2001) (Vanderkwaak *et al.*, 1999)
		6xHis	(Biermann *et al.*, 2001)
		NGR-motif	(Mizuguchi *et al.*, 2001)
		Random	(Fontana *et al.*, 2003)
	Penton-base	LDV-motif	(Wickham *et al.*, 1995)
	Globular fiber domain	C4 gp120	(Zerbini *et al.*, 2002)
	Protein IX	Poly-lysine RGD-motif	(Dmitriev *et al.*, 1998) (Vellinga *et al.*, 2004)

4.2. Adeno-associated virus

Adeno-associated virus (AAV) is a member of the parvovirus family, a single-stranded DNA virus that requires a helper virus such as adenovirus for replication. Wild-type AAV is able to infect non-dividing human cells and to stably integrate into a specific locus on chromosome 19 (McCarty *et al.*, 2004). Heparan sulphate proteoglycan (HSPG) has been reported to be the primary attachment receptor for AAV-2 (Summerford & Samulski, 1998). In addition, human fibroblast growth factor receptor 1 and $\alpha_V\beta_5$ integrin have been proposed to act as secondary receptors (Qing *et al.*, 1999, Summerford *et al.*, 1999). No pathology appears to be associated with infection by AAV, and the site that it integrates in does not encode an important gene. AAV contains two open reading frames, *rep* and *cap*, which encode for the 4 replication proteins and 3 capsid proteins, respectively. These replication proteins and capsid proteins can be provided *in trans*, thus allowing the generation of a recombinant AAV vector that only retains the 145 nt inverted terminal repeats, which are necessary for viral replication and integration (Rolling & Samulski, 1995). In contrast to wild-type AAV, recombinant virus carrying a therapeutic gene in place of the normal viral replicase and capsid genes loses its ability to integrate specifically. Moreover, in certain cell-types, it has been reported that recombinant AAV is not integrated into the host genome, but is maintained episomally (McCarty *et al.*, 2004). Recent advances have eliminated the need for helpervirus for the production of AAV virus by supplying AAV proteins and essential adenovirus helper genes *in trans*. Using co-transfection of cells with a recombinant AAV plasmid and appropriate helper plasmids, vector batches with very high titres can be obtained (Matsushita *et al.*, 1998, Xiao *et al.*, 1998).

Recombinant AAV vectors possess a number of attractive features which make them particularly useful for gene therapy. They lack any pathogenicity, are able to transduce a wide range of host cells and allow long-term expression of transgenes (Lai *et al.*, 2002). Recombinant AAV vectors have been used to deliver transgenes into a wide variety of proliferating and quiescent cells both *in vitro* and *in vivo*, including muscle, liver, lung, the central nervous system, eye and heart. Furthermore, AAV vectors are able to transduce cells from various different species including rat, mouse, human, primate, dog and rabbit. However, the broad host range also represents a limitation because recombinant AAV mediated gene transfer should be specific for the tissue or cell-type of interest in order to avoid transduction and possible integration of the recombinant genome in unwanted cells and tissues. Thus, clinical development of AAV for gene therapy requires the ability to target specific tissue types.

One way to modify the tropism of AAV is the use of capsid proteins from other AAV serotypes in the virus production system. This process is known as 'cross-packaging' and is basically similar to the process of pseudotyping (see above). In a recent study, AAV-2 based vectors were packaged into virions by using serotype-specific capsid proteins and it was found that AAV-1 was superior for efficient transduction of liver and muscle, followed by AAV-5, AAV-3, AAV-2, and AAV-4. In the retina, however, AAV-5 and AAV-4 were most efficient followed by AAV-1(Rabinowitz *et al.*, 2002). These differences in transduction profiles are likely related to distinct mechanisms of uptake and intracellular trafficking of the various serotypes. Additionally, different serotype-specific inverted terminal repeat elements could also influence transgene expression. Thus the use of different serotypes or cross-

packaging broadens the tissue tropism of recombinant AAV vectors. Furthermore, selective use of particular serotypes might allow specific targeting of tissues. For example, it has been reported that a recombinant AAV-2 genome packaged in an AAV-5 capsid (AAV2/5) transduced both photoreceptors and retinal pigment epithelial (RPE) cells. However, an AAV-2-encapsidated vector transduced primarily photoreceptors while an AAV-1-encapsidated vector transduced mainly RPE cells (Auricchio *et al.*, 2001).

The first attempt at genetically incorporating a ligand into the AAV capsid involved linking the gene encoding a scFv against human CD34 molecules to the AAV-2 VP2 gene (Yang *et al.*, 1998). The incorporation of the scFv at the N terminus of VP2 resulted in improved infectivity of haematopoietic progenitor cells and maintained transduction of normal HeLa cells. However, the system was dependent on co-expression of the wild-type VP1, VP2 and VP3 proteins and virus titres were extremely low. Subsequently, linker insertion mutagenesis was used to establish which parts of the capsid protein could be used for the insertion of short peptides. Using this approach several regions that are on the surface of the capsid were identified and were shown to yield viable virions (Girod *et al.*, 1999, Rabinowitz *et al.*, 1999, Shi *et al.*, 2001, Wu *et al.*, 2000b). Moreover, it was shown for the first time that the tropism of AAV could be changed by the insertion of specific ligands. Insertion of the serpin receptor ligand in VP2 resulted in enhanced infectivity in cells that expressed the serpin receptor. However, the serpin-tagged viruses continued to use HSPG as the primary receptor (Wu *et al.*, 2000b). Incorporation of a 14-amino acid ligand that contains the RGD-motif that recognizes integrins resulted in efficient infection of cells that were resistant to infection with wild-type AAV-2 (Girod *et al.*, 1999). Using deletions and insertions in putative loop regions of the capsid protein, Grifman et al showed that amino acid sequences around position 587/588 (Loop IV) in VP3 were involved in HSPG binding. Whereas a deletion of 6 amino acids (GNRQAA) from Loop IV abolished binding to heparin, replacement of this sequence with NGRAHA, a peptide that was selected by means of phage display and which is specific for CD13, resulted in restoration of heparin binding. Moreover, viruses containing the NGRAHA sequence were able to transduce CD13 expressing cells 10 to 20-fold better that wild-type AAV (Grifman *et al.*, 2001). In order to target vascular epithelial cells, Nicklin et al used a 7-mer peptide SIGYPLP that was selected by phage display for its specific binding to endothelial cells. Incorporation of the peptide in the AAV capsid at position 587 resulted in enhanced transduction of human epithelial cells as compared to wild-type AAV. Furthermore, these authors showed that the modified virus was unable to bind to heparin indicating that transduction of epithelial cells was independent of the presence of HSPGs (Nicklin *et al.*, 2001). This was an important finding since definitive retargeting of AAV to individual cell-types should be HSPG independent. HSPG-independent binding was also shown after the incorporation of an integrin-binding RGD-peptide. By using the RGD-integrin interaction as an alternative infection pathway, the ability of the virus to transduce several cell-types which are normally poorly infected by wild-type AAV could be dramatically improved (Shi & Bartlett, 2003). Recent developments include the incorporation of targeting peptides that have been selected either by phage display technology (White *et al.*, 2004, Work *et al.*, 2004a, Work *et al.*, 2002) or by using random peptide libraries displayed on AAV capsids to select for targeted vectors on specific cell-types (Muller *et al.*, 2003, Perabo *et al.*, 2003). Using the

latter technique, infectious AAV mutants were obtained which transduced target cells with an up to 100-fold increased efficiency in a receptor-specific manner and without interacting with the primary receptor for wild-type AAV (Perabo *et al.*, 2003). Given the complexity of the virus-cell interaction and the incomplete understanding of the effects of incorporating peptide sequences on the capsid structure, it seems more advantageous to screen a large library of random peptide-modified AAV than to generate a limited number of virus variants by the incorporation of peptides identified by the phage display technology.

Many receptor-ligand interactions involve non-linear, conformation-dependent binding domains. However, incorporation of targeting ligands into the capsid protein of AAV is limited to relatively small peptides (< 30 residues) consisting of linear receptor binding epitopes. This limitation may be due to the overlap of the VP1, VP2 and VP3 proteins in the *cap* ORF of AAV which makes it impossible to modify only one capsid protein across its entire sequence without also modifying the other ones. Only region, (residues 1-137 of VP1) allows for modification of only one capsid protein, but this region has been shown to contain a phopholipase A motif that is essential for viral infection. Nevertheless, it has been shown that a scFv against CD34 can be incorporated into recombinant capsids if it is fused to the N-terminus of VP2 and co-expressed with wild-type VP1, VP2 and VP3 (Yang *et al.*, 1998). These observations were used by Warrington and co-workers to device a system for producing AAV vectors in which expression of a modified capsid protein is combined with separate expression of the remaining two unmodified capsid proteins. The results showed that recombinant particles were formed as long as the VP3 capsid was present whereas the presence VP2 proved to be non-essential. Using this system, recombinant AAV particles containing large ligands (up to 30-kDa) inserted after residue 138 in VP1 and/or VP2 were generated (Warrington *et al.*, 2004). This system promises great flexibility for manipulating the composition of the AAV particle and may allow vector retargeting to receptors that require conformation-dependent peptide ligands. However, actual retargeting using this system has not yet been reported.

An alternative retargeting strategy for AAV was described which is based on the use of capsids incorporating the immunoglobulin-binding domain derived from protein A. By binding to (monoclonal) antibodies with defined specificities for cell surface molecules, the virus-antibody complexes can be targeted to specific cell-types. When coupled to antibodies against CD29, CD117 and CXCR4 such recombinant AAV vectors specifically transduced human haematopoietic cell lines, whereas no transduction was seen in the absence of antibodies (Ried *et al.*, 2002).

4.3. Retrovirus en lentivirus

Retroviruses are small enveloped RNA viruses that replicate through a DNA intermediate. The retrovirus genome encodes for three polyproteins, *gag*, *pol*, and *env*, which are required *in trans* for viral replication and packaging. All that is required for viral replication *in cis* are the 5' and 3' long-terminal repeats (LTRs), which contain promoter, polyadenylation, and integration sequences, a packaging site termed *psi*, and a tRNA binding site, as well as several additional sequences involved in reverse transcription. The genes encoding the three viral proteins can be removed, and heterologous genes and transcriptional regulatory sequences inserted. The retrovirus infects target

cells through a specific interaction between the viral envelope protein and a cell surface receptor on the target cell. The virus is then internalised, where it is uncoated and the RNA reverse-transcribed into proviral double-stranded DNA (dsDNA) by means of the virally encoded pol gene. The dsDNA is then transported to the nucleus, where it is stably integrated into the host genome. The ability of retroviruses to insert their genome into the host DNA allows for stable genetic modification of the host cell. The disadvantage of retroviral vectors is that they require cell division, in particular mitosis, for integration. Thus, the current retroviral vectors are better suited for ex vivo gene therapy in which isolated cells can be propagated in culture, genetically modified by retroviral infection, and then transplanted into a recipient patient. Exceptions to the requirement for cell division for infection are lentiviruses, such as human immunodeficiency virus (HIV), which appear to have acquired factors that allow for active transport of the DNA to the nucleus of nondividing cells. Many of the lentivirus vectors used in gene therapy are based on HIV. Typically, HIV vectors can accommodate fairly large inserts and can provide long-term expression through chromosomal integration. The limitations of using lentivirus vectors in clinical trials are the production of high-titre virus stocks and the safety concerns related to their origin from HIV.

Similar to other gene therapy vectors, targeting to specific cell-type remains one of the most desirable properties of retroviral and lentiviral vectors. This is of utmost importance since retroviral and lentiviral vectors that have the capacity to deliver transgenes into specific tissues are expected to be of great value for various gene therapy applications *in vivo*. Two different strategies to modify the surface of retroviral and lentiviral vectors have been developed, *i.e.*, (1) pseudotyping with heterologous viral glycoproteins that exploits the tropism of a given virus, and (2) engineering the viral glycoproteins by fusing or inserting heterologous polypeptides, in order to retarget the virus particles to a cell of interest for therapeutic gene delivery. Many heterologous viral glycoproteins can be incorporated into retroviral and lentiviral particles. Non-retroviral glycoproteins include those derived from vesiculoviruses, lyssaviruses, arenaviruses, hepadnaviruses, flaviviruses, paramyxoviruses, orthomyxoviruses, filoviruses, and alphaviruses (Verhoeyen & Cosset, 2004). The incorporation of heterologous proteins into virus particles may modify the properties of the pseudotyped vectors. However, as stated above, although it cannot be excluded that this type of retargeting may lead to changes in pathogenicity for the individual host, it does not represent an enhanced environmental risk over the use of the original viral vector. Therefore, pseudotyping will not be further considered here. For more information on pseudotyping of retroviral and lentiviral vectors the reader is referred to the literature (Lavillette *et al.*, 2001, Verhoeyen & Cosset, 2004).

Initial attempts to genetically modify the tropism of retroviral vectors consisted of the insertion of various ligands, such as growth factors, hormones, peptides or single-chain antibodies, in several locations on the viral surface glycoproteins. These modifications would allow the virus to bind cell-surface molecules different from the parent virus receptor. Direct targeting strategies rely on the idea that fusion activation of the chimeric envelope should be triggered by the interaction of the displayed ligand with its specific receptor on the target cell. However, so far, this concept has met with only limited success. The different types of modifications of the retroviral envelope glycoprotein include domain replacements (Barnett *et al.*, 2001, Benedict *et al.*, 1999, Kasahara *et al.*, 1994), peptide

insertions in pre-folded domains (Gollan & Green, 2002a, Gollan & Green, 2002b, Valsesia-Wittmann *et al.*, 1994, Wu *et al.*, 2000a), and display of polypeptides as additional folded domains (Chadwick *et al.*, 1999, Cosset *et al.*, 1995, Fielding *et al.*, 2000, Kayman *et al.*, 1999, Martin *et al.*, 2002, Maurice *et al.*, 1999, Maurice *et al.*, 2002, Russell *et al.*, 1993, Valsesia-Wittmann *et al.*, 1996). Although the great majority of these ligand-displaying envelope glycoproteins could specifically and efficiently bind the targeted cells, the infectivity of the corresponding viral particles was generally very poor, if not absent. It seems that although the fusogenic potential of chimeric glycoproteins incorporated on the viral particles is intact, in the absence of the retroviral receptor, the interaction of the displayed ligand with its target cell surface molecule is generally not able to activate fusion. This post-binding block in targeted retroviral-mediated gene transfer was shown to be caused by the inability of the chimeric envelope proteins to undergo a conformational change that is required for fusion (Benedict *et al.*, 1999, Zhao *et al.*, 1999). Thus, the poor fusion activity of chimeric glycoproteins is attributed to the loss of coupling between retargeted binding and fusion activation. Another potential problem of the use of chimeric glycoproteins is that their binding to new receptor molecules actually abolishes infection, even when the retroviral receptor is present at the surface of the targeted cells. It has been shown, for example, that retroviral envelope proteins that display epidermal growth factor (EGF) have greatly impaired infectivity in cells that express both EGF-receptors and retroviral receptors. Presumably, the EGF-receptors can sequester virus particles and/or traffic virus particles to cell compartments that are not compatible with the natural route of infection (Cosset *et al.*, 1995). The observation that vectors displaying EGF could efficiently bind EGF-receptor positive cells, but could not infect them, has been used to selectively infect EGF-receptor negative cells in a process known as 'inverse targeting' (Cosset *et al.*, 1995, Fielding *et al.*, 1998).

Despite the fact that many retroviral particles containing chimeric envelope glycoproteins are unable initiate fusion, in most cases they are still correctly targeted to host cells that express the corresponding receptor. This observation has been used for the construction of chimeric glycoproteins in which the ligand is separated from the viral glycoprotein by a protease cleavage site. If the cleavage site is recognized by a cellular matrix-associated protease, cleavage of the chimeric glycoprotein results in liberation of the authentic viral glycoprotein which is subsequently able to interact with its cognate receptor and to initiate fusion. This type of targeting, also known as 'protease-targeting', is dependent on the presence on the host cell of the correct receptors for the retroviral vector (Verhoeyen & Cosset, 2004).

Since direct targeting strategies using glycoproteins that are receptor-dependent for fusion are precluded by the specific fusion activation mechanism of retroviral glycoproteins, alternative mechanisms have been explored. The ability of the glycoproteins of some membrane-enveloped viruses to fuse in low-pH endosomes provides an alternative basis for targeting. Many types of receptor candidates for targeting can recycle from the cell surface and traffic into low-pH endosomes. Therefore, it is likely that the fusogenicity of the pH-dependent chimeric glycoproteins bound to various targeted cell surface molecules will be activated during endocytosis, even if these chimeras are not attached to their wild-type receptors. When an IgG-binding domain of protein A was inserted in the hemagglutinin (HA) of Influenza virus or the E2 envelope protein of Sindbis virus, gene delivery by

retroviral vectors carrying the HA or E2 chimeric glycoproteins could be retargeted in the presence of specific antibodies against the target cell-surface molecules (Hatziioannou *et al.*, 1999, Morizono *et al.*, 2001). As both proteins can efficiently pseudotype primate lentiviral vectors, these results may open new ways to *in vivo* applications in gene transfer areas.

4.4. Hybrid virus vectors

Hybrid virus vectors have been developed In order to combine the useful properties of specific gene therapy vectors. To achieve high-efficiency stable transduction *in vivo* combined with long-term expression, a hybrid adenovirus/retrovirus vector was constructed (Bilbao *et al.*, 1997). The system consisted of two adenovirus vectors, one that contained the entire genome of a retroviral vector, and a second that expressed the retroviral packaging functions. Co-infection of cells with the two vectors resulted in the generation of transient producer cells which generated retroviral particles that were able to infect neighbouring cells leading to stable integration of the retroviral vector into the genome of these cells. Animal experiments confirmed that the system was operative albeit with relatively poor efficiency. This was probably related to the fact that target cell proliferation is required for retroviral vectors to achieve stable integration.

Ideally, gene therapy of genetic disorders requires stable integration of appropriately regulated transgenes in human somatic cells. Although retroviruses and lentiviruses integrate at high efficiency, integration is almost random with a preference for active genes (Schroder *et al.*, 2002, Wu *et al.*, 2003). The wild-type AAV genome integrates at a specific position in human chromosome 19, but this ability is lost in AAV vectors since they are deleted for the *rep* gene in order to enhance their packaging size. Thus, similar to adenovirus, also AAV vectors do not normally integrate, or do so with varying efficiency depending on the host cell. In order to combine the site-specific integration capacity of AAV with the capacity of helper-dependent adenoviral vectors to accommodate large stretches of DNA, hybrid adenovirus/AAV (Ad-AAV) vector systems have been developed. High capacity adenovirus vectors that carried the inverted terminal repeats (ITRs) of AAV were generated by recombination between two first generation adenovirus vectors. The resulting vector contained an 11.6 kb expression cassette including the human γ-globin gene and upstream transcriptional control elements. Since the vector did not express the AAV *rep* gene, integration was random but the gene cassette was shown to be intact (Shayakhmetov *et al.*, 2002).

A system consisting of two helper-dependent adenovirus vectors, one carrying the AAV *rep* gene and the other containing the AAV ITRs was described by Recchia and co-workers (Recchia *et al.*, 1999). Although *rep* protein expression interfered with adenovirus replication, the vector was successfully amplified in producer cells. Importantly, after coinfection of hepatoma cells, successful transduction and specific integration at the authentic AAV integration site could be demonstrated. Recently this system has been optimised by generating a single adenovirus vector that carries the flanking ITRs of AAV and a copy of the AAV *rep* gene that is driven by an inducible promoter (Recchia *et al.*, 2004). After a single tail vein administration of the Ad/AAV vector, specific integrations were mapped and sequenced in livers of animals in which activation of *rep* expression was induced by drug treatment.

High cloning capacity combined with site specific integration and long term expression was also achieved with an adeno-associated virus/adenovirus (AAV-Ad) hybrid vector. In this case the AAV vector was provided *in cis* with adenoviral packaging signals. The AAV vector was packaged using an adenovirus helpervirus that complemented *in trans* AAV helper functions and adenoviral structural proteins. Remarkably, cross-packaging was able to overcome the limited cloning capacity of AAV vectors (Goncalves *et al.*, 2001, Goncalves *et al.*, 2002, Goncalves *et al.*, 2004). When combined with specific targeting, both the Ad-AAV and AAV-Ad system may hold great promise for future gene therapy applications.

4.5. Herpesvirus

Herpes simplex virus (HSV) is an enveloped virus carrying a linear dsDNA genome of approximately 150 kb, encoding over 80 viral proteins. The virus enters the cell by a complex fusion process involving several different envelope glycoproteins (Garner, 2003). Glycoproteins gD, gB and the gH/gL-complex are required for entry, while other glycoproteins such as gE, gI, and gM are also thought to be involved in membrane fusion events and intracellular trafficking. After delivery to the nucleus, the linear DNA circularises and a cascade of gene expression is initiated by the viral protein VP16, which is carried into the cells as part of the viral tegument. Newly synthesized viral particles are released from the infected cells by lysis. Many HSV proteins are nonessential for viral replication and can be deleted, allowing HSV to be used as a vector for the insertion of large exogenous coding regions (Burton *et al.*, 2002). Inactivation of one or more of the immediate early proteins, ICP0, ICP4, ICP22, and ICP27, results in a vector unable to replicate, except in a complementing cell line (Krisky *et al.*, 1998, Marconi *et al.*, 1996, Wu *et al.*, 1996). Deletion of the gene encoding ICP34.5 results in a virus that may replicate *in vitro*, but not in neurons *in vivo* (Andreansky *et al.*, 1997, Whitley *et al.*, 1993). However, the virus retains its ability to undergo lytic replication in rapidly dividing tumor cells (Markert *et al.*, 2000). HSV vectors have the advantages of being able to infect nondividing cells, establishing latency in some cell-types, and having the capacity to carry large regions of exogenous DNA.

Several strategies have been considered for modifying the tropism of HSV. Pseudotyped HSV vectors have been made using the vesicular stomatitis virus envelope glycoprotein (VSV-G). It was demonstrated that VSV-G chimeras containing the transmembrane domain of gD, or a truncated gB transmembrane domain, were efficiently incorporated into the viral envelope and that the wild-type VSV-G protein was incorporated rather less efficiently. However, only the latter intact VSV-G protein was able to partially rescue the gD-deficient phenotype, whereas the chimeric proteins were non-functional (Anderson *et al.*, 2000). Since binding of viral gB/gC to glycosaminoglycan (GAG) cell surface receptors is the first step in the entry cascade, targeted adsorption of virions might be achieved through the modification of gC and gB. This was tested by generating engineered virions in which gC was fused to erythropoietin (EPO) (Laquerre *et al.*, 1998). The viruses expressed the fusion proteins at their surface and showed greatly reduced GAG binding as well as the acquisition of specific binding to the new ligand, the EPO receptor. However, although the viruses were taken up by cells expressing the EPO-receptor, productive infection did not ensue, indicating that the natural route

of infection was not used. Successful targeting without loss of infectivity was shown using HSV amplicon vectors in which the heparan sulphate binding domain (HSBD) of gC was replaced by a hexameric histidine-tag as a model ligand. Packaging of amplicons by a gC-negative helpervirus resulted in gC-modified virus particles which showed increased binding to cells expressing a his-tag pseudoreceptor (Grandi *et al.*, 2004a, Grandi *et al.*, 2004b, Spear *et al.*, 2003). Recently, successful specific retargeting of HSV to hepatocytes was reported. An engineered HSV-1 mutant, which was deleted for gC and the heparan binding-binding domain of gB, and which expressed a fusion between gC and a 27 amino acid preS1 peptide of human Hepatitis B virus, was shown to bind specifically to a hepatoblastoma cell line resulting in productive infection (Argnani *et al.*, 2004). These results indicate that HSV-1 can productively infect cells through specific binding to a non-HSV-1 receptor. Modification of the viral entry/attachment glycoprotein D might present an alternative to engineering the cell attachment receptors gC and gB. However, this targeting strategy demands an intimate knowledge of crucial gD amino acid residues involved in receptor binding, and represents a formidable protein engineering challenge.

4.6. Negative-stranded RNA viruses

The negative-strand RNA viruses form a broad group of enveloped viruses whose genome consists of either one (paramyxoviruses, rhabdoviruses, filoviruses and bornaviruses), two (arenaviruses), three (bunyaviruses) or eight (orthomyxoviruses) RNA segments. The virus carries its own RNA-dependent RNA polymerase, which is responsible for transcription and replication of the viral genome in the infected cell. The genome of these viruses is found in both virions and infected cells to be complexed with the viral nucleoprotein as ribonucleoprotein (RNP) complexes. These RNP complexes, rather than naked viral RNA, are the actual templates recognized by the viral RNA polymerase. Replication involves the synthesis of a replicative intermediate consisting of a complementary copy of the genome, known as the antigenome, which is also encapsidated. The genome is also used as template by the viral RNA polymerase to synthesize the viral mRNAs, which, in contrast to the antigenomes, are capped and polyadenylated. The tissue tropism of negative-strand RNA viruses is mediated, at least in part, by their envelope proteins, which contain the receptor-binding sites and mediate viral entry into the cell. Some negative-strand viruses, such as rhabdoviruses and orthomyxoviruses, are endocytosed and require acidification of the endosome for infectivity. Others, such as paramyxoviruses, can fuse at neutral pH directly with the cell membrane.

The first reports dealing with the deliberate change of the tropism of negative-strand RNA viruses concerned the rhabdoviruses rabies virus and vesicular stomatitis virus (VSV). These viruses have a single envelope glycoprotein, the G protein that is responsible for infectivity of the virus. The observation that foreign glycoproteins expressed by recombinant rhabdoviruses could be efficiently incorporated into virus particles along with the G protein (Schnell *et al.*, 1996) and the subsequent finding that rhabdoviruses are able to form spikeless non-infectious particles in the absence of the G protein, suggested that these viruses could be used for the generation of viruses with an altered receptor specificity. It was shown that a hybrid HIV-1 envelope glycoprotein in which the cytoplasmic tail was replaced by that of the rabies virus G protein could be incorporated into the envelope of rabies

virus and that the pseudotyped virus particles specifically infected CD4+ cells, the natural target cells for HIV-1 (Mebatsion & Conzelmann, 1996). Using a similar approach, G-deficient rabies virus pseudotyped with CD4- and CXCR4-derived proteins selectively infected cells expressing HIV-1 envelope protein (Mebatsion *et al.*, 1997). Subsequently, a replication-competent recombinant VSV was constructed that expressed a chimeric HIV-VSV envelope protein from an extra transcription unit. This recombinant was shown to specifically infect CD4+ cells after treatment with anti-VSV serum to block infectivity via the G protein. Furthermore, CD4-specific infectivity could be neutralized with anti-HIV serum (Johnson *et al.*, 1997). Specific targeting of virus infected cells was accomplished by using a G-deficient VSV recombinant that expressed the HIV receptor CD4 and co-receptor CXCR4. This virus was unable to infect normal cells but did infect and kill cells that were previously infected with HIV-1 (Schnell *et al.*, 1997). The fusion (F) protein of the pneumovirus respiratory syncytium virus (RSV) was cloned in VSV and expressed in recombinant VSV-infected cells. The RSV-F glycoprotein was incorporated into virions, and functional studies revealed that the presence of this glycoprotein in the viral envelope allowed for infectivity that was independent of endosome acidification. This low-pH-independent infectivity was specific for RSV-F since incorporation of RSV-G did not allow for low-pH-independent infectivity (Kahn *et al.*, 1999).

Replication-competent rabies virus recombinants that expressed the gp160 envelope protein of HIV displayed HIV-like tropism and infected professional antigen-presenting cells (human dendritic cells) opening the potential use of rabies virus recombinants as vaccines (Foley *et al.*, 2002). That retargeting using this approach is not always successful was shown by the inability of VSV recombinants that expressed and incorporated high levels of Hepatitis C virus (HCV) glycoproteins to infect cell lines that supported HCV infection and replication (Buonocore *et al.*, 2002). Recently, preferential targeting of VSV to breast cancer cells was reported. In one approach, non-replicating and replicating VSV recombinants were created whose only surface protein was a Sindbis virus glycoprotein with severely reduced native binding that contained the Fc-binding domain of *Staphylococcus aureus* protein A. This virus was selectively targeted to cells that expressed the *Her2/neu* receptor in the presence of monoclonal antibodies directed against the *Her2/neu* receptor (Bergman *et al.*, 2003). Subsequently, a replication-competent VSV recombinant was created that expressed a chimeric Sindbis glycoprotein containing a single-chain antibody against the *Her2/neu* receptor. This recombinant was shown to be preferentially targeted to breast cancer cells that expressed the *Her2/neu* receptor (Bergman *et al.*, 2004).

In contrast to many other viruses, two separate envelope proteins, one for attachment (receptor recognition) and another for fusion are used by members of the *paramyxoviridae*. The attachment protein has hemagglutination (H) or hemagglutination plus neuraminidase (HN) activity whereas membrane fusion is mediated by the fusion (F) protein. This constellation allows for the specific modification of receptor recognition by a chimeric H or HN protein without compromising fusion. The first demonstration that large specificity domains can be displayed on the extracellular domain of the attachment protein without losing replication competence was reported for measles virus by Cattaneo an co-workers. Hybrid proteins consisting of the epidermal growth factor (EGF) or the insulin-like growth factor 1 (IGF1) linked to the extracellular carboxy-terminus of the measles virus Edmonston B

vaccine strain were produced. Replication competent measles virus displaying EGF or IGF1 efficiently entered cells that were lacking the natural measles virus receptor CD46 but which expressed either the EGF or IGF1 receptor (Schneider *et al.*, 2000). Subsequently, data from the same laboratory showed that also single-chain antibodies could be functionally displayed on the measles virus H protein. In this study, a scFv against carcinoembryonic antigen (CEA), which is highly over-expressed on the surface of a number of cancerous cells, was displayed on top of the H protein and the corresponding virus replicated efficiently in non-human CEA expressing cells, which the parental virus failed to infect (Hammond *et al.*, 2001). Similar results were reported for a measles virus recombinant that displayed scFv's against CD20, which is expressed on normal and neoplastic B cells, and CD38, a myeloma cell marker (Bucheit *et al.*, 2003, Peng *et al.*, 2003). A recombinant measles virus could even be targeted to cells expressing a specific peptide/MHC complex by using a high-affinity single-chain T-cell receptor (TCR). The efficiency of TCR-mediated virus entry was dependent on the number of peptide/MHC complexes expressed on the target cells (Peng *et al.*, 2004). Although targeting and fusion could be triggered by displaying ligands at the C-terminus of the wild-type H protein, the hybrid H proteins still triggered fusion via the natural measles virus receptors CD46 and SLAM, which are widely expressed on normal cells. Recently, the identification of specific amino acid residues which are responsible for the binding of the H protein to the CD46 and SLAM receptors (Nakamura *et al.*, 2004, Vongpunsawad *et al.*, 2004) resulted in the generation of truly retargeted measles virus recombinants which are blind to the native receptors CD46 and SLAM but which propagated efficiently and exclusively via alternative cellular receptors such as EGF or CD38 (Hadac *et al.*, 2004).

Strategies for changing the receptor-binding specificity of the influenza A virus have been explored (Patterson *et al.*, 1999). Problem is that the receptor-binding specificity and the membrane fusion function reside in one and the same protein, *i.e.*, the haemagglutinin (HA) protein. A scFv specific for the hapten NIP was inserted in HA to create a scFv-HA chimeric protein. This protein was shown to possess anti-NIP binding activity, but membrane fusion activity could not be demonstrated, suggesting that linking the scFv domain directly to HA inhibits its fusion function. Co-expression of the anti-NIP scFv-HA chimeric protein together with wild-type HA protein in Chinese hamster ovary cells resulted in fusion of these cells with NIP-conjugated red blood cells that lacked sialic acid, the natural ligand of the HA protein. These results indicate that the receptor-binding function of HA can be transferred to an adjacent molecule, and also changed in its specificity, without compromising its membrane fusion activity. However, to date, no results describing this application in the context of a recombinant Influenza virus have been reported.

4.7. Other viruses

Alphaviruses such as Sindbis virus and Semliki Forest virus (SFV) have received considerable attention for use as virus-based expression vectors. Attractive properties of alphavirus vectors include the rapid engineering of expression constructs, production of high-titered stocks of infectious particles, infection of non-dividing cells, and high expression levels (Liljestrom & Garoff, 1991, Lundstrom, 2003a). However, a major drawback is that these vectors lack target-cell specificity. For mammalian

cells at least one receptor is the high-affinity laminin receptor, whose wide distribution and highly conserved nature is probably responsible for the broad host-range of the virus. Cell-specific targeting of Sindbis virus vectors was achieved indirectly by using vectors that displayed the IgG-binding domain of *Staphylococcus aureus* protein A (Ohno *et al.*, 1997). When used in conjunction with monoclonal antibodies that react with cell-surface antigens, the protein A-envelope chimeric virus was able to efficiently infect cells which were minimally infected by the wild-type virus. Another strategy relied on the expression of a chimeric protein consisting of the Sindbis virus E2 glycoprotein and the human chorionic gonadotrpopin (hCG) glycoprotein, a hormone that is recognized by the luteinizing hormone / chorionic gonadotropin (LH/CG) receptor. The hCG-envelope chimeric Sindbis vector showed minimal infectivity against cell lines which lacked the LH/CG receptor on their surface, but could infect LH/CG receptor bearing cancer cell lines and transfer a reporter gene into these cells with high efficiency in a viral dose-dependent manner (Sawai & Meruelo, 1998).
Switching of species tropism was achieved by exchanging the ectodomains of the spike glycoprotein (S) of the coronaviruses mouse hepatitis virus (MHV) and feline infectious peritonitis virus (FIPV) (Haijema *et al.*, 2003, Kuo *et al.*, 2000). The chimeric virus fMHV (MHV containing the ectodomain of the S glycoprotein of the feline coronavirus) acquired the ability to infect feline cells and simultaneously lost the ability to infect murine cells. Similarly, the chimeric virus mFIPV (FIPV containing the ectodomain of the S glycoprotein of murine coronavirus) acquired the ability to infect murine cells and lost the ability to infect feline cells. This highly selective infectivity has been elegantly used for establishing a reverse genetics system for these coronaviruses by using the possibility to select recombinants after targeted RNA recombination.

Table 2. Overview of retargeting strategies	
Non-genetic:	
Pseudotyping / cross-packaging	Adenovirus, Retrovirus, AAV, Herpesvirus, SS(-)RNA[a]
Bispecific conjugates / bispecific antibodies	Adenovirus, AAV, SS(-)RNA
Genetic:	
Replacement of attachment protein gene with homologous gene from other serotype	Adenovirus, AAV
Replacement of part of attachment protein gene with corresponding part of other serotype	Adenovirus, AAV, Retrovirus
Replacement of attachment protein gene with attachment protein gene from other species	SS(-)RNA
Replacement of part of attachment protein gene with corresponding part of other species	SS(-)RNA, Coronavirus
Incorporation of short ligands in viral attachment proteins	Adenovirus, AAV, Retrovirus, Herpesvirus
Display of conformational domains on attachment protein	Adenovirus, AAV, Retrovirus, Herpesvirus, SS(-)RNA, Alphavirus
Display of scFv on attachment protein	AAV, Retrovirus, SS(-)RNA
Display of IgG-binding domain (protA) in combination with Mab's specific for cell surface markers	Retrovirus, SS(-)RNA, Alphavirus
Inverse targeting	Retrovirus
Protease targeting	Retrovirus

[a] Single-stranded negative-sense RNA viruses

5. CHIMERIC VIRUSES CONTAINING FOREIGN VIRAL SURFACE PROTEINS

Membrane glycoproteins drive the assembly and budding of enveloped viruses and play a key role in the initiation of the infection of target cells. Since they are exposed at the surface of the virus particle, these proteins are in most cases also major targets for the humoral - and sometimes also the cellular - immune response. Therefore, in terms of vaccine development, surface glycoproteins are prime candidates as immunogens for vaccination. However, in many cases, surface glycoproteins are also the primary determinants of the host range and tissue tropism of viruses. Therefore, the construction of chimeric viruses in which the replication properties of one virus are combined with the envelope proteins of another virus can result in viruses with properties which may differ from those of the parent viruses. For the construction of chimeras, certain constraints on the ability of the envelope of one virus to enclose the nucleocapsid of another virus may be present. Such constraints are evidenced by the failure to detect pseudotype formation. Generally, a strong and specific interaction between the nucleocapsid and the envelope proteins will minimize the likelihood of pseudotyping, whereas pleomorphic or loosely structured viruses seem to more easily tolerate envelope proteins from distantly related viruses. However, in some cases, the inability of pseudotyping with intact proteins can be overcome by generating chimeric surface proteins consisting of a fusion between the cytoplasmic tail and transmembrane regions of a surface protein of one virus and the extracellular region of a surface protein of another virus.

Since this report deals with changes in tropism, we will focus on chimeras between enveloped viruses which have been shown - or are at the least expected - to have acquired changes in the composition of their envelope proteins as a result of the addition or replacement of genes expressing such proteins. Few examples of non-enveloped chimeric viruses which are characterized by replacements of capsid proteins will also be discussed.

5.1. Chimeric poxviruses

One of the first, and probably the most well-known chimeric virus is the recombinant vaccinia virus VR-G, which expresses the rabies virus G protein, that was already developed in the 1980's (Kieny *et al.*, 1984, Wiktor *et al.*, 1984). The recombinant virus was applied in the form of baits for immunization of wild animals, especially foxes, and protective immunization with reduced incidence of rabies has been demonstrated in large field trials (Brochier *et al.*, 1996, Pastoret & Brochier, 1996). Recombinant poxvirus based vaccines have since been shown to be able to protect animals from a number of different diseases of veterinary importance. Examples include the use of poxvirus recombinants to protect cattle against vesicular stomatitis virus (Mackett *et al.*, 1985), rinderpest (Giavedoni *et al.*, 1991, Romero *et al.*, 1994, Verardi *et al.*, 2002, Yilma *et al.*, 1988) and rabies (Aspden *et al.*, 2002); raccoons and foxes against rabies (Blancou *et al.*, 1986, Blasco *et al.*, 2001, Brochier *et al.*, 1991, Masson *et al.*, 1999, Rupprecht *et al.*, 1988, Wiktor *et al.*, 1984); vampire bats against rabies (Aguilar-Setien *et al.*, 2002); mice and chickens against influenza virus (Chambers *et al.*, 1988, Qiao *et al.*, 2003, Sutter *et al.*, 1994b, Swayne *et al.*, 2000, Taylor *et al.*, 1988); chickens against Newcastle disease virus (Boursnell *et al.*, 1990a, Boursnell *et al.*, 1990b, Boursnell *et al.*, 1990c, Edbauer *et al.*, 1990, Letellier *et al.*, 1991, Taylor *et al.*, 1990) and Marek's disease (Lee *et al.*, 2003, Liu *et al.*, 1999);

and dogs against canine distemper virus (Taylor *et al.*, 1991). In all of these cases, the immunogenic protein(s) expressed by the recombinant poxvirus consisted of the major envelope glycoprotein(s) of the respective viral agents (sometimes in combination with additional non-structural proteins). In most cases, the expressed proteins were shown to be expressed at the surface of cells infected with the recombinant poxviruses suggesting that they were also present in the envelope of poxvirus particles which are released from infected cells by budding trough the cell membrane. Safety studies indicated that none of the recombinants had acquired a pathogenic phenotype.
Several poxviruses have been used for vaccination studies in humans. These include the NYVAC strain (Paoletti *et al.*, 1995, Tartaglia *et al.*, 1992) which is derived from the Copenhagen strain of vaccinia virus and the MVA strain (Meyer *et al.*, 1991, Sutter & Moss, 1992) which is a modified vaccinia virus Ankara strain. Also highly attenuated derivatives of avian poxviruses such as fowlpox virus (TROVAC) and canarypox virus (ALVAC) have been tested in extensive studies which have shown their safety in a variety of species including humans (Paoletti, 1996, Paoletti *et al.*, 1995). Vaccination studies in humans with recombinant poxviruses expressing viral surface proteins have been reported for the envelope proteins gp120 and gp160 of HIV (Bures *et al.*, 2000, Fleury *et al.*, 1996, Girard *et al.*, 1999, Graham, 2002, Graham *et al.*, 1992, Slobod *et al.*, 2004), the G protein of rabies virus (Cadoz *et al.*, 1992), and the major membrane glycoprotein of Epstein-Barr virus (Gu *et al.*, 1995). All recombinant poxvirus vaccine candidates that have been approved for human studies are unable to complete a productive cycle in mammalian cells and thus they are unable to produce infectious progeny.

5.2. Chimeric herpesviruses

Several herpesviruses have been used for the expression of genes encoding surface proteins of other viruses. The swine herpesvirus pseudorabies virus (PRV), that causes Aujeszky's disease in pigs, was used as a vector for the expression of chimeric viral membrane proteins composed of portions of the PRV envelope glycoprotein gIII (presently renamed gC) and of the HIV-1 envelope glycoproteins gp120 and gp41 (Whealy *et al.*, 1988). The glycosylated fusion proteins expressed by the recombinant viruses could be immunoprecipitated by polyvalent sera specific for gIII, as well as by sera from an AIDS patient. The levels of expression were lower than expected due primarily to instability or altered processing of the hybrid mRNA. Cleavage of chimeric proteins carrying the gp120-gp41 protease processing site could not be detected. PRV has also been used for the expression of the envelope glycoprotein E2 (previously E1) of classical swine fever virus. Chimeric viruses based on a live attenuated strain of PRV or a non-transmissible gD-mutant were both shown to be able to protect pigs against both swine fever and Aujeszky's disease (Peeters *et al.*, 1997, van Zijl *et al.*, 1991).
A chimeric PRV mutant which lacked the gene encoding the essential envelope glycoprotein B (gB) but expressed the homologous gene of bovine herpes virus type-1 (BHV-1) was generated and the effect of the gB exchange was analysed in swine, which is the natural host of PRV (Gerdts *et al.*, 2000). Animals infected with wild-type PRV showed high fever, marked respiratory symptoms but minimal neurological disorders. In contrast, animals infected with the chimeric PRV showed no respiratory symptoms and developed only mild fever. However, on day 5 after infection, all piglets

developed severe central nervous system (CNS) symptoms leading to death within 48 to 72 h. Detailed histological analyses showed that the chimeric PRV infected all regions of the nasal mucosa and subsequently spread to the CNS preferentially by the trigeminal route. In contrast, wild-type PRV primarily infected the olfactory epithelium and spread via the olfactory route. In the CNS, more viral antigen and significantly more pronounced histological changes resulting in more severe encephalitis were found after infection with the chimeric virus. These findings demonstrate that replacement of PRV gB by the homologous BHV-1 glycoprotein resulted in a dramatic increase in neurovirulence combined with an alteration in the route of neuroinvasion, indicating that gB is involved in determining neurotropism and neurovirulence of PRV.

The gIII (gC) gene of PRV was inserted into the tk gene region of BHV-1. The infectivity of the BHV-1 recombinant that expressed PRV gIII could be neutralized by polyclonal PRV antisera and by monoclonal antibodies to PRV gIII, indicating that both gIII proteins were present in the envelope of the recombinant virus (Kit *et al.*, 1992). BHV-1 was also used for the expression of the bRSV G protein (Kuhnle *et al.*, 1998). Immunoblots showed that the G protein contained N- and O-linked carbohydrates and that it was incorporated into the membrane of infected cells and into the envelope of recombinant virions. The latter was also demonstrated by neutralization of infectivity by monoclonal antibodies or polyclonal anti-BRSV G antisera and complement.

A herpesvirus of turkeys (HVT) recombinant containing the glycoprotein B (gB) gene of the closely related avian herpesvirus Marek's disease virus (MDV) has been constructed (Ross *et al.*, 1993). Replication of the recombinant in chick embryo fibroblasts (CEF) was comparable to that of wild-type HVT. However, the level of replication of the recombinant *in vivo* appeared to be lower than wild-type HVT. Pathogenicity tests showed that the recombinant was safe and did not cause lesions or other abnormalities. Both HVT and MDV have been used for the expression of the fusion (F) protein of Newcastle disease virus (NDV), either alone (Morgan *et al.*, 1993, Morgan *et al.*, 1992, Sakaguchi *et al.*, 1998, Sondermeijer *et al.*, 1993, Sonoda *et al.*, 2000) or in combination with the hemagglutinin-neuraminidase (HN) protein (Heckert *et al.*, 1996, Reddy *et al.*, 1996). The resulting recombinant viruses were all shown to be safe and to induce a protective immune response against Newcastle disease in chickens.

Oronasal vaccination of cats with a recombinant feline herpesvirus type 1 (FHV-1) that expressed the envelope glycoprotein gene from feline leukaemia virus (FeLV) provided significant protection against challenge with wild-type FeLV (Willemse *et al.*, 1996).

In order to evaluate whether canine herpesvirus (CHV) could be used as a live vector for the expression of heterologous immunogens, a recombinant canine herpesvirus (CHV) expressing the rabies virus G protein was constructed (Xuan *et al.*, 1998a). The G protein expressed by the recombinant CHV was processed and transported to the cell surface as in rabies virus infected cells, and showed the same biological activities such as low pH dependent cell fusion and hemadsorption. Dogs inoculated intranasally with the recombinant CHV produced higher titres of virus neutralizing antibodies against rabies virus than those inoculated with a commercial, inactivated rabies vaccine (Xuan *et al.*, 1998b).

An expression cassette encoding a chimeric protein consisting of the signal peptide of the E^{RNS} protein and the E2 envelope protein of bovine viral diarrhoea virus (BVDV) was incorporated into the genome of BHV-1. Penetration kinetics and single-step growth curves indicated that incorporation of the BVDV E2 glycoprotein in the BHV-1 envelope did not require BHV-1-specific signals but interfered with entry into target cells and egress of progeny virions. These results demonstrate that a pestivirus glycoprotein be can incorporated into the BHV-1 viral envelope (Schmitt *et al.*, 1999).

5.3. Chimeric negative-stranded RNA viruses

The negative-stranded RNA viruses include many important human as well as veterinary pathogens including influenza virus, mumps virus, measles virus, respiratory syncytial virus, parainfluenza virus, metapneumovirus, Ebola virus, rinderpest virus, Newcastle disease virus, rabies virus, and vesicular stomatitis virus. The advent of genetic engineering techniques for negative-stranded RNA viruses (Conzelmann, 1998, Garcia-Sastre, 1998, Neumann & Kawaoka, 2001, Palese *et al.*, 1996) has resulted in the development of a number of recombinant and chimeric viruses for vaccination purposes (Murphy & Collins, 2002, von Messling & Cattaneo, 2004).

As already discussed above, foreign glycoproteins expressed from an extra transcription unit by recombinant VSV viruses were efficiently incorporated into virus particles. For instance, the measles virus H or F protein were incorporated into the membrane envelope of VSV along with the VSV-G protein (Schnell *et al.*, 1996). Also, functional HA and NA proteins of influenza virus were incorporated with high efficiency in recombinant VSV (Kretzschmar *et al.*, 1997) and mice which were immunized with a VSV-HA recombinant were protected from challenge with influenza strain WSN (Roberts *et al.*, 1998). Deletion of the VSV-G gene resulted in non-infectious virus particles indicating that the HA protein could not functionally substitute for the VSV-G protein in the viral entry process. When the deletion mutant was grown in *trans*-complementing VSV-G expressing cells, the resulting phenotypically complemented VSV∆G-HA virions were able to induce a protective immune response against influenza virus but did not induce antibodies against the VSV vector (Roberts *et al.*, 1999).

Other examples of viral envelope glycoproteins which were successfully expressed and incorporated into recombinant VSV are the respiratory syncytial virus attachment protein (RSV-G) and fusion proteins (RSV-F) (Kahn *et al.*, 1999). Also in this case deletion of the VSV-G protein resulted in non-infectious particles (Kahn *et al.*, 2001).

The first truly chimeric single-stranded RNA virus with completely altered envelope and tropism consisted of a virus in which the reading frames of the measles virus H and F genes were substituted by a single reading frame encoding the VSV-G protein (Spielhofer *et al.*, 1998). In this chimeric virus, transcription and replication were mediated by the measles virus ribonucleoproteins, whereas the VSV-G envelope glycoprotein dictated the tropism as evidenced by the finding that the chimeric virus was able to infect a wider range of cells than measles virus. Remarkably, the matrix (M) protein of measles virus was not incorporated into progeny virus particles in the absence of the H and F proteins. When the G protein was substituted by a chimeric G/F protein consisting of the cytoplasmic domain of the measles virus F protein and the ectodomain of the VSV-G protein, incorporation of the

M protein was restored, indicating that the cytoplasmic tail of the F protein interacts with the M protein in the process of measles virus budding.

To investigate the importance of the rabies virus G protein in protection against rabies, a recombinant rabies virus was constructed in which the G ecto- and transmembrane domains were replaced with the corresponding regions of the VSV-G protein (rRV-VSV-G). Budding of the chimeric virus was delayed and infectious titres were reduced 10-fold compared with the parental rabies virus strain. Biochemical analysis showed equal replication rates of both viruses, and similar amounts of wild-type and chimeric G were present in the respective viral particles. Vaccination-challenge studies in mice showed that the chimeric virus rRV-VSV-G was unable to protect against virulent rabies virus, confirming that the rabies virus G protein is essential for successful vaccination (Foley *et al.*, 2000).

Three replication-competent rabies virus based vectors were constructed expressing either both hepatitis C virus (HCV) envelope proteins E1 and E2 or a modified version of E2 which contained the human CD4 transmembrane domain and the CD4 or rabies virus G protein cytoplasmic domain. Surface expression of HCV E2 resulted in efficient incorporation of the HCV envelope protein regardless of the presence of the rabies virus G cytoplasmic domain, which was described previously as a requirement for incorporation of the HIV-1 envelope protein into rabies virus particles (Mebatsion & Conzelmann, 1996). Killed and purified rabies virus particles containing HCV E2 were highly immunogenic in mice and were able to induce cellular responses against HCV E2, suggesting that recombinant rabies viruses are potentially useful vaccine vectors against important human viral diseases.

A chimeric rabies virus harbouring covalently linked gp120/gp41 envelope proteins of HIV-1 was recently described (McKenna *et al.*, 2003). In HIV-1 virions the gp120 and gp41 proteins are non-covalently associated resulting in the loss of gp120 during virus purification. Therefore, a chimeric HIV-1 *Env* protein which contains introduced cysteine residues that give rise to an intermolecular disulfide bridge between gp120 and gp41 was generated. This protein was fused in frame to the cytoplasmic domain of the rabies virus G protein and was shown to be efficiently incorporated into rabies virus particles. Immunogenicity of these particles was shown in a Rhesus macaque (McKenna *et al.*, 2004).

Glycoproteins substitutions were reported for the closely related pneumoviruses bovine respiratory syncytial virus (bRSV) and human respiratory syncytial virus (hRSV) (Buchholz *et al.*, 2000). The complete G and F genes of bRSV were replaced by their hRSV A2 counterparts. Whereas bRSV and hRSV grew more efficiently in bovine and human cells, respectively, the chimeric bRSV/A2 exhibited intermediate growth characteristics in a human cell line and grew better than either parent in a bovine line. The cytopathology induced by the chimera more closely resembled that of bRSV. Replication of bRSV was highly restricted in the respiratory tract of chimpanzees, a host that is highly permissive for hRSV. Interestingly, the bRSV/A2 chimeric virus was somewhat more competent than bRSV for replication in chimpanzees but remained highly restricted compared to hRSV. This showed that the substitution of the G and F glycoproteins alone was not sufficient to induce efficient replication in chimpanzees. Thus, the F and G proteins contribute to the host range restriction of bRSV but are not the major determinants of this phenotype.

Chimeric bRSV expressing glycoproteins of bovine parainfluenza virus type 3 bPIV3 instead of bRSV glycoproteins were reported by Stope and co-workers (Stope *et al.*, 2001). The G and F proteins of bRSV were replaced individually or together by the HN and/or F glycoproteins of bPIV3. Recombinant virus could not be recovered when the bRSV F protein was replaced the bPIV3 F protein. However, recovery of a chimeric virus with both glycoproteins replaced simultaneously, and of a chimeric virus with the bRSV G protein replaced by bPIV3 HN, was successful. The replication rates of both chimeras were similar to that of standard bRSV. These results indicate that the envelope glycoproteins derived from a member of the respirovirus genus can together functionally replace their homologs in a pneumovirus background.

Human parainfluenza virus (hPIV) type 1, 2 and 3 are significant causes of serious lower respiratory tract disease in infants and children. Promising live attenuated hPIV3 vaccine candidates are undergoing clinical evaluation but comparable vaccine candidates do not exist for hPIV1 and hPIV2. In order to develop vaccine candidates for hPIV1 and hPIV2, the reverse genetics system that is available for hPIV3 was used to construct chimeric viruses in which the protective F and HN proteins of hPIV3 were replaced by those of hPIV1 and hPIV2 (Skiadopoulos *et al.*, 1999, Skiadopoulos *et al.*, 2002b, Tao *et al.*, 1998, Tao *et al.*, 2000, Tao *et al.*, 2001, Tao *et al.*, 1999). The hPIV3-1 chimera replicated in tissue culture cells and in the respiratory tract of hamsters as efficiently as its hPIV1 and hPIV3 parents, which is remarkable because the HN and F glycoproteins share only 43 and 47% overall amino acid sequence identity. Furthermore, the hPIV3-1 chimera was found to possess biological properties derived from each of its parent viruses. Specifically, it required trypsin for efficient plaque formation in tissue culture, like its hPIV1 parent but unlike hPIV3. On the other hand, it caused an extensive cytopathic effect (CPE) in LLC-MK2 cultures which resembles that of its hPIV3 parent but differs from that of its non-cytopathic hPIV1 parent. This latter finding indicates that the genetic basis for the CPE of hPIV3 in tissue culture lies outside regions encoding the HN or F glycoproteins (Tao *et al.*, 1998). Using the strategy described above for hPIV3-1, attempts to recover recombinant chimeric hPIV3-hPIV2 isolates carrying the full-length PIV2 glycoproteins in a wild-type hPIV3 backbone failed. Viable hPIV3-2 chimeras were only recovered when chimeric HN and F proteins rather than complete hPIV2 F and HN proteins were used, indicating that the cytoplasmic tail of the HN or F glycoproteins of hPIV3 were required for successful recovery of hPIV3-2 chimeras. Although the hPIV3-2 chimeric viruses replicated efficiently *in vitro*, they were moderately to highly attenuated for replication in the respiratory tracts of hamsters, African green monkeys, and chimpanzees. This unexpected finding indicated that chimerization of the HN and F proteins of hPIV2 and hPIV3 itself specified an attenuation phenotype *in vivo*. Despite this attenuation, these viruses were highly immunogenic and protective against challenge with wild-type hPIV2 in hamsters and African green monkeys (Tao *et al.*, 2001). In order to generate a single virus capable of inducing immunity to both hPIV1 and hPIV2, the chimeric virus hPIV3-1 was used as a vector to express the HN protein of PIV2 from an extra gene cassette. The recombinant derivative, designated rPIV3-1.2HN, exhibited a level of temperature sensitivity and *in vitro* growth similar to that of its parental virus. The rPIV3-1.2HN virus was restricted in replication in both the upper and lower respiratory tracts of hamsters compared with hPIV3-1, identifying an attenuating effect of the hPIV2 HN insert in hamsters. rPIV3-1.2HN elicited serum

antibodies to both hPIV1 and hPIV2 and induced resistance against challenge with wild type hPIV1 or hPIV2. Thus, rPIV3-1.2HN, a virus attenuated solely by the insertion of the hPIV2 HN gene, functioned as a live attenuated bivalent vaccine candidate against both hPIV1 and hPIV2 (Tao *et al.*, 2001).
A chimeric recombinant hPIV3 virus containing bovine PIV3 F and HN glycoprotein genes in place of its own and the reciprocal recombinant consisting of bovine PIV3 bearing the hPIV3 F and HN genes were generated to assess the effect of glycoprotein substitution on replication of hPIV3 and bPIV3 in the upper and lower respiratory tract of rhesus monkeys (Schmidt *et al.*, 2000). hPIV3 bearing the bPIV3 F and HN genes was restricted in replication in rhesus monkeys to a level similar to that of its bPIV3 parent virus, indicating that the glycoprotein genes of bPIV3 are major determinants of its host range restriction. bPIV3 bearing the hPIV3 F and HN genes (b/hPIV3) replicated in rhesus monkeys to a level intermediate between that of hPIV3 and bPIV3, indicating that the F and HN genes make a significant contribution to the overall attenuation of bPIV3 for rhesus monkeys. However, other bPIV3 sequences also contribute to the attenuation phenotype in primates. The chimeric b/hPIV3 recombinant was used for the expression of the G and F proteins of RSV subgroup A and B. The level of replication of b/hPIV3-RSV chimeric viruses in the respiratory tract of rhesus monkeys was similar to that of their parent virus, and each of the chimeras induced a robust immune response to both RSV and hPIV3 (Schmidt *et al.*, 2002).
Along the same line, a chimeric virus was developed which was based on an attenuated temperature-sensitive recombinant bPIV3 strain in which the HN and F genes were replaced by those of hPIV1. The resulting chimeric b/hPIV3 was temperature sensitive for growth in Vero cells. The replication of the chimeric virus was restricted in the lungs of hamsters, albeit not as severely as was observed for the parent strain. The chimeric virus protected hamsters and rhesus monkeys against challenge with hPIV3 (Pennathur *et al.*, 2003). The chimeric b/h PIV3 was employed as a vector for RSV antigen expression. African green monkeys immunized with b/hPIV3 expressing either the native or soluble RSV F protein were protected from challenge with wild-type RSV and produced RSV neutralizing and RSV F-protein specific serum antibodies (Tang *et al.*, 2004).
Attenuated derivatives of hPIV3 have been used for the expression of measles virus HA in order to develop a vaccine that could be used for immunization of infants against measles and hPIV3. hPIV3(HA) chimeric viruses based on the cold-adapted hPIV3 strain cp45L replicated 5- to 10-fold less well than the parent virus in the upper respiratory tract of hamsters suggesting that the approximately 2-kb HA insert itself conferred attenuation. Nevertheless, the hPIV3(HA) recombinants induced a high level of resistance to replication of hPIV3 challenge virus in hamsters and induced very high levels of measles virus neutralizing antibodies (Durbin *et al.*, 2000). The chimeric recombinant virus rHPIV3-N(B)HA which expressed measles virus HA and which was attenuated due to the presence of the bPIV3 nucleocapsid (N) protein ORF in place of the hPIV3 ORF, replicated like its attenuated rHPIV3-N(B) parent virus *in vitro* and in the upper and lower respiratory tracts of rhesus monkeys, indicating that the insertion of the measles virus HA did not further attenuate rHPIV3-N(B) *in vitro* or *in vivo*. Monkeys immunized with rHPIV3-N(B)HA developed a vigorous immune response to both measles virus and HPIV3 (Skiadopoulos *et al.*, 2001). Recombinant hPIV3s bearing genes encoding HN of hPIV1, HN of hPIV2, or HA of measles virus replicated efficiently *in vitro*, including the

largest recombinant which contained three gene insertions. Viruses with inserts were restricted for replication in the respiratory tract of hamsters. Viruses containing two inserts were generally more attenuated than those with a single insert, and viruses with three inserts were over-attenuated for replication in hamsters. A recombinant hPIV3 bearing both the hPIV1 and the hPIV2 HN genes was attenuated, immunogenic, and protected immunized hamsters from challenge with hPIV1, hPIV2, and hPIV3 (Skiadopoulos *et al.*, 2002a).

A chimeric Newcastle disease virus (NDV) vaccine was constructed with an insertion of the HA gene from an avian influenza virus (AIV) H7N2 serotype. Recombinant virus particles exhibited hemagglutinating activity which could be inhibited by antisera against both NDV and AIV H7 indicating that the HA protein was incorporated into virus particles. The chimeric NDV-HA vaccine provided partial protection from virulent NDV and highly pathogenic AIV challenge (Swayne *et al.*, 2003).

Influenza A viruses are enveloped viruses containing a genome composed of eight strands of negative-sense RNA that encode ten viral proteins (Steinhauer & Skehel, 2002). Influenza viruses of aquatic birds have been proposed as the ancestors of all influenza virus subtypes existing in humans and other animals. They are classified into subtypes based on antigenic differences in the two surface glycoproteins, the haemagglutinin (HA) and neuraminidase (NA). Currently, 15 HA subtypes and nine NA subtypes of influenza A virus are known, and all of these subtypes can be found in aquatic birds. Genetic variation of the HA and/or NA genes resulting in the emergence of new influenza virus strains has frequently been recognized. The mechanism of variation can be either ‘genetic drift’, which occurs as a result of point mutations, or ‘genetic shift’, which occurs as a result of genetic reassortment between two strains of influenza A virus. Differences in the sialic acid binding specificity of the HA protein are involved in determining the tropism of influenza virus. Most HA proteins of avian and equine influenza viruses preferentially recognize receptors containing the α(2,3)- linkage of sialic acid to sugar chains, and those of human viruses the α(2,6)-linkage. However, these generalizations should not be considered an “all-or-none” phenomenon, as various residues of not only the HA protein but also of the NA protein may play a role in determining tropism (Kobasa *et al.*, 1999, Matrosovich *et al.*, 2000). Recently, the construction of influenza virus recombinants which expressed either intact NA of influenza type B or a chimeric protein consisting of the HA of influenza type A and B was described (Flandorfer *et al.*, 2003, Horimoto *et al.*, 2003). A type A virus possessing a chimeric HA in which the entire ectodomain of the type A HA molecule was replaced with that of the type B HA, protected mice from challenge by a wild-type B virus (Horimoto *et al.*, 2003). A type A/B chimeric virus carrying not only the chimeric (A/B) HA, but also the full-length type B NA instead of the type A NA, resulted in (A/B) HA/NA chimeric viruses possessing type B HA and NA ectodomains in the background of a type A virus. These chimeric viruses were attenuated in both cell culture and mice as compared with the wild-type A virus (Horimoto *et al.*, 2004).

5.4. Chimeric flaviviruses

The flaviviruses consist of nearly 80 viruses that are distributed worldwide. Most flaviviruses are arthropod-borne and have been classified on the basis of serological data, vector transmissibility and phylogenic analysis (Monath & Heinz, 1996). Many of the arthropod-borne flaviviruses are important

human pathogens responsible for diverse illnesses, including yellow fever (YF), West-Nile fever (WNF) Japanese encephalitis (JE), tick-borne encephalitis (TBE), and dengue fever. Flaviviruses are small enveloped viruses which contain a positive-strand RNA genome of ca. 11 kb. Translation of the genome results in the synthesis of a single polyprotein precursor that is co- and posttranslationally processed into three structural proteins, *i.e.*, capsid (C), pre-membrane (prM) and envelope (E) protein, and the nonstructural (NS) proteins NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. Many flaviviruses are neurotropic, exhibiting various degrees of neuroinvasiveness and neurovirulence in experimentally infected rodents and primates.

A live-attenuated YF virus vaccine, strain 17D, has been developed by serial passage of the wild type virus in animals and cultured cells. The live YF virus vaccine is used extensively throughout the world and has an impressive safety and efficacy record (Monath, 1999). The YF17D vaccine is the paradigm for other live attenuated flavivirus vaccines. Live-attenuated vaccines offer the potential advantages of a single dose and delivery of the complete repertoire of antigens that closely resemble the pathogen against which they are meant to protect. The live vaccine may induce a cytokine milieu similar to that following natural infection, stimulating innate immunity and induction of strong immunologic memory and a complete and long-lasting humoral and cell-mediated response. Attempts to develop safe and effective live-attenuated vaccines against dengue and tick-borne encephalitis have not yielded licensed products and a live attenuated vaccine against Japanese encephalitis is approved for use only in China. Recent advances in recombinant DNA technology have made it possible to explore a novel approach for developing live attenuated flavivirus vaccines against other flaviviruses. Full-length cDNA clones allow the construction of infectious virus bearing attenuating mutations or deletions incorporated in the viral genome. It is also possible to create chimeric flaviviruses in which the structural protein genes for the target antigens of a flavivirus are replaced by the corresponding genes of another flavivirus.

The majority of chimeric flaviviruses that have been constructed thus far are between two vector-borne flaviviruses. These chimeric flaviviruses have mainly been constructed in an attempt to develop vaccines against flaviviruses that cause disease in humans (Lai & Monath, 2003). Most of these chimeric viruses are derived from the infectious clone of YF17D (Rice *et al.*, 1989). YF17D has been used as a “vector” for engineering new vaccines against other flaviviruses, *e.g.*, chimeric vaccines against Japanese encephalitis virus (Chambers *et al.*, 1999, Monath *et al.*, 1999), against different serotypes of Dengue virus (Caufour *et al.*, 2001, Guirakhoo *et al.*, 2001, Guirakhoo *et al.*, 2002, Guirakhoo *et al.*, 2004, Guirakhoo *et al.*, 2000, Guirakhoo *et al.*, 1999, Van Der Most *et al.*, 2000) and against West-Nile virus (Arroyo *et al.*, 2001, Monath, 2001). Other examples include intertypic chimeric Dengue vaccines (Bray & Lai, 1991, Chen *et al.*, 1995, Pletnev & Men, 1998) and chimeric viruses against TBE using Dengue virus as a backbone (Pletnev *et al.*, 1992). In all chimeric flaviviruses generated thus far, either the C, prM, and E structural proteins or the prM and E structural proteins have been exchanged. Neutralizing antibodies specific for the prM, M and E structural flavivirus proteins provide the first line of defence. Flavivirus infections also induce cytotoxic T cells that recognize the structural proteins as well as several non-structural proteins in a type-specific or cross-reactive manner. Thus, by combining the structural genes (‘backbone’) of a highly safe vaccine

strain with the immunogenic proteins of another strain, new chimeric vaccines can be generated which should be safe for immunization of humans. Encouraging results from preclinical and clinical studies have shown that several chimeric flavivirus vaccines have the safety profile and satisfactory protective efficacy to warrant further evaluations in humans. However, some caution should be respected in view of recent results which show that the prM and E proteins may determine neuroinvasiveness of flaviviruses. A chimeric flavivirus (MOD/YF) was constructed consisting of the YF17D backbone in which the prM and E genes were replaced by those of the murine flavivirus Modoc virus. The MOD/YF virus, like the parent strain MOD from which it had acquired the prM and E structural proteins, but unlike YF, proved to be neuroinvasive in SCID mice (Charlier *et al.*, 2004). Animals developed neurological symptoms about 15 days after inoculation and died shortly thereafter. The distribution of MOD/YF in the brain of infected mice was similar to that observed in MOD-infected mice. These observations provide compelling evidence that the determinants of neuroinvasiveness of Modoc virus are entirely located in the envelope proteins prM and E. However, it should be noted that SCID mice are severely immunocompromized.

5.5. Other chimeric viruses

Classical swine fever virus (CSFV), bovine diarrhoea virus (BVDV) and border disease virus (BVD) belong to the Pestivirus genus of the Flaviviruses. Chimeric CSFV viruses in which the gene encoding either envelope protein E^{RNS} or envelope protein E2 was replaced by the homologous gene of BVDV were generated. The recombinant with the E2 replacement showed a slower growth rate on swine kidney cell line SK6 than the recombinant with the E^{RNS} replacement or the parent strain. Replacement of E^{RNS} or E2 did not alter cell tropism for SK6 or foetal bovine epithelial cells. Vaccination of pigs resulted in complete protection against a lethal CSFV challenge, and both chimeras induced E2 or E^{RNS} antibodies which could be discriminated from those induced after wild-type virus infection (de Smit *et al.*, 2001, van Gennip *et al.*, 2000). An inverse chimeric virus, *i.e.*, a chimeric BVDV recombinant in which the E2 gene was replaced by the E2 gene of CSFV was recently described. Efficient growth of the chimeric virus could only be demonstrated on porcine cells and, in contrast to the parental BVDV strain, the chimeric virus only inefficiently infected and propagated in bovine cells. These results indicated that the tropism of the virus was dictated by the origin of E2 protein (Reimann *et al.*, 2004). This conclusion was corroborated by the results obtained with another chimeric pestivirus, *i.e.*, a recombinant BVDV in which the E2 gene was replaced by the E2 gene from BDV. BVDV isolates infect cultured Madin-Darby bovine kidney (MDBK) cells as efficiently as sheep kidney cells. In contrast, BDV propagates poorly in MDBK cells but infects sheep cells very efficiently. As expected, the chimeric BVDV-E2(BDV) virus retained the ability to multiply in sheep cells but experienced a remarkable reduction in its ability to propagate and form plaques in MDBK cells (Liang *et al.*, 2003).

Arteriviruses are enveloped, positive-strand RNA viruses for which the two major envelope proteins GP5 and M occur as disulfide-linked heterodimers. In order to assess whether these proteins are involved in tropism, the ectodomains of GP5 and M were replaced by their homologous counterparts from related viruses. Using an equine arteritis virus (EAV) infectious cDNA clone, chimeras in which

the ectodomain of GP5 was replaced by the corresponding ectodomain of GP5 from the arteriviruses porcine reproductive and respiratory syndrome virus (PRRSV) or mouse lactate-dehydrogenase elevating virus (LDV) were generated. Although the two viable GP5 chimeras were attenuated, they were still able to infect baby hamster kidney (BHK-21) and rabbit kidney (RK-13) cells, which can be infected by EAV, but not by either PRRSV or LDV. This implies that the ectodomain of the major glycoprotein GP5 is not the main determinant of EAV tropism in cell culture (Dobbe *et al.*, 2001). Similar results were reported for a chimeric PRRSV in which the ectodomain of the M protein was replaced by the corresponding domain of LDV, EAV or a PRRSV-isolate from the US. Characterization of the chimeric viruses revealed that they had retained their ability to infect porcine cells and had not acquired tropism for cells susceptible to the viruses from which the foreign ectodomains were derived (Verheije *et al.*, 2002).

Coronaviruses are able to generate diseases in a number of different of animals. Transmissible gastroenteritis coronavirus (TGEV) is a coronavirus of swine. Whereas some TGEV strains mainly cause enteric disease, others cause severe respiratory disease. Recombinant TGEV strains were generated by a double crossover in the S gene that resulted in replacement of most of the S gene of a attenuated respiratory strain by the S gene of an virulent enteric isolate. The recombinant virus was virulent and showed an enteric tropism demonstrating that a change in the tropism and virulence of TGEV can be engineered by sequence changes in the S gene (Sanchez *et al.*, 1999).

Mouse hepatitis virus (MHV), another member of the coronaviruses, provides a useful model to study virus-induced neurological disorders. Both the MHV membrane (M) protein and the spike (S) glycoprotein have been implicated as determinants of viral pathogenesis. Chimeric viruses were produced in which the M gene of MHV-A59, a mildly neurovirulent strain, was substituted with the M gene of MHV-2, a non-neurovirulent strain, by targeted recombination. Viruses were then analysed for their biological properties following intracerebral inoculation. Chimeric viruses had a phenotype similar to MHV-A59 indicating that the replacement of the M gene is insufficient to produce a phenotype that lacks encephalitis similar to MHV-2 (Lavi *et al.*, 1998). By using targeted RNA recombination to introduce the S gene of the highly neurovirulent strain MHV-4 into the genome of MHV-A59, the role of the S gene in neurovirulence has been addressed (Phillips *et al.*, 1999). In cell culture, the chimeric virus containing the MHV-4 S gene exhibited a small-plaque phenotype and replicated to low levels, similar to wild-type MHV-4. However, intracranial inoculation of mice revealed a marked alteration in pathogenesis. Relative to wild-type controls, the MHV-4 S gene chimera exhibited a dramatic increase in virulence and viral antigen staining and inflammation in the central nervous system, without an apparent increase in the level of viral replication in the brain. These studies demonstrated that the S gene of a neurovirulent strain is sufficient to confer a highly neurovirulent phenotype to a mildly neurovirulent virus. Further mapping of the neurovirulence determinants indicated that interactions of multiple regions of the MHV S protein, including the hypervariable region of the S1 subunit, act in concert to allow for efficient infection of, and virulence in, the murine central nervous system (Phillips *et al.*, 2001, Phillips & Weiss, 2001). Recombinant MHVs expressing chimeric S glycoproteins have also been used to map the receptor binding domain of the S protein (Tsai *et al.*, 2003).

Few examples are available of chimeric viruses from non-enveloped viruses other than the chimeric adenovirus and AAV recombinants discussed above. Porcine circovirus type 2 (PCV2) is the primary causative agent of postweaning multisystemic wasting syndrome (PMWS), whereas the ubiquitous porcine circovirus type 1 (PCV1) is nonpathogenic for pigs. A chimeric PCV1-2 virus was constructed which contained the PCV2 capsid gene cloned in the backbone of the nonpathogenic PCV1 genome. Similarly, a reciprocal chimeric PCV2-1 virus was constructed by replacing the PCV2 capsid gene with that of PCV1. The PCV1, PCV2, and chimeric PCV1-2 and PCV2-1 viruses were all shown to be infectious in PK-15 cells. The chimeric PCV1-2 virus was attenuated in pigs and was able to induce protective immunity against wild-type PCV2 (Fenaux *et al.*, 2004, Fenaux *et al.*, 2003).
Natural poliovirus isolates that proved to be recombinants between type-3, -2 and -1 (Swayne *et al.*, 2003) or between type-3 and -2 (Blomqvist *et al.*, 2003) polioviruses have been isolated from healthy vaccinees. Although the recombinants expressed a chimeric VP1 capsid protein and showed altered antigenic properties when probed with monoclonal antibodies, they retained their type-3 serotype since the virus reacted with polyclonal sera raised against type 3 but not against type 2 or 1.

Table 3. Characteristics of major animal virus families				
Family	**Genome**[a]	**Envelope**	**Entry route**	**Representative(s)**
dsDNA viruses				
Adenoviruses	Linear (34-42)	No	Endocytosis	Human adenovirus type-2
Herpesviruses	Linear (120-230)	Yes	Surface	Herpes simplex virus
Papovaviruses	Circular (8)	No	Endocytosis	Human papillomavirus type-16
Polyomaviruses	Circular (5)	No	Endocytosis	SV40
Poxviruses	Linear (130-375)	Yes	Endocytosis	Vaccinia virus
ssDNA viruses				
Parvoviruses	Linear (5)	No	Endocytosis	Human parvovirus B19 Adeno-associated virus
Circoviruses	Circular (2)	No	Endocytosis	Chicken anemia virus
Reverse-transcribing viruses				
Retroviruses	Linear (7-11)	Yes	Endocytosis / Surface	HIV, murine leukaemia virus
Hepadnaviruses	Circular (3)	Yes	Endocytosis	Hepatitis B virus
Negative-stranded RNA viruses				
Arenaviruses	2 linear (5-7)	Yes	Endocytosis	Lassa fever
Bornaviruses	linear (9)	Yes	Endocytosis	Borna disease virus
Bunyaviruses	3 linear (10-23)	Yes	Endocytosis	Hantaan virus
Filoviruses	linear (19)	Yes	Endocytosis	Ebola virus
Orthomyxoviruses	8 linear (12-15)	Yes	Endocytosis	Influenza A virus
Paramyxoviruses	linear (15-16)	Yes	Surface	Respiratory syncytial virus, Sendai virus, parainfluenza
Rhabdoviruses	linear (11-15)	Yes	Endocytosis	Rabies virus, vesicular stomatitis virus
Positive-stranded RNA viruses				
Arteriviruses	Linear (13)	Yes	Endocytosis	Equine arteritis virus
Astroviruses	Linear (7-8)	No	Endocytosis	Human astrovirus type-1
Caliciviruses	Linear (8)	No	Endocytosis	Norwalk virus
Coronaviruses	Linear (20-33)	Yes	Endocytosis / Surface(?)	Mouse hepatitis virus, SARS-Coronavirus
Flaviviruses	Linear (10-12)	Yes	Endocytosis	Yellow fever virus, Dengue
Picornaviruses	Linear (7-8)	No	Endocytosis / Surface(?)	Poliovirus, foot-and-mouth disease virus
Togaviruses	Linear (10-12)	yes	Endocytosis	Semlike forest virus, Sindbis virus
dsRNA viruses				
Reoviruses	10-12 Linear (18-30)	No	Surface(?)	African horse sickness virus, Rotavirus, Bluetongue virus
Birnaviruses	2 Linear (6)	No	Endocytosis	Infectious bursal desease virus

[a] number of segments, conformation, (size in kb)

6. VIRAL FACTORS AND HOST FACTORS THAT DETERMINE HOST-RANGE AND TROPISM

In order for a virus to be successful, it has to be able to enter a host, establish a productive infection, and produce sufficient progeny virions to allow transmission to a new host. A number of obstacles are encountered along this way. These obstacles occur at two levels, *i.e.*, at the cellular level, and the organismal level. At the cellular level, the virus first has to find a way to recognize and bind to a suitable host cell. Subsequently, the virus has to enter the cell and it has to be able to complete its full repertoire of genome replication, protein synthesis, virus assembly and release. Furthermore, it has to deal with antiviral responses that are mounted by the infected cell. At the organismal level, apart from physical barriers, the virus has to cope with different types of innate and adaptive immune responses in order to survive long enough to allow successful transmission to a new host. Although this is a formidable task for any virus, many viruses have succeeded in doing so, each in their own specific ways. However, success generally does not come instantly. Only by a process of constant adaptation and by co-evolution of viruses with their hosts, this goal has been achieved - not in the least due to the genetic plasticity of viruses. Below, an overview is given of both viral as well as host factors that are involved in different host-pathogen interactions and which may determine host-range and tropism.

6.1. Receptor and co-receptor usage

Binding of viruses to cells is mediated by the interaction between viral attachment or entry proteins and cell surface components (‘receptors’). Three different types of cellular receptors can be distinguished, *i.e.*, (1) low-affinity receptors, (2) high-affinity receptors, and (3) co-receptors. Low affinity receptors usually consist of cell surface carbohydrates such as sialic acid or glycans. High-affinity receptors and co-receptors generally consist of specific cell surface proteins which are involved in cell-cell interactions and in the recognition of extracellular components such as cytokines, growth factors, hormones, etc. Some viruses need co-receptors for infectivity since the primary receptor is only used to capture the virus but does not mediate viral entry. Binding to the primary receptor triggers exposure of envelope protein domains that mediate downstream interactions resulting in virus entry. Examples of post-attachment entry coreceptors include CCR5 and CXCR4 for HIV-1 (Deng *et al.*, 1996, Feng *et al.*, 1996, Reeves *et al.*, 2004) and CD81 for hepatitis C virus (Cormier *et al.*, 2004). Viruses from the same family can bind to many different cellular receptors, for instance retroviruses. On the other hand, the same surface molecule can serve as receptor for several different viruses, for instance sialic acid. Generally speaking, viruses which recognize a cell surface molecule that is present on a wide variety of cells have a broad tropism, whereas viruses which recognize a surface molecule that is highly cell-type specific have a narrow tropism.
The structure of viral entry proteins varies from ‘spikes’ which consist of a stem and a globular head, such as those of reoviruses, adenoviruses, orthomyxoviruses and paramyxoviruses, to relatively ‘flat’ structures, such as those of the alphaviruses. The topologies of the non-enveloped reovirus and adenovirus attachment proteins show overall similarities, as do the envelope proteins from enveloped viruses, such as orthomyxoviruses and paramyxoviruses. The structures of the envelope proteins from flaviviruses and alphaviruses are similar to each other, which - in combination with similar functions -

might indicate the existence of a common ancestor for these proteins. Viral envelope proteins have been divided into two classes based on (1) their mechanism of action, (2) whether the protein is cleaved, and (3) whether the protein is complexed with other viral proteins. Envelope proteins that contain coiled-coils, such as those encoded by orthomyxo-, paramyxo-, retro-, filo-, and coronaviruses, have been designated class I fusion proteins and those of alphaviruses and flaviviruses have been designated class II fusion proteins (Dimitrov, 2004). Many class I envelope proteins are proteolytically cleaved to yield membrane-anchored subunits which contain N-terminal fusion peptides. However, the spike proteins of some coronaviruses are not cleaved and remain trimeric throughout the fusion process. Class II envelope proteins are not proteolytically cleaved and have internal, rather than N-terminal fusion peptides. Activation of the fusogenic potential of class II envelope proteins is accomplished by the proteolytic cleavage of an accessory membrane glycoprotein (prM) that is complexed with the fusion protein (Heinz & Allison, 2001). Activation of class I proteins is dependent on a conformational change that is induced either by low pH, *e.g.*, HA of influenza virus, or by protein-protein interactions by another attachment protein upon binding of the latter protein to the cellular receptor, *e.g.*, activation of the F protein by the HN protein in paramyxoviruses. The G proteins of the rhabdoviruses VSV and rabies virus have characteristics in common with both class I and class II fusion proteins. They have an internal fusion peptide, are not cleaved, and are not complexed with other proteins at the virion surface.

6.2. Entry and transport

Viruses use two main routes to enter the cell, *i.e.*, the endocytic and non-endocytic route (Dimitrov, 2004). The endocytic route is by uptake and transport in clathrin-coated vesicles, non-clathrin coated pits, or caveolae, or by macropinocytosis. The non-endocytic route involves direct fusion of the viral envelope and the cell membrane at neutral pH. Viruses that use endocytosis contain entry proteins which undergo a low-pH dependent conformational change that triggers fusion and penetration. Endocytosis is crucial to these viruses because of the acidification occurring within the endosomal pathway. It is becoming clear that many viruses without a strict low-pH step for entry can also enter cells via endocytosis, *e.g.*, HIV-1. In order to exit the endosome and gain access to the cytosol, enveloped viruses such as alphaviruses and influenza virus fuse with the endosomal membrane, whereas non-enveloped viruses such as adenovirus, reovirus, rhinovirus or parvovirus do so either by a pore-forming mechanism or by lysing the endosomal membrane.

The strict dependence on low-pH is an important limitation that prevents virions from fusion with the plasma membrane under normal conditions. When artificially introduced into the cytoplasm by direct fusion at the plasma membrane by acidifying the medium, some viruses fail to initiate genome replication and protein synthesis in certain cell-types. This has, for instance, been shown for SFV and VSV using baby hamster kidney (BHK) cells and Chinese hamster ovary (CHO) cells. Whereas BHK cells were infected efficiently following fusion in endosomes or at the plasma membrane, CHO cells were only infected following fusion from endocytic organelles (Marsh & Bron, 1997). Also, targeted adsorption of engineered herpes virions in which gC was fused to EPO resulted in internalisation by cells expressing the EPO-receptor, but productive infection did not ensue, indicating that the natural

route of infection was not used (Laquerre *et al.*, 1998).These examples indicate that in certain cell-types a barrier other than the plasma membrane can prevent infection. One possible barrier would be the endocytic pathway which provides a trafficking mechanism to bring viral particles to the correct sites in the cytoplasm where replication can proceed.
Trafficking depends on specific post-internalisation membrane sorting and, in some cases, direct interactions of virus particles with the cytoskeleton (Sodeik, 2000). Two endocytic trafficking pathways exist in the cell, *i.e.*, a recycling pathway and a lysosome-targeting pathway (Gruenberg, 2001, Sieczkarski & Whittaker, 2002). The internalised vesicle acquires properties that are defined temporally and are thus termed ‘early‘ and ‘late’ endosomes. The early endosome is a major sorting station where internalised cargo can either be delivered back to the plasma membrane (the recycling pathway) or can progress to the late endosome. Late endosomes contain internal vesicles (multi-vesicular bodies) and differ from early endosomes in that they have a significantly lower pH. Late endosomes subsequently progress to lysosomes, which are characterized by the presence of degradative proteases and hydrolases which are delivered by the trans-Golgi network.
Virions, subviral particles, viral genome-protein complexes and viral proteins are transported within the host cell cytoplasm during and after viral entry from the plasma membrane to the site of viral replication, and during egress from the sites of synthesis of viral components and virus assembly to the plasma membrane. This cytoplasmic transport depends on a complex network of three cytosolic filaments, *i.e.*, microtubules, intermediate filaments and microfilaments. For viral macromolecular complexes, there are two different ways to manoeuvre inside a cell, *i.e.*, (1) inside a cellular or virally modified membrane, or (2) directly within the cytosol. In the first case, the vesicle interacts with the cytoskeleton, whereas in the second a viral component binds to the cytosolic transport machinery (Sodeik, 2000). Viruses that are released into the medium after cell lysis presumably do not require specific transport mechanisms. However, many enveloped viruses assemble at internal membranes or the plasma membrane. Although integral viral membrane proteins are transported using host membrane traffic, little is known about how viral genomes or capsids are targeted to specific organelles for assembly. Once the viruses have budded into the lumen of secretory or endocytic organelles, transport to the cell surface seems to use the same mechanisms as that of host components.

6.3. Nuclear import

The genomes of most DNA viruses as well as retroviruses and lentiviruses are delivered to the nucleus where they are either integrated into the host chromosome or maintained as episomal DNA. Before the viral genome can enter the nucleus, the capsid that encloses the genome is either partially disassembled in the cytosol or is docked at the nuclear pore complex. The nuclear pore complex spans the double membrane of the nuclear envelope and controls macromolecule transport into and from the nucleus. Cellular factors, such as importins and karyopherins, which interact with nuclear localization sequences are recruited that facilitate capsid disassembly and import of the viral nucleic acid-protein complex into the nucleus (Greber & Fassati, 2003). Lentiviruses uncoat and reverse transcribe their RNA genome into DNA within the cytoplasm. They then recoat the DNA and drive it

into the nucleus using multiple factors, including redundant nuclear localisation signals, import receptors, conformational changes and unknown cellular factors. For unknown reasons, retroviruses are unable to enter the nucleus similar to lentiviruses and are dependent on breakdown of the nuclear envelope during mitosis in order to access the host chromatin. Large DNA viruses, such as adenovirus and herpesviruses, dissociate their DNA from the capsid prior to nuclear import. Small DNA viruses are thought to maintain their genome in an encapsidated state until they arrive in the nucleus.
For influenza virus, the available evidence suggests that the nucleoprotein and the polymerase complex accompany the RNA into the nucleus where they are required for transcription and replication (Whittaker *et al.*, 2000). Since, at least for some viruses, nuclear import requires specific interactions between viral proteins or viral structural components and host cell factors, these interactions may be utilized by the host cell to restrict access to the nucleus, thereby inhibiting viral replication (see below).

6.4. Interference with interferon responses

Infected cells respond to viral infections by mounting anti-viral responses. The most powerful anti-viral defence mechanism is mediated by interferons (Goodbourn *et al.*, 2000, Pestka *et al.*, 2004).
Interferons are cytokines that are produced upon viral infection in many different cell-types. Type I interferons consist of interferon-α (IFN-α) which is predominantly produced by leukocytes, and IFN-β which is synthesized by most cell-types but particularly by fibroblasts. Type II interferon consists of IFN-γ and is synthesized in response to the recognition of infected cells by activated T lymphocytes and natural killer (NK) cells. Type I interferons are induced by double-stranded RNA (dsRNA) as a result of viral replication and transcription. Interferon production results in transcriptional activation of many cellular genes which results in the production of proteins that actively participate in the inhibition of viral replication. One protein that is upregulated by type I interferons and which is also directly activated by dsRNA is the dsRNA-dependent protein kinase R (PKR). Activated PKR is able to phosphorylate the translation-initiation factor eIF-2α which leads to a general inhibition of protein synthesis in infected cells. Furthermore, type I interferons activate the 2'-5'- oligoadenylate cyclase (OAS) system which results in the synthesis of 2'-5'-oligoadenylates which bind with high affinity to endoribonuclease L (RNaseL) and induces its activation via dimerization. Activated RNaseL cleaves both messenger RNA (mRNA) and ribosomal RNA (rRNA) in the cytoplasm of the cell, leading to an inhibition of protein synthesis. Other antiviral activities of interferons include the induction of Mx proteins which confer resistance to infection by several RNA viruses, caspases which are involved in apoptosis, and anti-proliferative responses which inhibit cell growth and thereby replication of some viruses.
The induction of interferons not only results in antiviral activities within infected cells. Interferons are secreted by infected cells and are able to trigger an antiviral state in surrounding cells by a process known as interferon-signalling. Binding of IFN-α/β to their cognate receptors on the surface of cells results in activation of the Jak/STAT signalling pathway. The ultimate outcome of this signalling is the transcriptional activation of target genes that encode proteins involved in different types of antiviral activities such as the ones described above.

In order to successfully infect and replicate in their hosts, viruses have developed means to circumvent the interferon response either by (1) inhibiting the production of interferon, (2) interfering with interferon signalling, or (3) by directly inhibiting interferon-induced antiviral effector molecules (Goodbourn *et al.*, 2000, Levy & Garcia-Sastre, 2001, Sen, 2001, Taniguchi & Takaoka, 2002). The ability of viruses to cope with the cellular interferon response may be an important factor that determines viral tropism. This has recently been shown for members of the negative-stranded RNA viruses. Many of these viruses are able to counteract IFN-α/β responses by expressing non-essential accessory proteins such as the V (respiroviruses, rubulaviruses), C (respiroviruses) or NS proteins (pneumoviruses, influenza virus) that antagonize the intracellular signalling pathways downstream of the activated IFN-α/β receptor (Bossert & Conzelmann, 2002, Didcock *et al.*, 1999a, Didcock *et al.*, 1999b, Garcia-Sastre *et al.*, 1998a, Garcia-Sastre *et al.*, 1998b, Garcin *et al.*, 2001, Garcin *et al.*, 2000, Garcin *et al.*, 1999, Garcin *et al.*, 2002, Huang *et al.*, 2003, Komatsu *et al.*, 2000, Park *et al.*, 2003a, Park *et al.*, 2003b, Schlender *et al.*, 2000, Young *et al.*, 2000). The ability of these viruses to interfere with the antiviral interferon response has been shown to be species specific and in some cases also organ specific. These observations indicate that host range and tissue tropism can be determined by viral factors that are able to interfere with the interferon response. This conclusion is corroborated by experiments using knock-out mice which are unable to mount an interferon response. For instance, ablation of the IFN-α/β response altered the apparent cell and tissue tropism of Sindbis virus and rendered cells from the macrophage-dendritic cell lineage permissive to infection (Ryman *et al.*, 2000). Also, replication of influenza virus strain A/WSN/33, which is normally restricted to the lungs of wild-type mice, was shown to be systemic in STAT1-/- knock-out mice (Garcia-Sastre *et al.*, 1998a). Another example concerns the poxvirus strain MVA. MVA is a highly attenuated vaccine strain that has lost multiple genes involved in immunomodulation including two viral proteins that function as soluble receptors for IFN-α/β and IFN-γ. However, it still expresses two proteins, K3L and E3L that function within the infected cell to block interferon-induced antiviral proteins such as PKR and OAS. Whereas MVA replicated to high titres in primary chicken embryofibroblasts (CEF) and BHK-21 cells, an MVA-ΔE3L deletion mutant was unable to replicate in CEF cells, while replication in BHK-21 cells was unaffected. This host range phenotype in CEF cells was associated with deficient viral protein and DNA synthesis and enhanced production of IFN-α/β (Hornemann *et al.*, 2003).
Since the specific protein-protein interactions that underlie the different mechanisms involved in blocking the interferon response may be tissue or species specific, it is conceivable that the (in)ability of many viruses to interfere with the interferon-response of the host is an important determinant for virus tropism in general. In other words, the tropism of a virus will, among others, be determined by whether or not it is able to successfully circumvent the interferon response in a given cell-type or host. This would also predict that virus mutants or recombinants could arise which have gained the ability to block the interferon response of an otherwise non-permissive host, thereby extending their host range. This has, for instance, been shown for SV5, a virus that is able to block interferon signalling in human, monkey and canine cells, but not in murine cells. In certain murine cells this virus is able to establish a low-grade persistent infection in which the viruses fluxes between active and repressed states in response to local interferon production. Mutants that were better able to replicate in murine cells were

selected and one isolate with a single amino acid substitution in the V protein was shown to be able to block interferon signalling in murine cells (Young *et al.*, 2001). Indeed, it cannot be excluded that emerging viral zoonotic diseases such as Nipah and Hendra virus infections in humans are the result of host range extension based on the ability of these viruses to evade the host immune response (Rodriguez & Horvath, 2004).

6.5. Interference with general immune responses

In order for viruses to survive in the presence of an active immune response, they have developed mechanisms to evade the host's immune system or to actively interfere with the immune response. These mechanisms include, (1) downregulation of expression of viral gene products (*e.g.*, during persistence or latency), (2) infection of areas with little surveillance by the immune system (*e.g.*, neurons), (3) infection of immune cells (T-cells and B-cells), thereby inhibiting their immune functions, and (4) expression of viral gene products that directly interfere with the immune response.
With respect to the latter class: interference with the antiviral interferon response has been discussed above. Other strategies include down-regulation of MHC class I antigen presentation, interference with the action of cytokines and chemokines, and the inhibition of apoptosis.
Viral interference with MHC Class I antigen processing and presentation can take place at virtually every step along the pathway from the generation of peptides to their display by MHC class I molecules at the cell surface (Fruh *et al.*, 1999, Hewitt, 2003, Yewdell & Bennink, 1999) (Fig. 1). Most peptides presented by class I molecules are produced from a cytosolic pool of proteins by the action of proteasomes. Some viral proteins interfere with peptide generation by blocking proteasome digestion. Import of cytosolic peptides into the endoplasmic reticulum (ER) via the peptide transporter TAP can be blocked, both on the cytosolic side and the luminal side, by specific viral proteins. Other viral proteins can redirect the newly synthesized class I molecules to the cytosol for proteasomal destruction. Still other viral proteins interact with class I molecules, resulting in their retention in the ER or Golgi-complex or in targeting from the ER to lysosomes for destruction. Lysosomal targeting of class I molecules by viral proteins can also occur from the trans-Golgi complex or from the cell surface (Fig. 1).

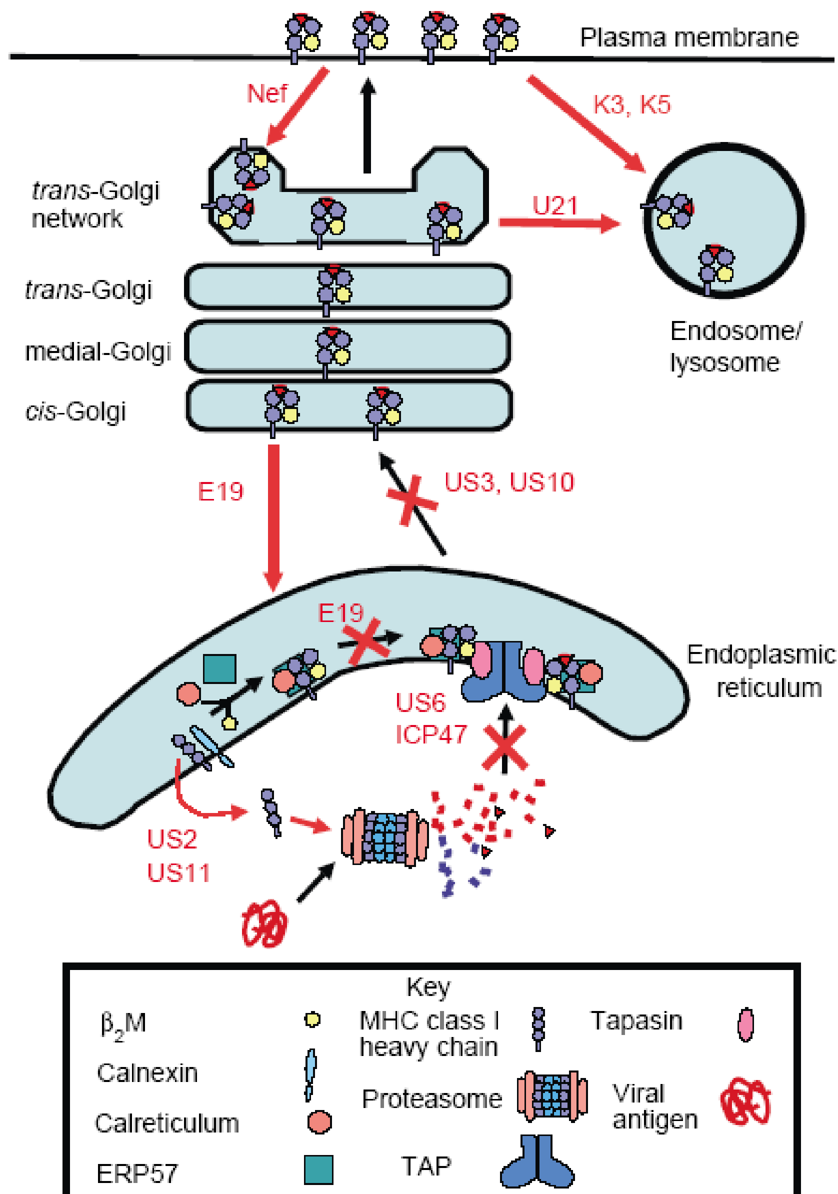

Figure 1. Viral inhibition of the MHC class I antigen presentation pathway.

Viruses have evolved proteins that inhibit the MHC class I pathway at virtually every step. The human Cytomegalovirus (hCMV) proteins US2 and US11 dislocate the MHC class I heavy chain into the cytosol where it is degraded by the proteasome. HSV ICP47 and hCMV US6 inhibit peptide translocation by TAP. Adenovirus protein E19 inhibits MHC class I association with TAP and also inhibits trafficking by retrieving MHC class I molecules from the *cis*-Golgi. Similarly, hCMV US3 and US10 inhibit the ER export of MHC class I molecules. The human herpesvirus 7 U21 protein diverts MHC class I molecules to the lysosome. The HIV-1 Nef protein down-regulates MHC class I molecules from the plasma membrane, sequestering them in the *trans*-Golgi network. Finally, K3 and K5, encoded by Kaposi's sarcoma-associated herpesvirus, down-regulate MHC class I molecules and sort them into the late endocytic pathway where they are degraded.

Source: Hewitt, E.W. *Immunology* (2003) **110**:163-169.

Cytokines and chemokines (chemoattractant cytokines) play a key role in the initiation and regulation of the innate and adaptive immune responses. In order to cope with these responses, viruses have developed mechanisms to interfere with the production, activity and signal transduction of cytokines and chemokines. Apart from proteins that interfere with antigen presentation (see above) and inflammation, viral immunomodulatory proteins mainly consist of homologs of cellular cytokines ('virokines'), and cytokine receptors ('viroceptors'). Viral homologs of host genes involved in the immune system are mainly found in large DNA viruses (herpesviruses and poxviruses) and their existence suggests that viruses have 'stolen' genes from the host. Virokines are generally secreted and mimic host molecules such as cytokines thereby redirecting the immune response for the benefit of the virus. Viroceptors can be either secreted or localized in the cell surface and act as 'decoys' to sequester cellular cytokines thereby inhibiting their action (Alcami & Koszinowski, 2000, Haig, 2001, Johnston & McFadden, 2003, Johnston & McFadden, 2004, Lorenzo *et al.*, 2001, Moss & Shisler, 2001, Ploegh, 1998, Seet *et al.*, 2003). In view of the immunomodulatory functions of many herpesviruses and poxviruses genes it is not surprising to see that deletion of such genes results in an attenuated phenotype. Indeed, the attenuated phenotype of the poxvirus vaccine vectors MVA has been shown to be due to the loss of several of the genes encoding immunomodulatory virokines and viroceptors (Blanchard *et al.*, 1998). The interactions of virokines, viroceptors and other viral immunomodulatory proteins may be species specific, as has for instance been shown for the IFN-γ binding proteins of vaccinia virus and ectromelia virus (Mossman *et al.*, 1995). Therefore, they represent another class of factors that determine tissue and/or host tropism of viruses.
In addition to inducing immune and inflammatory responses, infection by most viruses triggers apoptosis or programmed death of the infected cell. This interferes with the objective of most viruses to generate large numbers of progeny virions and to spread these virions to new cells, tissues and hosts. Therefore, viruses have evolved strategies to modulate the process of apoptosis and to use it to their advantage either by inhibiting apoptosis to allow for the production of sufficient progeny, or to trigger apoptosis in order to kill the host cell at the end of the viral infectious cycle (Aubert & Jerome, 2003, Benedict *et al.*, 2002, Roulston *et al.*, 1999, Shisler & Moss, 2001). Cell death by apoptosis may be advantageous for the virus since the entire cellular content, including progeny virions, is packaged into apoptotic bodies that are rapidly taken up by surrounding cells. This process suppresses the inflammatory response and allows the infection to spread. Since apoptosis is intimately linked with the function and regulation of the immune system, the ability of viruses to inhibit apoptosis could profoundly alter the immune response. Viral anti-apoptotic proteins could protect infected cells from apoptosis induced by cytotoxic lymphocytes. They could also alter antigen cross-presentation and the priming of the immune response, or modulate the expression of danger signals from the sites of infection. Since the interaction of viral factors with apoptotic and/or pro-apoptitic host cell factors may be cell-type or species specific, they may also be involved in determining the tissue and host tropism of viruses.

6.6. Host restriction factors

Although the adaptive and innate immune responses are primarily responsible for providing protection against viral pathogens, it is becoming increasingly clear that dominant inhibitory host gene products can also have an important role in controlling susceptibility to viral infection. These post-entry host cell barriers or 'restriction factors' determine whether or not a virus is able to replicate in a given cell or host. The best studied restriction factors are those governing replication of retroviruses (Bieniasz, 2003, Goff, 2004a, Goff, 2004b). The prototype retrovirus restriction factor is *Fv*1 which controls susceptibility of mice to leukemia induced by the Friend strain of murine leukemia virus (MLV). Several *Fv* genes modify immune responses to MLV and only exert their action upon inoculation of animals. However, two dominant genes, *Fv*1 and *Fv*4, are active in cultured cells derived from mice and exhibit substantial resistance to MLV infection. Remarkably, the *Fv*1 gene shows clear sequence similarity to the retroviral *gag* gene. Although the exact molecular mechanism by which *Fv* exerts its effect is still not completely elucidated, the available evidence indicates that it is fundamentally a trafficking block. *Fv*4 appeared to be a homologue of an *env* gene of a defective provirus that encodes an envelope protein which blocks the receptor for ecotropic MLV (Goff, 2004b, Ikeda & Sugimura, 1989). An *Fv*1-like activity is also present in human cells and is called Restriction factor-1 (*Ref1*). *Ref1* seems to act earlier than *Fv*1 in the course of infection by blocking reverse transcription of the retroviral RNA. Cell lines from rhesus macaque were found to be unable to support infection by HIV-1, although they were very permissive for the simian immunodeficiency virus of macaques (SIVmac). The factor that mediates resistance to HIV-1 and/or SIVmac in primates is termed lentivirus susceptibility factor-1 (*Lv1*). Recently, it was shown that *Ref1* and *Lv1* are species specific variants of tripartite interaction motif 5α (TRIM5α), a cytoplasmic component that blocks HIV-1 infection in rhesus macaque cells (Goff, 2004b, Hatziioannou *et al.*, 2004, Stremlau *et al.*, 2004).

An exciting mechanism of viral resistance against cellular defence mechanisms is represented by the interaction between the accessory protein *Vif* of HIV-1 and the cellular APOBEC3G protein (Harris *et al.*, 2003, Mangeat *et al.*, 2003, Sheehy *et al.*, 2002). The latter protein is present in human T lymphocytes and inhibits HIV-1 through a remarkable mode of action. APOBEC3G is a cytidine deaminase that is active on single-stranded DNA and converts cytidine to uridine. In infected cells the enzyme is incorporated into virion particles of HIV-1 *Vif*-mutants and upon infection of naïve cells it induces the deamination of single-stranded viral DNA that is synthesized by reverse transcriptase. This destroys HIV-1 replication through hypermutation of the genome. The *Vif* protein circumvents this host defence mechanism by binding to APOBEC3G, thereby targeting it for proteasomal degradation. Thus, unlike HIV-1 *Vif*-mutants, wild-type HIV-1 can replicate in human cells because it is able to interfere with the action of APOBEC3G. A single amino acid at position 128 of human and African green monkey APOBEC3G governs the virus-specific sensitivity of these proteins to *Vif*-mediated inhibition. This phenotype correlates with the ability of *Vif* to bind APOBEC3G and interfere with its incorporation into virions (Mangeat *et al.*, 2004). These results indicate that the interaction between *Vif* and APOBEC3G is an important determinant of the tropism of primate lentiviruses. Recently, it was shown that APOBEC3G is also able to inhibit replication of hepatitis B virus (HBV), suggesting that

this is the reason why 95% of individuals infected with HBV manage to escape chronic infection with the virus (Turelli *et al.*, 2004).

Host cell factors that are required for the production of progeny virus are also potential factors of virus restriction. Cyclophilin A (CypA), for example, is a cellular protein that is encapsidated in HIV-1 virions as a result of its interaction with the viral capsid protein. Encapsidation of CypA in virions seems to be a requirement for successful replication, since HIV-1 virions produced in the absence of CypA fail to establish infection of target cells because of a block at an early post-entry step. Incorporation of CyPA thus seems to relief the restriction of HIV-1 replication by inhibiting binding of cellular restriction factors. The observation that critical amino acid residues, which are involved in binding of the MLV capsid protein to the *Ref1* and *Fv*1 encoded TRIM5α proteins, overlap with those for CypA binding, raises the interesting possibility that CypA could compete with binding of TRIM5α (Goff, 2004b, Towers & Goff, 2003, Towers *et al.*, 2003).

Mx proteins are interferon-inducible proteins with antiviral activity against a wide variety of viruses (Lee & Vidal, 2002). The prototype Mx protein, Mx1, was identified in a mouse strain that proved to be resistant to influenza virus infection. Mx1 accumulates in the nucleus and inhibits influenza virus transcription, thereby inhibiting influenza virus multiplication (Staeheli *et al.*, 1983). A number of Mx proteins have been identified in higher eukaryotes including fish, birds and mammals. MxA, the human homolog of Mx1, accumulates in the cytoplasm and interferes with multiplication of orthomyxoviruses (Frese *et al.*, 1995, Marschall *et al.*, 2000, Pavlovic *et al.*, 1990), paramyxoviruses (Zhao *et al.*, 1996), bunyaviruses (Frese *et al.*, 1996) and togaviruses (Landis *et al.*, 1998). Recent evidence indicates that nuclear MxA suppresses influenza virus transcription by interacting with the polymerase protein, PB2, and the nucleoprotein, NP (Turan *et al.*, 2004). MxA also inhibits multiplication of hepatitis B virus, a DNA virus, by blocking export of viral mRNA from the nucleus (Gordien *et al.*, 2001). The observation that some Mx proteins act in the nucleus whereas others act in the cytoplasm suggests that Mx proteins may interfere with translocation of viral components between the nucleus and the cytoplasm.

6.7. Viral host range genes

In some cell-types, poxviruses fail to complete their reproductive cycle, a phenomenon known as host range restriction. Studies using mutant viruses have identified viral host range (hr) genes that rescue virus growth in otherwise restrictive cells. However, up to now, the cellular mechanism of host range restriction remains largely unknown. One of the exceptions is E3L of vaccinia virus, which binds to double-stranded RNA and downregulates PKR-mediated apoptosis (Xiang *et al.*, 2002). Another vaccinia virus protein, K1L, is required for activation of a host-encoded intermediate transcription factor in RK13 cells (Sutter *et al.*, 1994a). A third example is myxoma virus M11L, which was shown to be an anti-apoptotic protein that prevents the release of mitochondrial cytochrome C (Everett *et al.*, 2002). The cowpox virus protein CP77 was shown to rescue growth in HeLa cells of a vaccinia virus deletion mutant that lacked the hr genes K1L and C7L. CP77 allowed translation of viral intermediate genes which was blocked in the mutant virus (Hsiao *et al.*, 2004). Although most studies suggest that hr genes possess anti-apoptotic activities that antagonize host restriction, other studies suggest that apoptosis is a cellular stress response associated with host restriction.

An interesting host range restriction phenotype of influenza virus was shown to be determined by a single amino acid at position 627 in the PB2 gene The host range restriction phenotype was first characterized by efficient replication in avian tissue and failure to produce plaques in mammalian Madin-Darby canine kidney (MDCK) cells (Subbarao *et al.*, 1993). Recently, the same mutation was shown to be associated with pathogenicity since a single amino acid substitution from glutamic acid to lysine at position 627 of PB2 converted a nonlethal human H5N1 influenza A isolate into a lethal virus for mice. The mutation did not affect viral tropism among cells of different mouse organs but enhanced its ability to support efficient viral replication in mouse cells in general (Shinya *et al.*, 2004). Since overexpression of PB2 abrogates the antiviral activity of the Mx protein (Jin *et al.*, 1998), it is tempting to speculate that this mutation correlates with sensitivity for the Mx protein. However, the mouse strain used in the above study lacked a functional Mx protein.

Determinants of host range restriction have been shown to be polygenic for the Kansas strain of bovine PIV3 which showed a 100-1000 fold restricted replication in the respiratory tract of non-human primates compared to human PIV3. Both the N and P genes were the major determinants of host restriction suggesting that there may be multiple mechanism by which this restriction was achieved (Skiadopoulos *et al.*, 2003). Restriction of measles virus replication in a mouse epithelial cell line could be trans-complemented by overexpression of either the N-P-L or N-P functional protein complexes but not by P-L complexes or individual N, P or L proteins. Moreover, the use of human/mouse somatic hybrid cell lines indicated that the restriction phenotype could be complemented by (a) human cellular factor(s), possibly by stabilizing the viral polymerase templates (Vincent *et al.*, 2002).

Experiments with host range restricted isolates of minute parvovirus of mice (MVM) suggested that the activity of complexes formed by the non-structural (NS1 and NS2) polypeptides and recruited cellular factors restrict parvovirus DNA amplification in a cell-type-dependent manner and that NS functions may in addition determine MVM host range acting at postencapsidation steps of viral maturation (Rubio *et al.*, 2001). In addition to the NS proteins, also the capsid proteins (VP1 and VP2) of parvoviruses have been implicated in determining host range restriction (Bloom *et al.*, 1993, Horiuchi *et al.*, 1994). This tropism is not mediated via specific cellular receptors but by interactions with intracellular factors. The nature of these factors is unknown but most data point to a stage beyond the conversion of the single-stranded DNA genome by host cell DNA polymerase (Tijssen, 1999).

6.8. Host proteases required for processing of viral proteins

Many viral proteins require endoproteolytic cleavage for activation of their biological functions. One particular class is represented by viral surface (‘spike’) proteins that are involved in cell binding and entry (Klenk & Garten, 1994). In most cases, cleavage activation of viral spike proteins is required for the exposure of a highly hydrophobic protein domain that is able to induce fusion of the viral envelope with the plasma membrane. This fusion process can be triggered either by a low-pH or by specific protein-protein interactions. Examples of viral proteins that are activated by endoproteolytic cleavage are the HA protein of orthomyxoviruses (*e.g.*, influenza virus), the F protein of paramyxoviruses (*e.g.*, Sendai virus), the envelope protein of retroviruses and lentiviruses (*e.g.*, HIV-1), the E2 protein of togaviruses (*e.g.*, Semliki forest virus), the S protein of some coronaviruses (*e.g.*, Mouse hepatitis

virus), the gB protein of some herpesviruses (*e.g.*, human cytomegalovirus) and the GP protein of arenaviruses (*e.g.*, lymphocytic choriomeningitis virus). For some viruses, it is not the cleavage of the envelope protein itself but rather cleavage of a chaperone protein – for example, pE2 with pE1 in alphaviruses and prM with pE in flaviviruses - that results in a conformational change that leads to dissociation of the complex and subsequent activation of the envelope protein (Heinz & Allison, 2001). Cellular proteases that are involved in activation of viral entry proteins belong to a large family of ubiquitous calcium-dependent serine endoproteases, termed ‘subtilisin-like proprotein convertases’ (SPCs), of which furin (or SPC1) is the best studied (Nakayama, 1997). Furin is expressed in most cell-types and traffics between the trans-Golgi network (TGN), the endocytic pathway and the cell surface (Molloy *et al.*, 1999). It cleaves after a stretch of poly-basic amino acids containing the minimal recognition motif R-X-R/K-R, which is a common motif in many viral entry proteins. Other proteins that are involved in activation of viral entry proteins include trypsin-like endoproteases which are secreted by a limited number of cell-types mainly in the respiratory tract (Gotoh *et al.*, 1990).

Proteolytic activation of entry proteins can be an important determinant of tissue tropism and pathogenicity. This has been clearly shown, for instance, for NDV of which a large number of strains vary widely in their virulence for chickens (Nagai *et al.*, 1976). The F proteins of virulent and avirulent NDV strains differ in the amino acid sequence of the cleavage site. The F proteins of virulent strains are cleaved in almost all cell-types by a ubiquitous furin-like protease. In contrast, the F protein of avirulent strains is activated by trypsin-like proteases which are present in only a few cell-types. Thus, infection with avirulent strains results in a local infection that usually does not result in overt disease. On the other hand, infection with a virulent strain results in virus spread throughout the organism and results in fatal systemic infection (Nagai, 1995). Proteolytic cleavage has also been found to be the primary determinant for organ tropism and pathogenicity of Sendai virus in mice. The F protein of Sendai virus is cleaved by an organ-specific protease in the lungs. When other organs are infected, replication terminates after one cycle of replication because appropriate proteases which can cleave the F protein are lacking (Tashiro *et al.*, 1990). Cleavage of the HA protein is also an important determinant of influenza virus tropism and pathogenesis (Steinhauer, 1999). Pathogenicity for chickens correlates directly with the ability of these viruses to produce cleaved HA in infected cells. Cleavage depends on the presence of a sequence of basic residues in the HA protein. A notable exception is the HA protein of the WSN strain of influenza. This mouse-adapted strain shows multicyclic trypsin-independent replication in cell culture. Remarkably, this phenotype correlates with the presence of the NA protein instead of the HA protein. It appears that the NA protein of WSN has the property of a plasminogen-binding protein, thereby concentrating this plasmin precursor at the surface of viral envelopes and infected-cell membranes. After conversion to plasmin, the proteolytic activity of plasmin cleaves the adjacent HA protein thereby activating its fusion function (Goto & Kawaoka, 1998). The above examples illustrate that cleavage properties of viral entry proteins and the tissue distribution of activating proteases in the host are major factors for virus tropism and pathogenicity.

The proteins of viruses from families such as the picornaviruses and flaviviruses are produced from a single large precursor protein (polyprotein) that is processed to yield the ultimate functional viral

proteins. For other viruses, at least part of the genome is expressed as a polyprotein, *e.g.*, the polymerase proteins of coronaviruses and arteriviruses, the non-structural proteins of caliciviruses and togaviruses, and the both structural and non-structural proteins of retroviruses and lentiviruses. Processing of the viral proteins occurs *in cis* and *in trans* by virus encoded proteases and by host encoded proteases (Dougherty & Semler, 1993). The great diversity in both virus-encoded as well as cellular proteases and the fact that many viral proteins are dependent on proteolytic cleavage for maturation, would suggest that aberrant proteolytic processing of viral proteins may be responsible for host range restriction and differences in tissue and host tropism of viruses. However, examples indicative of such restrictions are almost completely lacking in the literature. This suggests that either this type of restriction has been overlooked, or that it does not play a significant role in determining tropism. One of the few examples is a report dealing with the restricted replication of caprine arthritis-encephalitis virus (CAEV), a natural lentivirus of goats, in ovine fibroblasts. Whereas the mature envelope protein gp135-SU was readily detectable in CAEV-infected permissive cells (macrophages), it was absent in CAEV-infected non-permissive fibroblasts. Instead, a novel protein doublet of 60 kDa was present in these cells. This 60 kDa doublet was derived from gp135-SU by a specific proteolytic cleavage and could be immunoprecipitated with a polyclonal CAEV-specific serum. Thus, restricted replication of CAEV in non-permissive cells was shown to be correlated with a specific but abnormal proteolytic cleavage of the envelope glycoprotein of this virus (Chebloune *et al.*, 1996).

6.9. Host transcription factors

Viruses are highly dependent on the biosynthetic machinery of the host cell for multiplication. Whereas RNA viruses are mainly dependent on translational processes, DNA viruses and retroviruses are also dependent on transcriptional processes since they are unable to generate their own mRNA’s. Especially the small DNA viruses such as polyomaviruses, papillomaviruses, parvoviruses, hepadnaviruses, retroviruses - and to a lesser extent adenoviruses - are highly dependent on host transcription factors for their replication. Since transcription of cellular genes is highly cell-type specific, replication of such viruses may also be restricted to certain cell-types or tissues. Therefore, the availability of the correct host transcription factors may be an important factor that determines the tropism of a virus.

Transcription of the proviral genome of retroviruses and lentiviruses is largely determined by host transcription factors that interact with the enhancer and promoter sequences in the long terminal repeats (LTRs). Many retroviruses have adapted to allow optimal replication in certain cell-types. Therefore, it is not surprising to see that cell-type specific replication of many retroviruses is governed by transcriptional constraints (Grimm & Nordeen, 1999, Hallberg & Grundstrom, 1988, Maury *et al.*, 2000, Wahlers *et al.*, 2002). These observations have been used for transcriptional targeting of retroviral gene therapy vectors by replacing various enhancer/promoter elements in the LTRs with cell- or tissue-specific promoter/enhancer sequences (Ferrari *et al.*, 1995, Grande *et al.*, 1999, Logg *et al.*, 2002, Lotti *et al.*, 2002). In order to restrict transgene expression to certain cell-types (especially cancer cells), transcriptional targeting has also been used - either alone or in conjunction with transductional targeting - for adenovirus gene therapy vectors (Glasgow *et al.*, 2004a, Nettelbeck *et*

al., 2000). Cell-type specific promoters have not only been used for the tissue or specific-specific expression of transgenes (Banerjee *et al.*, 2004, Barnett *et al.*, 2002b, Frauli *et al.*, 2003, Nicklin *et al.*, 2003, Reynolds *et al.*, 2001, Work *et al.*, 2004b, Zhu *et al.*, 2004a, Zhu *et al.*, 2004b) but also for the generation of conditional replicating adenovirus vectors in which replication is dependent on tissue specific promoters (Rivera *et al.*, 2004).

Hepatitis B virus (HBV, hepadnaviruses) infects hepatocytes of humans and primates, causing acute and chronic liver disease. Despite the small size of the genome of HBV (3.2 kb) it contains four promoters, each regulating the production of distinct transcripts. However, each promoter is regulated by the viral enhancer which is located at the proximal end of the genome. The HBV enhancer has been shown to interact with liver-enriched transcription factors suggesting that these interactions are at least partly responsible for the specific liver tropism of the virus (Ori & Shaul, 1995, Tang & McLachlan, 2001).

The human polyomavirus JC virus is the etiological agent of a brain disease called progressive multifocal leukoencephalopathy (PML). The virus selectively destroys oligodendrocytes, leading to demyelation and loss of brain function. The JC virus early promoter directs cell-specific expression of the viral replication factor large T-antigen. T-antigen represses the JC virus early promoter in glioma cells. In contrast T-antigen induced strong activation of the early promoter in non-glial cells. This differential activity suggests that transcriptional regulation constitutes a major mechanism of the tropism of this virus (Kim *et al.*, 2000, Krebs *et al.*, 1995).

Because of their limited coding capacity, parvoviruses rely heavily on cellular factors for their replication. Replication of the parvovirus MVM depends on cellular functions expressed transiently during the S-phase of the cell cycle (Deleu *et al.*, 1999) and productive infection also depends on the cell-type and differentiation state of the host cell. Activation of the P4 promoter which drives expression of the viral regulatory NS proteins is a key step in the life cycle of MVM. Using transgenic mice, activation of P4 was shown to be developmentally regulated. Activation occurred during embryogenesis and in the adult in a cell-type specific and differentiation dependent pattern that was consistent with transcriptional control (Davis *et al.*, 2003).

Human papillomavirus (HPV) has a restricted tropism for human epithelial cells and the viral life cycle is tightly linked to the differentiation program of the infected keratinocyte. This dependence seems to be dictated by viral transcription rather than viral DNA replication (Desaintes & Demeret, 1996). Transcription and replication are controlled by the long control region (LCR) that contains a tissue-specific enhancer, the E6/E7 promoter, and the origin of replication. Furthermore, the viral E2 protein plays an important role in the viral lifecycle since it regulates both transcription of E6/E7 and replication. E2 regulates expression of E6 and E7 separately and this regulation is cell-type dependent (Rapp *et al.*, 1997).

These are only a few examples of restriction of virus replication by host transcription factors. They show that some viruses have specialized in achieving optimal replication in certain cell-types or tissues by making use of available transcription factors. However, at the same time this has limited their ability to replicate in other cell-types or tissues.

6.10. Other factors

Not only host transcription factors but also host translation factors may be involved in determining virus tropism. This has, for instance, been shown for picornaviruses which make use of a complex structure in the 5’ untranslated region of the viral RNA - known as the internal ribosome entry site (IRES) - for translation of their genome. The IRES regulates the initiation of translation by coordinating binding of initiation factors and IRES-specific trans-acting factors (Jackson & Kaminski, 1995, Pestova *et al.*, 2001). Mutations in the IRES, or substitution of IRES sequences by those from another virus, can impair viral RNA translation and virus growth in particular cell-types, suggesting that cell-specific IRES-binding proteins are involved in virus tropism and pathogenicity (La Monica & Racaniello, 1989, Pilipenko *et al.*, 2001, Shiroki *et al.*, 1997, Svitkin *et al.*, 1988, Yanagiya *et al.*, 2003).

The human parvovirus B19 virus has been shown to possess a limited host range, with erythroid progenitor cells being the main target cells supporting B19 replication. Synthesis of the NS1 nonstructural protein occurs in both permissive and nonpermissive cells, whereas synthesis of the VP1 and VP2 capsid proteins seems to be restricted to erythroid cells. In nonpermissive cells, the NS1 protein is overexpressed and the NS1 RNAs are the predominant RNA species. However, the VP1 and VP2 proteins are not detectable, although the corresponding mRNAs are synthesized. Transient transfection assays indicated that the 3' untranslated region (UTR) of mRNAs coding for the capsid proteins repressed their own mRNA translation. The 3' UTR did not affect nuclear export or mRNA stability and the mRNAs did not associate with ribosomes at all. These results indicate that in nonpermissive cells, the 3' UTR of the capsid protein mRNAs repressed capsid protein synthesis at the translational level by inhibiting ribosome loading (Pallier *et al.*, 1997).

Other factors which are related to fysiological and metabolic processes may also determine tropism by differentially affecting virus replication. For instance, an important difference between avian and human influenza A viruses is the optimum temperature of their polymerase activity which is related to the site of replication and body temperature (42°C in bird intestine vs 34°C in human lung).

7. POTENTIAL RISKS ASSOCIATED WITH THE USE OF RECOMBINANT AND CHIMERIC VIRUSES

Changes in cell or tissue tropism of recombinant or chimeric viruses may be intentional or unintentional. For gene therapy and cancer gene therapy applications, changes in tropism are often intentional because retargeting of viral vectors to specific cell-types or tissues is often desirable in order to improve the efficacy of such vectors. For vaccination purposes, recombinant and chimeric viruses are developed that express foreign viral surface proteins with the aim to establish an immune response against these foreign proteins. If a foreign viral surface protein is incorporated into virion particles, such recombinant and chimeric viruses may possess a new tropism that differs from that of the vector virus. These changes in tropism are generally unintentional. As discussed above (section 6) unintentional changes in tropism may also result from post-entry interactions.

In some cases, changes in tropism as a result of the incorporation of a heterologous surface protein are not to be expected, for instance in the case of chimeric herpesviruses or poxviruses. Since multiple envelope proteins are involved in the entry process of these viruses, replacement of one of them - or the presence of an additional heterologous envelope protein - will probably not lead to a change in tropism. In other cases, however, a change in tropism may be expected or even predicted, for instance for chimeric parainfluenza viruses in which the HN and F proteins of one strain have been exchanged by the HN and F proteins of another strain. Also for other viruses which make use of only one or two entry proteins, exchange of these proteins (or the addition of an extra entry protein) is likely to result in an alteration in tropism. Generally, the chance of inducing a change in tropism is high in 'simple' viruses with a small genome size and a single or a limited number of entry proteins (such as most small RNA viruses), and low in 'complex' viruses with a large genome size and multiple entry proteins (such as herpesviruses and poxviruses). However, this may also be dependent on the phylogenetic relationship between the donor virus and vector virus. It has been observed, for instance, that replacement of the gB envelope glycoprotein of a swine herpesvirus by the homologous gB protein from a bovine herpesvirus resulted in an altered tropism (Gerdts *et al.*, 2000).

In order to change the tropism of a recombinant or chimeric virus, the entry protein of the donor virus has to be incorporated correctly in the chimeric virus particle and it has to be functional in the new environment. Since different viruses may have different entry mechanisms and different intracellular transport routes, not all entry proteins can be exchanged by another entry protein. This seems to be particularly true for retroviruses (see section 4.3). Thus, both functional and physical requirements determine whether the entry protein of the donor virus will be successful in changing the tropism of a chimeric virus. Generally, the replacement or addition of entry proteins from closely related donor viruses will probably be more successful than from distantly related donor viruses.

7.1. Increase in pathogenicity and virulence

Virus infection of host cells inevitably results in interference with normal cellular processes. To what extent these interactions may or may not disrupt cellular processes is dependent on specific characteristics of different viruses. Generally speaking, large disturbances in the cellular metabolism

will lead to disease - especially when more cell-types or tissues are concerned - whereas minor disturbances may go unnoticed. The major risk associated with the use of viruses in general, and with recombinant or chimeric viruses in particular, is their ability to cause disease (pathogenicity). Disease occurs when the host suffers from damage that interferes with homeostasis. In this respect, damage includes cell, tissue and organ damage. Damage can be mediated by either the pathogen or the host and is dependent on the immune status of the host. Damage in hosts that mount weak immune responses is primarily caused by the pathogen, whereas damage in hosts that mount strong immune responses is primarily caused by host-mediated inflammatory responses. Hence, disease is a complex outcome which can arise because of pathogen-mediated damage, host-mediated damage, or both. Whether or not a viral infection will result in disease is dependent on the pathogenicity and virulence of the virus. Here we will use the definitions put forward by Casadevall and Pirofski, *i.e.*, pathogenicity is defined as the capacity of a virus to cause damage in a host, and virulence as the relative capacity of a virus to cause damage in a host (Casadevall & Pirofski, 1999). There is considerable confusion regarding what is meant by virulence. This confusion stems from the fact that virulence is often considered to be a property of the virus. However, virulence is only expressed in susceptible hosts. For example, virulent viruses are non-virulent in hosts with specific immunity. Also, viruses which are extremely virulent for one host may be completely non-virulent for another. Thus, virulence is not a separate characteristic of the virus but a complex and dynamic phenomenon that includes both host and viral factors (Casadevall & Pirofski, 2001). Virulence depends on a variety of viral factors such as tropism and replication capacity, on host factors such as age, sex, species, and immune status, and on other factors such as dose, route of entry, etc. Clearly, virulence and pathogenicity are closely linked and can hardly be separated when dealing with virus infections because differences in pathogenicity are often directly correlated with differences in virulence.

Once it has been established that a recombinant or chimeric virus has acquired a new or extended tropism (either intentionally or unintentionally), the question is whether this will lead to (an increase in) pathogenicity. Pathogenicity of viruses is primarily determined by their virulence, which – amongst others - is determined by their tropism. Changes in tropism may not only be the result of changes in receptor binding, but also of changes in susceptibility to host defence mechanisms. Thus, when dealing with recombinant viruses and chimeric viruses of which the tropism has been modified, a potential increase in pathogenicity and virulence is a serious concern. Whether or not a recombinant or chimeric virus with an altered tropism has an increased virulence and pathogenicity compared to the parent (vector) virus, may be determined by several factors. These factors are summarized in Table 4, and can be roughly divided into 4 categories, *i.e.*, (1) properties of the vector virus, (2) changes in tropism that relate to receptor interactions, (3) changes in tropism that relate to antiviral responses, and (4) host factors. It should be emphasized that a change in tropism of a recombinant or chimeric virus does not automatically lead to an increase in virulence or pathogenicity. In many cases the reverse seems to be more likely, since changes in tropism of a virus may be accompanied by changes in fitness. The concept of fitness is difficult to define but it relates to the ability of a virus to replicate and spread *in vivo*. Fitness is determined by factors such as tropism, replication efficiency, stability, susceptibility to the immune response, etc. Normally, a given virus has reached an optimal

fitness in its natural host by a constant process of adaptation and co-evolution. However, when the virus is introduced into a new host (or host cell line) its replication capacity is generally sub-optimal. Thus, by changes in - or exchange of – (a) surface protein(s), the resulting recombinant or chimeric virus may have a lower fitness, for instance because of an altered genome size, interference with transcription and/or replication, or because individual proteins are not optimally adapted to each other in the new and forced constellation. Indeed, a reduction in fitness is frequently observed in chimeric viruses based on small viruses where the impact of gene addition or replacement is relatively high (Flandorfer *et al.*, 2003, Foley *et al.*, 2000, Horimoto *et al.*, 2003, Schmidt *et al.*, 2000, Skiadopoulos *et al.*, 2002a, Tao *et al.*, 2000). However, it has also been observed in larger viruses such as coronaviruses (Haijema *et al.*, 2003, Kuo *et al.*, 2000).

The virulence of the vector virus, and whether or not the recombinant or chimeric virus is replication competent, may be the most important factors that determine the risks associated with the use of such viruses. For any application involving recombinant or chimeric viruses, the safest strategy would be to use a virus that combines specific desirable properties with optimal safety. This has, for instance been achieved with chimeric live vaccines based on the Yellow fever vaccine strain YF17D. By exchanging the structural proteins of this strain with those of other flaviviruses, new vaccine candidates have been developed that are highly safe (see section 5.4). Nevertheless, it has been shown that a YF17D recombinant in which the prM and E genes were replaced by those of the mouse flavivirus Modoc virus, resulted in neurovirulence in SCID mice (Charlier *et al.*, 2004). Although this seems worrying at first sight, it must be kept in mind that SCID mice lack a functional immune system and thus are unable to mount an immune response against the invader. Indeed, such mice can be killed by many conventional viruses. Other examples of highly safe chimeric viruses include poxvirus recombinants which are used as vaccines to protect animals and humans from disease. Although such recombinants show a complete lack of virulence, infection of a human being with the vaccinia-rabies recombinant virus (which is generally considered to be very safe), has been reported. The woman, who was pregnant and suffered from a chronic skin disease, was bitten by her dog which was vaccinated with the vaccinia-rabies recombinant (Rupprecht *et al.*, 2001). This incident does not question the safety of the vaccine, it just illustrates that the condition of the host is also an important contributing factor that determines pathogenesis of recombinant or chimeric viruses.

Despite our growing knowledge of the interactions between cells and viruses, our knowledge of the cell- and tissue-distribution of viral receptors is often far from complete. It is not always clear, for instance, whether a co-receptor is involved in the entry process or not. Often, the cell- or tissue-distribution of a specific receptor or co-receptor is not exactly known. Furthermore, there may be other receptors which may be used by the same entry protein but which have not yet been identified. The situation is even worse when the receptor for the new entry protein is unknown. In this case, the outcome of an infection with a recombinant or chimeric virus may be unpredictable. In a worst case scenario, the new or expanded tropism of a recombinant virus would result in infection of cell-types or tissues which are normally not infected by the vector virus due to the absence of the receptor. If these cell-types or tissues fulfil an important or even an essential biological function - and replication of the recombinant or chimeric virus in these cells destroys these functions - than this could result in severe

pathogenesis. The acquisition of neurotropism by a virus that normally does not replicate in the brain is an example of increase in virulence. This has for instance been observed for a chimeric swine herpesvirus in which the gB envelope glycoprotein gene was replaced by the gB gene of a bovine herpesvirus (Gerdts *et al.*, 2000). This example shows that extra precautions should be taken when entry proteins are being used that may change or extend the tropism of chimeric viruses to vulnerable tissues or cell types such as neurons and cells of the immune system.

Changes in tropism are not necessarily determined by changes in entry proteins. As discussed above (section 6), the ability of viruses to antagonize antiviral responses, either at the cellular or the organismal level, may also be responsible for differences in tropism. Therefore, recombinant viruses that are able to circumvent antiviral responses may be expected to be more virulent than their parent strains. Indeed, the attenuated phenotype of many viruses after serial passage in cell culture is often accompanied by a loss of the ability to antagonize antiviral responses of the host (Blanchard *et al.*, 1998). It is becoming more and more clear that, in order to be successful, virtually every virus uses one or more viral proteins to interfere with the host immune response. Our knowledge about these proteins and their mode of action has long been limited to large DNA viruses such as herpesviruses and poxviruses. More recently, detailed studies have given us new insights in the mechanisms used by many other viruses. It may be expected that many more exciting examples of interference mechanisms will be uncovered in the near future.

7.2. Changes in host tropism

For any virus, recognition of the correct receptor is a prerequisite for successful infection of the host cell. Thus, the interaction between viral entry proteins and cellular receptors is the primary determinant of virus tropism. However, as discussed above (section 6) many viral and cellular factors determine whether replication of different viruses in specific cell types will be successful or not. Nevertheless, sometimes recognition of the receptor is the only factor that determines the tropism of a virus. This has, for instance, been shown for poliovirus. Whereas the natural host range of poliovirus is restricted to humans, expression of the poliovirus receptor in transgenic mice rendered these animals susceptible to poliovirus infection and pathogenesis (Koike *et al.*, 1991, Ren *et al.*, 1990). This indicates that also the acquisition of a new receptor binding specificity by changes in viral entry proteins may result in an extended host range. Host range extension can be determined by very subtle changes in viral entry proteins. It has been shown that even single amino acid changes in viral entry proteins can result in host range extension. For instance, a change from a leucine to serine at position 155 in gp85 of an avian retrovirus extended the host range from avian to non-avian cell-types, including human, dog, cat, mouse, rat and hamster (Rainey *et al.*, 2003). A change in host tropism was also obtained by exchanging the ectodomains of the spike glycoprotein (S) of the coronaviruses MHV and FIPV (Haijema *et al.*, 2003, Kuo *et al.*, 2000).

The biological behaviour of recombinant and chimeric viruses in tissue culture cells may not always reflect their behaviour in the intact organism. Many viruses such as, for instance, the swine herpesvirus pseudorabies virus are able to replicate in human tissue culture cells but are unable to cause disease in humans (Boldogkoi *et al.*, 2002). Thus, although the above examples show that a

change in host specificity is possible at the cellular level, it remains questionable whether such viruses will also be able to establish a productive infection and cause pathogenicity in the intact organism. Most recombinant and chimaeric virus which are developed for fundamental studies of virus-host interactions have not been tested in vivo. It would be interesting to study the biological behaviour of chimeric viruses with an extended host range in their natural host and in their new host. Such studies might give us valuable information about viral factors and host factors that determine the outcome of an infection.
Apart from receptor interactions also other factors may determine host tropism. For instance, viral genes involved in antagonizing the cellular antiviral interferon response, such as the those employed by the negative-stranded RNA viruses. In order to study the function of the V protein of NDV, mutants defective in the expression of the V protein, were generated. These mutants grew poorly in both embryonated chicken eggs and chicken embryo fibroblasts (CEFs) compared to wild-type NDV. However, replacement of the NDV V gene by the NS1 gene of influenza A virus restored growth to wt levels in embryonated chicken eggs and CEFs. These data indicate that for viruses infecting avian cells, the NDV V protein and the influenza NS1 protein are functionally interchangeable. Remarkably, in human cells, the titre of wt NDV was 10 times lower than that of the NDV V-/NS1 recombinant, whereas the level of interferon secreted by human cells infected with wt NDV was much higher than that secreted by cells infected with the NS1-expressing NDV (Park *et al.*, 2003a). These results indicate that the interferon antagonist activity of the NDV V protein is species specific and that substitution of the V protein by the NS1 protein of Influenza A virus allows for much more efficient replication in human cells. A direct correlation between a mutation in the V protein that extended its antagonistic activity and the ability to replicate in cells from another species was also shown for another paramyxovirus, SV5 (Young *et al.*, 2001). Anti-viral responses of the host operate at different levels and, therefore, it is difficult to predict whether a non-human virus that acquires the ability to infect human cells as a result of an interaction with the cellular interferon pathway, will also be successful in establishing an infection in humans. Perhaps the emergence of influenza A virus infections in humans is partly based on changes in the NS1 protein that allow it to antagonize the cellular interferon system (Geiss *et al.*, 2002).
Another important aspect that needs mentioning, is the ability of recombinant and chimeric viruses to become successful at the population level. Crossing the species barrier is one thing, but in order to maintain itself in the population, a virus must be able to reach a certain level of reproduction and transmission (May *et al.*, 2001). Successful jumps from one species to another happen only occasionally in nature, indicating that it is generally an unlikely event. However, once it has occurred it may have severe consequences as is the case, for instance, for HIV, influenza A virus, and SARS coronavirus (Louz *et al.*, 2005, Ludwig *et al.*, 2003, Palese, 2004). It should be noted that there is no evidence that genetically modified recombinant or chimeric viruses (or classically modified viruses such as influenza virus reassortants) made the jump from the lab to the field by infection of personnel or accidental escape from the contained environment. Many laboratories have made lots of genetically modified viruses in the past but apparently these did not give rise to unpredicted and undesired properties.

Due to our limited knowledge of the relationship between tropism and pathogenicity of viruses, research in this area should be stimulated and scientific developments in this area should be closely monitored in order to be able to make a realistic judgement of the risks associated with the use of recombinant or chimeric viruses.

7.3. Immunopathology

The main actors in the cellular arm of the immune system are CD4+ helper T-cells (Th) and CD8+ cytotoxic T-cells (CTL). Th cells can be classified on the basis of their cytokine production either as Th1 cells that produce IL-2, IL-12 and IFN-γ, or Th2 cells that produce predominantly IL-4, IL-5, IL-10, and IL-13 (Seder & Paul, 1994). Furthermore, cytokines produced by one Th subset can block the production or activity of cytokines produced by the other subset. Thus, the type of immune response - and in particular the type of effector T cells that are induced after infection or vaccination - is not only determined by the immunogen but also by the cytokine microenvironment that influences the development of naïve Th cells into Th1 or Th2 cells. This differential development can have profound implications for disease susceptibility since efficient clearance of a viral infection may require a type 1 response for one virus, and a type 2 response for another. It has been shown that vaccinia virus recombinants that express either the F or G protein of RSV, prime for different Th-responses in BALB/c mice. Whereas expression of the F protein primed for CTL and a Th1 response, expression of the G protein primed for a Th2 response (Alwan & Openshaw, 1993). This difference in the type of immune response may be affected by the type of cytokines that are induced after vaccination. Indeed, the use of recombinant vaccinia virus to study the *in vivo* effects of mouse cytokines has demonstrated that the course of infection can be modified and biased toward either an antiviral effect by Th1 cytokines, such as IL-2, IL-12 and IFN-γ, or enhanced virus virulence by Th2 cytokines, such as IL-4 and IL-10 (Aung *et al.*, 1999, Ramshaw *et al.*, 1997, Sharma *et al.*, 1996, van Den Broek *et al.*, 2000). Also other studies, using different viral infection models, have shown that overexpression or systemic administration of IL-4 impedes the development of virus-specific CTL activity, causing a delay in viral clearance, although infected mice generally survive infection (Bembridge *et al.*, 1998, Fischer *et al.*, 1997, Moran *et al.*, 1996). Furthermore, IL-4 not only suppresses primary anti-viral cell-mediated immune responses, but it may also inhibit memory responses, since infection of immunized mice with a recombinant ectromelia virus that expressed IL-4 resulted in significant mortality (Jackson *et al.*, 2001). These examples indicate that coexpression of immunogenic proteins and specific cytokines by a viral vector in order to modify the type of immune response against a viral infection, may not always result in the desired outcome. It also shows that it is extremely important to have a detailed knowledge of the different types of immune regulatory functions and effector functions that control viral diseases.

7.4. Conclusions

Although the main factors that may contribute to potential changes in tropism are systematically identified and summarized in this report, it is still difficult to draw general conclusions with respect to actual risks associated with the use of recombinant or chimeric viruses. This is mainly due to the great variety in possible combinations of vector viruses and donor genes/proteins, and our limited knowledge of the involvement of the many different factors that may influence tropism. Therefore, the question whether or not a recombinant or chimeric virus with an extended tropism will have an increased pathogenicity compared to the vector virus cannot easily be answered in general. Also, it is not always feasible to determine this experimentally, *e.g.*, for human applications. However, by trying to answer a number of specific questions ('checklist'), in case-by-case situations it may be possible to come up with an expert judgement which may form the basis for rational precautionary safety measures. These questions include, but may not be limited to the following:

Properties of the vector virus

- Is the vector virus replication competent?
- Is the vector virus associated with disease in humans or animals?
- Is the vector virus attenuated?
- If so, is the attenuating modification or the attenuation mechanism know?
- Is the attenuation mechanism preserved in the recombinant or chimeric virus?

Properties of the donor gene(s) and/or protein(s)

- Can the gene(s) or protein(s) of the donor virus interfere with the attenuation mechanism of the vector virus?
- Is (are) the donor protein(s) of the recombinant or chimeric virus an addition or a replacement?
- Is (are) the donor protein(s) incorporated in recombinant or chimeric virion particles?
- Is (are) the donor protein(s) functional in the recombinant or chimeric virion particles?

Entry mechanism of vector virus and donor virus

- Is the entry mechanism of the vector virus and the donor virus similar or distinct?
- What is the function in the entry process of the entry protein(s) of the vector virus?
- Do(es) the donor protein(s) fulfil a function in the entry process?
- Has (any one of) the donor protein(s) the same function as the entry protein(s) of the vector virus?

Phylogenetic relationship between vector virus and donor virus

- Do the donor and vector virus belong to the same species?
- Do the donor and vector virus belong to the same genus?
- Do the donor and vector virus belong to the same subfamily, family or order?

Host specificity and receptor usage of vector virus

- What is (are) the natural host(s) of the vector virus?
- What is the host range of the vector virus?
- What is the receptor for the vector virus?
- What is the tissue distribution of the receptor for the vector virus in the target species?
- Does the vector virus make use of a co-receptor?
- What is the tissue distribution of the co-receptor (if any) for the vector virus in the target species?

Host specificity and receptor usage of donor virus

- What is (are) the natural host(s) of the donor virus?
- What is the host range of the donor virus?
- What is the receptor for the donor virus?
- What is the tissue distribution of the receptor for the donor virus in the target animal?
- Does the donor virus make use of a co-receptor?
- What is the tissue distribution of the co-receptor (if any) for the donor virus in the target animal?

Post-entry interactions

- Is the tropism of the vector virus exclusively determined by receptor interactions or also by post-entry interactions?
- Is (are) the donor protein(s) involved in post-entry interactions that could extend the tropism of the recombinant or chimeric virus?

Possible changes in fitness

- Is there any evidence for a reduction or increase in fitness of the recombinant or chimeric virus as a result of the insertion of the donor gene(s) or protein(s)?
- Is there any reason to believe that insertion of the donor gene(s) or protein(s) will result in a reduction or increase in fitness of the recombinant or chimeric virus?

Antiviral responses

- Is (any one of) the donor protein(s) known to be involved in counteracting anti-viral responses of the target cells?
- Is (any one of) the donor protein(s) known to be involved in counteracting anti-viral responses of the host?

Whereas it may be possible to answer several - or even most - of the above questions, some will probably be difficult to answer. Nevertheless, by using this or an even more detailed checklist, it should be possible to identify the main factors that determine the risks associated with a given vector-donor combination. This will allow the identification of the weakest link(s) in the containment regimen

for a given application, and it will allow the application of specific safety measures in order to limit potential risks as much as possible. In view of our past experience, it seems that recombinant and chimeric viruses can generally be handled safely using the hitherto prescribed containment facilities. Nevertheless, one should be aware of the fact that new combinations may behave differently from what may be expected. Therefore, appropriate safety measures should always be observed.

Table 4. Risk factors associated with changes in tropism of recombinant and chimeric viruses

Properties of vector

1. The virus is replication competent
2. Pathogenicity and virulence of the vector virus

Changes in tropism relating to receptor interactions

1. The virus has acquired an extended cell- and tissue-tropism
2. The virus has acquired a new cell- and tissue-tropism
3. The virus has acquired a new host range
4. The virus has acquired specific tropism for vulnerable target cells (*e.g.*, immune cells, neurons)
5. The virus has an unknown tropism due to an unknown receptor specificity
6. The virus has an unknown tropism due to an unknown receptor distribution

Changes in tropism relating to antiviral responses

1. The virus has acquired superior interferon antagonistic activity
2. The virus has lost sensitivity to the antiviral host response
3. The virus has acquired interferon antagonistic activity that is specific for another species
4. The virus has acquired factors that allow it to interfere with the general immune response
5. The virus expresses a cytokine that subverts the antiviral immune response

Host factors

1. The host is naïve to new class of viruses
2. The host is immunocompromized

8. REFERENCES

Aguilar-Setien, A., Leon, Y. C., Tesoro, E. C., Kretschmer, R., Brochier, B. & Pastoret, P. P. (2002). Vaccination of vampire bats using recombinant vaccinia-rabies virus. *J Wildl Dis* **38**, 539-44.

Alcami, A. & Koszinowski, U. H. (2000). Viral mechanisms of immune evasion. *Trends Microbiol* **8**, 410-8.

Almazan, F., Gonzalez, J. M., Penzes, Z., Izeta, A., Calvo, E., Plana-Duran, J. & Enjuanes, L. (2000). Engineering the largest RNA virus genome as an infectious bacterial artificial chromosome. *Proc Natl Acad Sci U S A* **97**, 5516-21.

Alwan, W. H. & Openshaw, P. J. (1993). Distinct patterns of T- and B-cell immunity to respiratory syncytial virus induced by individual viral proteins. *Vaccine* **11**, 431-7.

Anderson, D. B., Laquerre, S., Ghosh, K., Ghosh, H. P., Goins, W. F., Cohen, J. B. & Glorioso, J. C. (2000). Pseudotyping of glycoprotein D-deficient herpes simplex virus type 1 with vesicular stomatitis virus glycoprotein G enables mutant virus attachment and entry. *J Virol* **74**, 2481-7.

Andreansky, S., Soroceanu, L., Flotte, E. R., Chou, J., Markert, J. M., Gillespie, G. Y., Roizman, B. & Whitley, R. J. (1997). Evaluation of genetically engineered herpes simplex viruses as oncolytic agents for human malignant brain tumors. *Cancer Res* **57**, 1502-9.

Argnani, R., Boccafogli, L., Marconi, P. C. & Manservigi, R. (2004). Specific targeted binding of herpes simplex virus type 1 to hepatocytes via the human hepatitis B virus preS1 peptide. *Gene Ther* **11**, 1087-98.

Arroyo, J., Miller, C. A., Catalan, J. & Monath, T. P. (2001). Yellow fever vector live-virus vaccines: West Nile virus vaccine development. *Trends Mol Med* **7**, 350-4.

Aspden, K., van Dijk, A. A., Bingham, J., Cox, D., Passmore, J. A. & Williamson, A. L. (2002). Immunogenicity of a recombinant lumpy skin disease virus (neethling vaccine strain) expressing the rabies virus glycoprotein in cattle. *Vaccine* **20**, 2693-701.

Aubert, M. & Jerome, K. R. (2003). Apoptosis prevention as a mechanism of immune evasion. *Int Rev Immunol* **22**, 361-71.

Aung, S., Tang, Y. W. & Graham, B. S. (1999). Interleukin-4 diminishes CD8(+) respiratory syncytial virus-specific cytotoxic T-lymphocyte activity in vivo. *J Virol* **73**, 8944-9.

Auricchio, A., Kobinger, G., Anand, V., Hildinger, M., O'Connor, E., Maguire, A. M., Wilson, J. M. & Bennett, J. (2001). Exchange of surface proteins impacts on viral vector cellular specificity and transduction characteristics: the retina as a model. *Hum Mol Genet* **10**, 3075-81.

Balliet, J. W. & Bates, P. (1998). Efficient infection mediated by viral receptors incorporated into retroviral particles. *J Virol* **72**, 671-6.

Banerjee, N. S., Rivera, A. A., Wang, M., Chow, L. T., Broker, T. R., Curiel, D. T. & Nettelbeck, D. M. (2004). Analyses of melanoma-targeted oncolytic adenoviruses with tyrosinase enhancer/promoter-driven E1A, E4, or both in submerged cells and organotypic cultures. *Mol Cancer Ther* **3**, 437-49.

Barnett, A. L., Davey, R. A. & Cunningham, J. M. (2001). Modular organization of the Friend murine leukemia virus envelope protein underlies the mechanism of infection. *Proc Natl Acad Sci U S A* **98**, 4113-8.

Barnett, B. G., Crews, C. J. & Douglas, J. T. (2002a). Targeted adenoviral vectors. *Biochim Biophys Acta* **1575**, 1-14.

Barnett, B. G., Tillman, B. W., Curiel, D. T. & Douglas, J. T. (2002b). Dual targeting of adenoviral vectors at the levels of transduction and transcription enhances the specificity of gene expression in cancer cells. *Mol Ther* **6**, 377-85.

Bartlett, J. S., Kleinschmidt, J., Boucher, R. C. & Samulski, R. J. (1999). Targeted adeno-associated virus vector transduction of nonpermissive cells mediated by a bispecific F(ab'gamma)2 antibody. *Nat Biotechnol* **17**, 181-6.

Bembridge, G. P., Lopez, J. A., Cook, R., Melero, J. A. & Taylor, G. (1998). Recombinant vaccinia virus coexpressing the F protein of respiratory syncytial virus (RSV) and interleukin-4 (IL-4) does not inhibit the development of RSV-specific memory cytotoxic T lymphocytes, whereas priming is diminished in the presence of high levels of IL-2 or gamma interferon. *J Virol* **72**, 4080-7.

Benedict, C. A., Norris, P. S. & Ware, C. F. (2002). To kill or be killed: viral evasion of apoptosis. *Nat Immunol* **3**, 1013-8.

Benedict, C. A., Tun, R. Y., Rubinstein, D. B., Guillaume, T., Cannon, P. M. & Anderson, W. F. (1999). Targeting retroviral vectors to CD34-expressing cells: binding to CD34 does not catalyze virus-cell fusion. *Hum Gene Ther* **10**, 545-57.

Bergman, I., Whitaker-Dowling, P., Gao, Y. & Griffin, J. A. (2004). Preferential targeting of vesicular stomatitis virus to breast cancer cells. *Virology* **330**, 24-33.
Bergman, I., Whitaker-Dowling, P., Gao, Y., Griffin, J. A. & Watkins, S. C. (2003). Vesicular stomatitis virus expressing a chimeric Sindbis glycoprotein containing an Fc antibody binding domain targets to Her2/neu overexpressing breast cancer cells. *Virology* **316**, 337-47.
Bian, H., Fournier, P., Moormann, R., Peeters, B. & Schirrmacher, V. (2005). Selective gene transfer in vitro to tumor cells via recombinant Newcastle disease virus. *Cancer Gene Ther* **12**, 295-303.
Biederer, C., Ries, S., Brandts, C. H. & McCormick, F. (2002). Replication-selective viruses for cancer therapy. *J Mol Med* **80**, 163-75.
Bieniasz, P. D. (2003). Restriction factors: a defense against retroviral infection. *Trends Microbiol* **11**, 286-91.
Biermann, V., Volpers, C., Hussmann, S., Stock, A., Kewes, H., Schiedner, G., Herrmann, A. & Kochanek, S. (2001). Targeting of high-capacity adenoviral vectors. *Hum Gene Ther* **12**, 1757-69.
Bilbao, G., Feng, M., Rancourt, C., Jackson, W. H., Jr. & Curiel, D. T. (1997). Adenoviral/retroviral vector chimeras: a novel strategy to achieve high-efficiency stable transduction in vivo. *Faseb J* **11**, 624-34.
Blanchard, T. J., Alcami, A., Andrea, P. & Smith, G. L. (1998). Modified vaccinia virus Ankara undergoes limited replication in human cells and lacks several immunomodulatory proteins: implications for use as a human vaccine. *J Gen Virol* **79**, 1159-67.
Blancou, J., Kieny, M. P., Lathe, R., Lecocq, J. P., Pastoret, P. P., Soulebot, J. P. & Desmettre, P. (1986). Oral vaccination of the fox against rabies using a live recombinant vaccinia virus. *Nature* **322**, 373-5.
Blasco, E., Lambot, M., Barrat, J., Cliquet, F., Brochier, B., Renders, C., Krafft, N., Bailly, J., Munier, M., Pastoret, P. P. & Aubert, M. F. (2001). Kinetics of humoral immune response after rabies VR-G oral vaccination of captive fox cubs (Vulpes vulpes) with or without maternally derived antibodies against the vaccine. *Vaccine* **19**, 4805-15.
Blomqvist, S., Bruu, A. L., Stenvik, M. & Hovi, T. (2003). Characterization of a recombinant type 3/type 2 poliovirus isolated from a healthy vaccinee and containing a chimeric capsid protein VP1. *J Gen Virol* **84**, 573-80.
Bloom, M. E., Berry, B. D., Wei, W., Perryman, S. & Wolfinbarger, J. B. (1993). Characterization of chimeric full-length molecular clones of Aleutian mink disease parvovirus (ADV): identification of a determinant governing replication of ADV in cell culture. *J Virol* **67**, 5976-88.
Boldogkoi, Z., Bratincsak, A. & Fodor, I. (2002). Evaluation of pseudorabies virus as a gene transfer vector and an oncolytic agent for human tumor cells. *Anticancer Res* **22**, 2153-9.
Bonnet, M. C., Tartaglia, J., Verdier, F., Kourilsky, P., Lindberg, A., Klein, M. & Moingeon, P. (2000). Recombinant viruses as a tool for therapeutic vaccination against human cancers. *Immunol Lett* **74**, 11-25.
Bossert, B. & Conzelmann, K. K. (2002). Respiratory syncytial virus (RSV) nonstructural (NS) proteins as host range determinants: a chimeric bovine RSV with NS genes from human RSV is attenuated in interferon-competent bovine cells. *J Virol* **76**, 4287-93.
Boursnell, M. E., Green, P. F., Campbell, J. I., Deuter, A., Peters, R. W., Tomley, F. M., Samson, A. C., Chambers, P., Emmerson, P. T. & Binns, M. M. (1990a). Insertion of the fusion gene from Newcastle disease virus into a non-essential region in the terminal repeats of fowlpox virus and demonstration of protective immunity induced by the recombinant. *J Gen Virol* **71**, 621-8.
Boursnell, M. E., Green, P. F., Campbell, J. I., Deuter, A., Peters, R. W., Tomley, F. M., Samson, A. C., Emmerson, P. T. & Binns, M. M. (1990b). A fowlpox virus vaccine vector with insertion sites in the terminal repeats: demonstration of its efficacy using the fusion gene of Newcastle disease virus. *Vet Microbiol* **23**, 305-16.
Boursnell, M. E., Green, P. F., Samson, A. C., Campbell, J. I., Deuter, A., Peters, R. W., Millar, N. S., Emmerson, P. T. & Binns, M. M. (1990c). A recombinant fowlpox virus expressing the hemagglutinin-neuraminidase gene of Newcastle disease virus (NDV) protects chickens against challenge by NDV. *Virology* **178**, 297-300.
Boyer, J. C. & Haenni, A. L. (1994). Infectious transcripts and cDNA clones of RNA viruses. *Virology* **198**, 415-26.
Bray, M. & Lai, C. J. (1991). Construction of intertypic chimeric dengue viruses by substitution of structural protein genes. *Proc Natl Acad Sci U S A* **88**, 10342-6.

Brochier, B., Aubert, M. F., Pastoret, P. P., Masson, E., Schon, J., Lombard, M., Chappuis, G., Languet, B. & Desmettre, P. (1996). Field use of a vaccinia-rabies recombinant vaccine for the control of sylvatic rabies in Europe and North America. *Rev Sci Tech* **15**, 947-70.
Brochier, B., Kieny, M. P., Costy, F., Coppens, P., Bauduin, B., Lecocq, J. P., Languet, B., Chappuis, G., Desmettre, P., Afiademanyo, K. & *et al.* (1991). Large-scale eradication of rabies using recombinant vaccinia-rabies vaccine. *Nature* **354**, 520-2.
Bruett, L. & Clements, J. E. (2001). Functional murine leukemia virus vectors pseudotyped with the visna virus envelope show expanded visna virus cell tropism. *J Virol* **75**, 11464-73.
Bucheit, A. D., Kumar, S., Grote, D. M., Lin, Y., von Messling, V., Cattaneo, R. B. & Fielding, A. K. (2003). An oncolytic measles virus engineered to enter cells through the CD20 antigen. *Mol Ther* **7**, 62-72.
Buchholz, U. J., Granzow, H., Schuldt, K., Whitehead, S. S., Murphy, B. R. & Collins, P. L. (2000). Chimeric bovine respiratory syncytial virus with glycoprotein gene substitutions from human respiratory syncytial virus (HRSV): Effects on host range and evaluation as a live-attenuated HRSV vaccine. *J Virol* **74**, 1187-1199.
Buonocore, L., Blight, K. J., Rice, C. M. & Rose, J. K. (2002). Characterization of vesicular stomatitis virus recombinants that express and incorporate high levels of hepatitis C virus glycoproteins. *J Virol* **76**, 6865-72.
Bures, R., Gaitan, A., Zhu, T., Graziosi, C., McGrath, K. M., Tartaglia, J., Caudrelier, P., El Habib, R., Klein, M., Lazzarin, A., Stablein, D. M., Deers, M., Corey, L., Greenberg, M. L., Schwartz, D. H. & Montefiori, D. C. (2000). Immunization with recombinant canarypox vectors expressing membrane-anchored glycoprotein 120 followed by glycoprotein 160 boosting fails to generate antibodies that neutralize R5 primary isolates of human immunodeficiency virus type 1. *AIDS Res Hum Retroviruses* **16**, 2019-35.
Burton, E. A., Fink, D. J. & Glorioso, J. C. (2002). Gene delivery using herpes simplex virus vectors. *DNA Cell Biol* **21**, 915-36.
Cadoz, M., Strady, A., Meignier, B., Taylor, J., Tartaglia, J., Paoletti, E. & Plotkin, S. (1992). Immunisation with canarypox virus expressing rabies glycoprotein. *Lancet* **339**, 1429-32.
Casadevall, A. & Pirofski, L. (2001). Host-pathogen interactions: the attributes of virulence. *J Infect Dis* **184**, 337-44.
Casadevall, A. & Pirofski, L. A. (1999). Host-pathogen interactions: redefining the basic concepts of virulence and pathogenicity. *Infect Immun* **67**, 3703-13.
Caufour, P. S., Motta, M. C., Yamamura, A. M., Vazquez, S., Ferreira, II, Jabor, A. V., Bonaldo, M. C., Freire, M. S. & Galler, R. (2001). Construction, characterization and immunogenicity of recombinant yellow fever 17D-dengue type 2 viruses. *Virus Res* **79**, 1-14.
Chadwick, M. P., Morling, F. J., Cosset, F. L. & Russell, S. J. (1999). Modification of retroviral tropism by display of IGF-I. *J Mol Biol* **285**, 485-94.
Chambers, T. J., Nestorowicz, A., Mason, P. W. & Rice, C. M. (1999). Yellow fever/Japanese encephalitis chimeric viruses: construction and biological properties. *J Virol* **73**, 3095-101.
Chambers, T. M., Kawaoka, Y. & Webster, R. G. (1988). Protection of chickens from lethal influenza infection by vaccinia-expressed hemagglutinin. *Virology* **167**, 414-21.
Charlier, N., Molenkamp, R., Leyssen, P., Paeshuyse, J., Drosten, C., Panning, M., De Clercq, E., Bredenbeek, P. J. & Neyts, J. (2004). Exchanging the yellow fever virus envelope proteins with modoc virus prM and E proteins results in a chimeric virus that is neuroinvasive in SCID mice. *J Virol* **78**, 7418-7426.
Chebloune, Y., Sheffer, D., Karr, B. M., Stephens, E. & Narayan, O. (1996). Restrictive type of replication of ovine/caprine lentiviruses in ovine fibroblast cell cultures. *Virology* **222**, 21-30.
Chen, W., Kawano, H., Men, R., Clark, D. & Lai, C. J. (1995). Construction of intertypic chimeric dengue viruses exhibiting type 3 antigenicity and neurovirulence for mice. *J Virol* **69**, 5186-90.
Conzelmann, K. K. (1998). Nonsegmented negative-strand RNA viruses: genetics and manipulation of viral genomes. *Annu Rev Genet* **32**, 123-62.
Conzelmann, K. K. & Meyers, G. (1996). Genetic engineering of animal RNA viruses. *Trends Microbiol* **4**, 386-93.
Cormier, E. G., Tsamis, F., Kajumo, F., Durso, R. J., Gardner, J. P. & Dragic, T. (2004). CD81 is an entry coreceptor for hepatitis C virus. *Proc Natl Acad Sci U S A* **101**, 7270-4.
Cosset, F. L., Morling, F. J., Takeuchi, Y., Weiss, R. A., Collins, M. K. & Russell, S. J. (1995). Retroviral retargeting by envelopes expressing an N-terminal binding domain. *J Virol* **69**, 6314-22.
Cripe, T. P., Dunphy, E. J., Holub, A. D., Saini, A., Vasi, N. H., Mahller, Y. Y., Collins, M. H., Snyder, J. D., Krasnykh, V., Curiel, D. T., Wickham, T. J., DeGregori, J., Bergelson, J. M. & Currier, M. A.

(2001). Fiber knob modifications overcome low, heterogeneous expression of the coxsackievirus-adenovirus receptor that limits adenovirus gene transfer and oncolysis for human rhabdomyosarcoma cells. *Cancer Res* **61**, 2953-60.
Curiel, D. T. (1999). Strategies to adapt adenoviral vectors for targeted delivery. *Ann N Y Acad Sci* **886**, 158-71.
Davis, C., Segev-Amzaleg, N., Rotem, I., Mincberg, M., Amir, N., Sivan, S., Gitelman, I. & Tal, J. (2003). The P4 promoter of the parvovirus minute virus of mice is developmentally regulated in transgenic P4-LacZ mice. *Virology* **306**, 268-79.
de Smit, A. J., Bouma, A., van Gennip, H. G., de Kluijver, E. P. & Moormann, R. J. (2001). Chimeric (marker) C-strain viruses induce clinical protection against virulent classical swine fever virus (CSFV) and reduce transmission of CSFV between vaccinated pigs. *Vaccine* **19**, 1467-76.
Deleu, L., Pujol, A., Faisst, S. & Rommelaere, J. (1999). Activation of promoter P4 of the autonomous parvovirus minute virus of mice at early S phase is required for productive infection. *J Virol* **73**, 3877-85.
Deng, H., Liu, R., Ellmeier, W., Choe, S., Unutmaz, D., Burkhart, M., Di Marzio, P., Marmon, S., Sutton, R. E., Hill, C. M., Davis, C. B., Peiper, S. C., Schall, T. J., Littman, D. R. & Landau, N. R. (1996). Identification of a major co-receptor for primary isolates of HIV-1. *Nature* **381**, 661-6.
Desaintes, C. & Demeret, C. (1996). Control of papillomavirus DNA replication and transcription. *Semin Cancer Biol* **7**, 339-47.
Didcock, L., Young, D. F., Goodbourn, S. & Randall, R. E. (1999a). Sendai virus and simian virus 5 block activation of interferon-responsive genes: importance for virus pathogenesis. *J Virol* **73**, 3125-33.
Didcock, L., Young, D. F., Goodbourn, S. & Randall, R. E. (1999b). The V protein of simian virus 5 inhibits interferon signalling by targeting STAT1 for proteasome-mediated degradation. *J Virol* **73**, 9928-33.
Dimitrov, D. S. (2004). Virus entry: molecular mechanisms and biomedical applications. *Nat Rev Microbiol* **2**, 109-22.
Dmitriev, I., Krasnykh, V., Miller, C. R., Wang, M., Kashentseva, E., Mikheeva, G., Belousova, N. & Curiel, D. T. (1998). An adenovirus vector with genetically modified fibers demonstrates expanded tropism via utilization of a coxsackievirus and adenovirus receptor-independent cell entry mechanism. *J Virol* **72**, 9706-13.
Dobbe, J. C., van der Meer, Y., Spaan, W. J. & Snijder, E. J. (2001). Construction of chimeric arteriviruses reveals that the ectodomain of the major glycoprotein is not the main determinant of equine arteritis virus tropism in cell culture. *Virology* **288**, 283-94.
Dougherty, W. G. & Semler, B. L. (1993). Expression of virus-encoded proteinases: functional and structural similarities with cellular enzymes. *Microbiol Rev* **57**, 781-822.
Durbin, A. P., Skiadopoulos, M. H., McAuliffe, J. M., Riggs, J. M., Surman, S. R., Collins, P. L. & Murphy, B. R. (2000). Human parainfluenza virus type 3 (PIV3) expressing the hemagglutinin protein of measles virus provides a potential method for immunization against measles virus and PIV3 in early infancy. *J Virol* **74**, 6821-31.
Edbauer, C., Weinberg, R., Taylor, J., Rey-Senelonge, A., Bouquet, J. F., Desmettre, P. & Paoletti, E. (1990). Protection of chickens with a recombinant fowlpox virus expressing the Newcastle disease virus hemagglutinin-neuraminidase gene. *Virology* **179**, 901-4.
Efstathiou, S. & Minson, A. C. (1995). Herpes virus-based vectors. *Br Med Bull* **51**, 45-55.
Engelstadter, M., Buchholz, C. J., Bobkova, M., Steidl, S., Merget-Millitzer, H., Willemsen, R. A., Stitz, J. & Cichutek, K. (2001). Targeted gene transfer to lymphocytes using murine leukaemia virus vectors pseudotyped with spleen necrosis virus envelope proteins. *Gene Ther* **8**, 1202-6.
Enjuanes, L., Sola, I., Alonso, S., Escors, D. & Zuniga, S. (2005). Coronavirus reverse genetics and development of vectors for gene expression. *Curr Top Microbiol Immunol* **287**, 161-97.
Evans, D. J. (1999). Reverse genetics of picornaviruses. *Adv Virus Res* **53**, 209-28.
Everett, H., Barry, M., Sun, X., Lee, S. F., Frantz, C., Berthiaume, L. G., McFadden, G. & Bleackley, R. C. (2002). The myxoma poxvirus protein, M11L, prevents apoptosis by direct interaction with the mitochondrial permeability transition pore. *J Exp Med* **196**, 1127-39.
Everts, B. & van der Poel, H. G. (2005). Replication-selective oncolytic viruses in the treatment of cancer. *Cancer Gene Ther* **12**, 141-61.
Fenaux, M., Opriessnig, T., Halbur, P. G., Elvinger, F. & Meng, X. J. (2004). A chimeric porcine circovirus (PCV) with the immunogenic capsid gene of the pathogenic PCV type 2 (PCV2) cloned into the genomic backbone of the nonpathogenic PCV1 induces protective immunity against PCV2 infection in pigs. *J Virol* **78**, 6297-303.

Fenaux, M., Opriessnig, T., Halbur, P. G. & Meng, X. J. (2003). Immunogenicity and pathogenicity of chimeric infectious DNA clones of pathogenic porcine circovirus type 2 (PCV2) and nonpathogenic PCV1 in weanling pigs. *J Virol* **77**, 11232-43.
Feng, Y., Broder, C. C., Kennedy, P. E. & Berger, E. A. (1996). HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* **272**, 872-7.
Ferrari, G., Salvatori, G., Rossi, C., Cossu, G. & Mavilio, F. (1995). A retroviral vector containing a muscle-specific enhancer drives gene expression only in differentiated muscle fibers. *Hum Gene Ther* **6**, 733-42.
Fielding, A. K., Chapel-Fernandes, S., Chadwick, M. P., Bullough, F. J., Cosset, F. L. & Russell, S. J. (2000). A hyperfusogenic gibbon ape leukemia envelope glycoprotein: targeting of a cytotoxic gene by ligand display. *Hum Gene Ther* **11**, 817-26.
Fielding, A. K., Maurice, M., Morling, F. J., Cosset, F. L. & Russell, S. J. (1998). Inverse targeting of retroviral vectors: selective gene transfer in a mixed population of hematopoietic and nonhematopoietic cells. *Blood* **91**, 1802-9.
Fischer, J. E., Johnson, J. E., Kuli-Zade, R. K., Johnson, T. R., Aung, S., Parker, R. A. & Graham, B. S. (1997). Overexpression of interleukin-4 delays virus clearance in mice infected with respiratory syncytial virus. *J Virol* **71**, 8672-7.
Flandorfer, A., Garcia-Sastre, A., Basler, C. F. & Palese, P. (2003). Chimeric influenza A viruses with a functional influenza B virus neuraminidase or hemagglutinin. *J Virol* **77**, 9116-23.
Fleury, B., Janvier, G., Pialoux, G., Buseyne, F., Robertson, M. N., Tartaglia, J., Paoletti, E., Kieny, M. P., Excler, J. L. & Riviere, Y. (1996). Memory cytotoxic T lymphocyte responses in human immunodeficiency virus type 1 (HIV-1)-negative volunteers immunized with a recombinant canarypox expressing gp 160 of HIV-1 and boosted with a recombinant gp160. *J Infect Dis* **174**, 734-8.
Fodor, E., Devenish, L., Engelhardt, O. G., Palese, P., Brownlee, G. G. & Garcia-Sastre, A. (1999). Rescue of influenza A virus from recombinant DNA. *J Virol* **73**, 9679-82.
Foley, H. D., McGettigan, J. P., Siler, C. A., Dietzschold, B. & Schnell, M. J. (2000). A recombinant rabies virus expressing vesicular stomatitis virus glycoprotein fails to protect against rabies virus infection. *Proc Natl Acad Sci U S A* **97**, 14680-5.
Foley, H. D., Otero, M., Orenstein, J. M., Pomerantz, R. J. & Schnell, M. J. (2002). Rhabdovirus-based vectors with human immunodeficiency virus type 1 (HIV-1) envelopes display HIV-1-like tropism and target human dendritic cells. *J Virol* **76**, 19-31.
Fontana, L., Nuzzo, M., Urbanelli, L. & Monaci, P. (2003). General strategy for broadening adenovirus tropism. *J Virol* **77**, 11094-104.
Frauli, M., Ribault, S., Neuville, P., Auge, F. & Calenda, V. (2003). Adenoviral-mediated skeletal muscle transcriptional targeting using chimeric tissue-specific promoters. *Med Sci Monit* **9**, BR78-84.
Frese, M., Kochs, G., Feldmann, H., Hertkorn, C. & Haller, O. (1996). Inhibition of bunyaviruses, phleboviruses, and hantaviruses by human MxA protein. *J Virol* **70**, 915-23.
Frese, M., Kochs, G., Meier-Dieter, U., Siebler, J. & Haller, O. (1995). Human MxA protein inhibits tick-borne Thogoto virus but not Dhori virus. *J Virol* **69**, 3904-9.
Fruh, K., Gruhler, A., Krishna, R. M. & Schoenhals, G. J. (1999). A comparison of viral immune escape strategies targeting the MHC class I assembly pathway. *Immunol Rev* **168**, 157-66.
Gall, J., Kass-Eisler, A., Leinwand, L. & Falck-Pedersen, E. (1996). Adenovirus type 5 and 7 capsid chimera: fiber replacement alters receptor tropism without affecting primary immune neutralization epitopes. *J Virol* **70**, 2116-23.
Garcia-Castro, J., Segovia, J. C., Garcia-Sanchez, F., Lillo, R., Gomez-Navarro, J., Curiel, D. T. & Bueren, J. A. (2001). Selective transduction of murine myelomonocytic leukemia cells (WEHI-3B) with regular and RGD-adenoviral vectors. *Mol Ther* **3**, 70-7.
Garcia-Sastre, A. (1998). Negative-strand RNA viruses: applications to biotechnology. *Trends Biotechnol* **16**, 230-5.
Garcia-Sastre, A., Durbin, R. K., Zheng, H., Palese, P., Gertner, R., Levy, D. E. & Durbin, J. E. (1998a). The role of interferon in influenza virus tissue tropism. *J Virol* **72**, 8550-8.
Garcia-Sastre, A., Egorov, A., Matassov, D., Brandt, S., Levy, D. E., Durbin, J. E., Palese, P. & Muster, T. (1998b). Influenza A virus lacking the NS1 gene replicates in interferon-deficient systems. *Virology* **252**, 324-30.
Garcin, D., Curran, J., Itoh, M. & Kolakofsky, D. (2001). Longer and shorter forms of Sendai virus C proteins play different roles in modulating the cellular antiviral response. *J Virol* **75**, 6800-7.
Garcin, D., Curran, J. & Kolakofsky, D. (2000). Sendai virus C proteins must interact directly with cellular components to interfere with interferon action. *J Virol* **74**, 8823-30.

Garcin, D., Latorre, P. & Kolakofsky, D. (1999). Sendai virus C proteins counteract the interferon-mediated induction of an antiviral state. *J Virol* **73**, 6559-65.
Garcin, D., Marq, J. B., Strahle, L., le Mercier, P. & Kolakofsky, D. (2002). All four Sendai Virus C proteins bind Stat1, but only the larger forms also induce its mono-ubiquitination and degradation. *Virology* **295**, 256-65.
Garner, J. A. (2003). Herpes simplex virion entry into and intracellular transport within mammalian cells. *Adv Drug Deliv Rev* **55**, 1497-513.
Geiss, G. K., Salvatore, M., Tumpey, T. M., Carter, V. S., Wang, X., Basler, C. F., Taubenberger, J. K., Bumgarner, R. E., Palese, P., Katze, M. G. & Garcia-Sastre, A. (2002). Cellular transcriptional profiling in influenza A virus-infected lung epithelial cells: the role of the nonstructural NS1 protein in the evasion of the host innate defense and its potential contribution to pandemic influenza. *Proc Natl Acad Sci U S A* **99**, 10736-41.
Gerdts, V., Beyer, J., Lomniczi, B. & Mettenleiter, T. C. (2000). Pseudorabies virus expressing bovine herpesvirus 1 glycoprotein B exhibits altered neurotropism and increased neurovirulence. *J Virol* **74**, 817-27.
Giavedoni, L., Jones, L., Mebus, C. & Yilma, T. (1991). A vaccinia virus double recombinant expressing the F and H genes of rinderpest virus protects cattle against rinderpest and causes no pock lesions. *Proc Natl Acad Sci U S A* **88**, 8011-5.
Girard, M., Habel, A. & Chanel, C. (1999). New prospects for the development of a vaccine against human immunodeficiency virus type 1. An overview. *C R Acad Sci III* **322**, 959-66.
Girod, A., Ried, M., Wobus, C., Lahm, H., Leike, K., Kleinschmidt, J., Deleage, G. & Hallek, M. (1999). Genetic capsid modifications allow efficient re-targeting of adeno-associated virus type 2. *Nat Med* **5**, 1052-6.
Glasgow, J. N., Bauerschmitz, G. J., Curiel, D. T. & Hemminki, A. (2004a). Transductional and transcriptional targeting of adenovirus for clinical applications. *Curr Gene Ther* **4**, 1-14.
Glasgow, J. N., Kremer, E. J., Hemminki, A., Siegal, G. P., Douglas, J. T. & Curiel, D. T. (2004b). An adenovirus vector with a chimeric fiber derived from canine adenovirus type 2 displays novel tropism. *Virology* **324**, 103-16.
Goff, S. P. (2004a). Genetic Control of Retrovirus Susceptibility in Mammalian Cells. *Annu Rev Genet* **38**, 61-85.
Goff, S. P. (2004b). Retrovirus restriction factors. *Mol Cell* **16**, 849-59.
Goff, S. P. & Berg, P. (1976). Construction of hybrid viruses containing SV40 and lambda phage DNA segments and their propagation in cultured monkey cells. *Cell* **25**, 227-232.
Gollan, T. J. & Green, M. R. (2002a). Redirecting retroviral tropism by insertion of short, nondisruptive peptide ligands into envelope. *J Virol* **76**, 3558-63.
Gollan, T. J. & Green, M. R. (2002b). Selective targeting and inducible destruction of human cancer cells by retroviruses with envelope proteins bearing short peptide ligands. *J Virol* **76**, 3564-9.
Goncalves, M. A., Pau, M. G., de Vries, A. A. & Valerio, D. (2001). Generation of a high-capacity hybrid vector: packaging of recombinant adenoassociated virus replicative intermediates in adenovirus capsids overcomes the limited cloning capacity of adenoassociated virus vectors. *Virology* **288**, 236-46.
Goncalves, M. A., van der Velde, I., Janssen, J. M., Maassen, B. T., Heemskerk, E. H., Opstelten, D. J., Knaan-Shanzer, S., Valerio, D. & de Vries, A. A. (2002). Efficient generation and amplification of high-capacity adeno-associated virus/adenovirus hybrid vectors. *J Virol* **76**, 10734-44.
Goncalves, M. A., van der Velde, I., Knaan-Shanzer, S., Valerio, D. & de Vries, A. A. (2004). Stable transduction of large DNA by high-capacity adeno-associated virus/adenovirus hybrid vectors. *Virology* **321**, 287-96.
Gonzalez, R., Vereecque, R., Wickham, T. J., Facon, T., Hetuin, D., Kovesdi, I., Bauters, F., Fenaux, P. & Quesnel, B. (1999a). Transduction of bone marrow cells by the AdZ.F(pK7) modified adenovirus demonstrates preferential gene transfer in myeloma cells. *Hum Gene Ther* **10**, 2709-17.
Gonzalez, R., Vereecque, R., Wickham, T. J., Vanrumbeke, M., Kovesdi, I., Bauters, F., Fenaux, P. & Quesnel, B. (1999b). Increased gene transfer in acute myeloid leukemic cells by an adenovirus vector containing a modified fiber protein. *Gene Ther* **6**, 314-20.
Goodbourn, S., Didcock, L. & Randall, R. E. (2000). Interferons: cell signalling, immune modulation, antiviral response and virus countermeasures. *J Gen Virol* **81**, 2341-64.
Goossens, P. H., Havenga, M. J., Pieterman, E., Lemckert, A. A., Breedveld, F. C., Bout, A. & Huizinga, T. W. (2001). Infection efficiency of type 5 adenoviral vectors in synovial tissue can be enhanced with a type 16 fiber. *Arthritis Rheum* **44**, 570-7.

Gordien, E., Rosmorduc, O., Peltekian, C., Garreau, F., Brechot, C. & Kremsdorf, D. (2001). Inhibition of hepatitis B virus replication by the interferon-inducible MxA protein. *J Virol* **75**, 2684-91.
Goto, H. & Kawaoka, Y. (1998). A novel mechanism for the acquisition of virulence by a human influenza A virus. *Proc Natl Acad Sci U S A* **95**, 10224-8.
Gotoh, B., Ogasawara, T., Toyoda, T., Inocencio, N. M., Hamaguchi, M. & Nagai, Y. (1990). An endoprotease homologous to the blood clotting factor X as a determinant of viral tropism in chick embryo. *Embo J* **9**, 4189-95.
Graham, B. S. (2002). Clinical trials of HIV vaccines. *Annu Rev Med* **53**, 207-21.
Graham, B. S., Belshe, R. B., Clements, M. L., Dolin, R., Corey, L., Wright, P. F., Gorse, G. J., Midthun, K., Keefer, M. C., Roberts, N. J., Jr. & *et al.* (1992). Vaccination of vaccinia-naive adults with human immunodeficiency virus type 1 gp160 recombinant vaccinia virus in a blinded, controlled, randomized clinical trial. The AIDS Vaccine Clinical Trials Network. *J Infect Dis* **166**, 244-52.
Grande, A., Piovani, B., Aiuti, A., Ottolenghi, S., Mavilio, F. & Ferrari, G. (1999). Transcriptional targeting of retroviral vectors to the erythroblastic progeny of transduced hematopoietic stem cells. *Blood* **93**, 3276-85.
Grandi, P., Spear, M., Breakefield, X. O. & Wang, S. (2004a). Targeting HSV amplicon vectors. *Methods* **33**, 179-86.
Grandi, P., Wang, S., Schuback, D., Krasnykh, V., Spear, M., Curiel, D. T., Manservigi, R. & Breakefield, X. O. (2004b). HSV-1 virions engineered for specific binding to cell surface receptors. *Mol Ther* **9**, 419-27.
Greber, U. F. & Fassati, A. (2003). Nuclear import of viral DNA genomes. *Traffic* **4**, 136-43.
Grifman, M., Trepel, M., Speece, P., Gilbert, L. B., Arap, W., Pasqualini, R. & Weitzman, M. D. (2001). Incorporation of tumor-targeting peptides into recombinant adeno-associated virus capsids. *Mol Ther* **3**, 964-75.
Grimm, S. L. & Nordeen, S. K. (1999). A composite enhancer element directing tissue-specific expression of mouse mammary tumor virus requires both ubiquitous and tissue-restricted factors. *J Biol Chem* **274**, 12790-6.
Gruenberg, J. (2001). The endocytic pathway: a mosaic of domains. *Nat Rev Mol Cell Biol* **2**, 721-30.
Gu, S. Y., Huang, T. M., Ruan, L., Miao, Y. H., Lu, H., Chu, C. M., Motz, M. & Wolf, H. (1995). First EBV vaccine trial in humans using recombinant vaccinia virus expressing the major membrane antigen. *Dev Biol Stand* **84**, 171-7.
Guirakhoo, F., Arroyo, J., Pugachev, K. V., Miller, C., Zhang, Z. X., Weltzin, R., Georgakopoulos, K., Catalan, J., Ocran, S., Soike, K., Ratterree, M. & Monath, T. P. (2001). Construction, safety, and immunogenicity in nonhuman primates of a chimeric yellow fever-dengue virus tetravalent vaccine. *J Virol* **75**, 7290-304.
Guirakhoo, F., Pugachev, K., Arroyo, J., Miller, C., Zhang, Z. X., Weltzin, R., Georgakopoulos, K., Catalan, J., Ocran, S., Draper, K. & Monath, T. P. (2002). Viremia and immunogenicity in nonhuman primates of a tetravalent yellow fever-dengue chimeric vaccine: Genetic reconstructions, dose adjustment, and antibody responses against wild-type dengue virus isolates. *Virology* **298**, 146-159.
Guirakhoo, F., Pugachev, K., Zhang, Z., Myers, G., Levenbook, I., Draper, K., Lang, J., Ocran, S., Mitchell, F., Parsons, M., Brown, N., Brandler, S., Fournier, C., Barrere, B., Rizvi, F., Travassos, A., Nichols, R., Trent, D. & Monath, T. (2004). Safety and efficacy of chimeric yellow Fever-dengue virus tetravalent vaccine formulations in nonhuman primates. *J Virol* **78**, 4761-75.
Guirakhoo, F., Weltzin, R., Chambers, T. J., Zhang, Z. X., Soike, K., Ratterree, M., Arroyo, J., Georgakopoulos, K., Catalan, J. & Monath, T. P. (2000). Recombinant chimeric yellow fever-dengue type 2 virus is immunogenic and protective in nonhuman primates. *Journal of Virology* **74**, 5477-5485.
Guirakhoo, F., Zhang, Z. X., Chambers, T. J., Delagrave, S., Arroyo, J., Barrett, A. D. & Monath, T. P. (1999). Immunogenicity, genetic stability, and protective efficacy of a recombinant, chimeric yellow fever-Japanese encephalitis virus (ChimeriVax-JE) as a live, attenuated vaccine candidate against Japanese encephalitis. *Virology* **257**, 363-72.
Hadac, E. M., Peng, K. W., Nakamura, T. & Russell, S. J. (2004). Reengineering paramyxovirus tropism. *Virology* **329**, 217-25.
Haig, D. M. (2001). Subversion and piracy: DNA viruses and immune evasion. *Res Vet Sci* **70**, 205-19.
Haijema, B. J., Volders, H. & Rottier, P. J. (2003). Switching species tropism: an effective way to manipulate the feline coronavirus genome. *J Virol* **77**, 4528-38.

Hallberg, B. & Grundstrom, T. (1988). Tissue specific sequence motifs in the enhancer of the leukaemogenic mouse retrovirus SL3-3. *Nucleic Acids Res* **16**, 5927-44.
Hammond, A. L., Plemper, R. K., Zhang, J., Schneider, U., Russell, S. J. & Cattaneo, R. (2001). Single-chain antibody displayed on a recombinant measles virus confers entry through the tumor-associated carcinoembryonic antigen. *J Virol* **75**, 2087-96.
Harris, R. S., Bishop, K. N., Sheehy, A. M., Craig, H. M., Petersen-Mahrt, S. K., Watt, I. N., Neuberger, M. S. & Malim, M. H. (2003). DNA deamination mediates innate immunity to retroviral infection. *Cell* **113**, 803-9.
Hatziioannou, T., Delahaye, E., Martin, F., Russell, S. J. & Cosset, F. L. (1999). Retroviral display of functional binding domains fused to the amino terminus of influenza hemagglutinin. *Hum Gene Ther* **10**, 1533-44.
Hatziioannou, T., Perez-Caballero, D., Yang, A., Cowan, S. & Bieniasz, P. D. (2004). Retrovirus resistance factors Ref1 and Lv1 are species-specific variants of TRIM5alpha. *Proc Natl Acad Sci U S A* **101**, 10774-9.
Havenga, M. J., Lemckert, A. A., Grimbergen, J. M., Vogels, R., Huisman, L. G., Valerio, D., Bout, A. & Quax, P. H. (2001). Improved adenovirus vectors for infection of cardiovascular tissues. *J Virol* **75**, 3335-42.
Havenga, M. J., Lemckert, A. A., Ophorst, O. J., van Meijer, M., Germeraad, W. T., Grimbergen, J., van Den Doel, M. A., Vogels, R., van Deutekom, J., Janson, A. A., de Bruijn, J. D., Uytdehaag, F., Quax, P. H., Logtenberg, T., Mehtali, M. & Bout, A. (2002). Exploiting the natural diversity in adenovirus tropism for therapy and prevention of disease. *J Virol* **76**, 4612-20.
Haviv, Y. S., Blackwell, J. L., Kanerva, A., Nagi, P., Krasnykh, V., Dmitriev, I., Wang, M., Naito, S., Lei, X., Hemminki, A., Carey, D. & Curiel, D. T. (2002). Adenoviral gene therapy for renal cancer requires retargeting to alternative cellular receptors. *Cancer Res* **62**, 4273-81.
Heckert, R. A., Riva, J., Cook, S., McMillen, J. & Schwartz, R. D. (1996). Onset of protective immunity in chicks after vaccination with a recombinant herpesvirus of turkeys vaccine expressing Newcastle disease virus fusion and hemagglutinin-neuraminidase antigens. *Avian Dis* **40**, 770-7.
Heinz, F. X. & Allison, S. L. (2001). The machinery for flavivirus fusion with host cell membranes. *Curr Opin Microbiol* **4**, 450-5.
Hewitt, E. W. (2003). The MHC class I antigen presentation pathway: strategies for viral immune evasion. *Immunology* **110**, 163-9.
Horimoto, T., Iwatsuki-Horimoto, K., Hatta, M. & Kawaoka, Y. (2004). Influenza A viruses possessing type B hemagglutinin and neuraminidase: potential as vaccine components. *Microbes and Infection* **6**, 579-583.
Horimoto, T., Takada, A., Iwatsuki-Horimoto, K., Hatta, M., Goto, H. & Kawaoka, Y. (2003). Generation of influenza A viruses with chimeric (type A/B) hemagglutinins. *J Virol* **77**, 8031-8.
Horiuchi, M., Goto, H., Ishiguro, N. & Shinagawa, M. (1994). Mapping of Determinants of the Host-Range for Canine Cells in the Genome of Canine Parvovirus Using Canine Parvovirus Mink Enteritis Virus Chimeric Viruses. *J Gen Virol* **75**, 1319-1328.
Hornemann, S., Harlin, O., Staib, C., Kisling, S., Erfle, V., Kaspers, B., Hacker, G. & Sutter, G. (2003). Replication of modified vaccinia virus Ankara in primary chicken embryo fibroblasts requires expression of the interferon resistance gene E3L. *J Virol* **77**, 8394-407.
Hsiao, J. C., Chung, C. S., Drillien, R. & Chang, W. (2004). The cowpox virus host range gene, CP77, affects phosphorylation of eIF2 alpha and vaccinia viral translation in apoptotic HeLa cells. *Virology* **329**, 199-212.
Huang, Z., Krishnamurthy, S., Panda, A. & Samal, S. K. (2003). Newcastle disease virus V protein is associated with viral pathogenesis and functions as an alpha interferon antagonist. *J Virol* **77**, 8676-85.
Hubel, K., Dale, D. C. & Liles, W. C. (2002). Therapeutic use of cytokines to modulate phagocyte function for the treatment of infectious diseases: current status of granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, macrophage colony-stimulating factor, and interferon-gamma. *J Infect Dis* **185**, 1490-501.
Ikeda, H. & Sugimura, H. (1989). Fv-4 resistance gene: a truncated endogenous murine leukemia virus with ecotropic interference properties. *J Virol* **63**, 5405-12.
Jackson, R. J. & Kaminski, A. (1995). Internal initiation of translation in eukaryotes: the picornavirus paradigm and beyond. *RNA* **1**, 985-1000.
Jackson, R. J., Ramsay, A. J., Christensen, C. D., Beaton, S., Hall, D. F. & Ramshaw, I. A. (2001). Expression of mouse interleukin-4 by a recombinant ectromelia virus suppresses cytolytic lymphocyte responses and overcomes genetic resistance to mousepox. *J Virol* **75**, 1205-10.

Jin, H. K., Yamashita, T., Ochiai, K., Haller, O. & Watanabe, T. (1998). Characterization and expression of the Mx1 gene in wild mouse species. *Biochem Genet* **36**, 311-22.
Johnson, J. E., Schnell, M. J., Buonocore, L. & Rose, J. K. (1997). Specific targeting to CD4+ cells of recombinant vesicular stomatitis viruses encoding human immunodeficiency virus envelope proteins. *J Virol* **71**, 5060-8.
Johnston, J. B. & McFadden, G. (2003). Poxvirus immunomodulatory strategies: current perspectives. *J Virol* **77**, 6093-100.
Johnston, J. B. & McFadden, G. (2004). Technical knockout: understanding poxvirus pathogenesis by selectively deleting viral immunomodulatory genes. *Cell Microbiol* **6**, 695-705.
Kafri, T. (2004). Gene delivery by lentivirus vectors an overview. *Methods Mol Biol* **246**, 367-90.
Kahn, J. S., Roberts, A., Weibel, C., Buonocore, L. & Rose, J. K. (2001). Replication-competent or attenuated, nonpropagating vesicular stomatitis viruses expressing respiratory syncytial virus (RSV) antigens protect mice against RSV challenge. *J Virol* **75**, 11079-87.
Kahn, J. S., Schnell, M. J., Buonocore, L. & Rose, J. K. (1999). Recombinant vesicular stomatitis virus expressing respiratory syncytial virus (RSV) glycoproteins: RSV fusion protein can mediate infection and cell fusion. *Virology* **254**, 81-91.
Kanerva, A. & Hemminki, A. (2004). Modified adenoviruses for cancer gene therapy. *Int J Cancer* **110**, 475-80.
Kanerva, A., Mikheeva, G. V., Krasnykh, V., Coolidge, C. J., Lam, J. T., Mahasreshti, P. J., Barker, S. D., Straughn, M., Barnes, M. N., Alvarez, R. D., Hemminki, A. & Curiel, D. T. (2002). Targeting adenovirus to the serotype 3 receptor increases gene transfer efficiency to ovarian cancer cells. *Clin Cancer Res* **8**, 275-80.
Kanerva, A., Zinn, K. R., Chaudhuri, T. R., Lam, J. T., Suzuki, K., Uil, T. G., Hakkarainen, T., Bauerschmitz, G. J., Wang, M., Liu, B., Cao, Z., Alvarez, R. D., Curiel, D. T. & Hemminki, A. (2003). Enhanced therapeutic efficacy for ovarian cancer with a serotype 3 receptor-targeted oncolytic adenovirus. *Mol Ther* **8**, 449-58.
Kang, Y., Stein, C. S., Heth, J. A., Sinn, P. L., Penisten, A. K., Staber, P. D., Ratliff, K. L., Shen, H., Barker, C. K., Martins, I., Sharkey, C. M., Sanders, D. A., McCray, P. B., Jr. & Davidson, B. L. (2002). In vivo gene transfer using a nonprimate lentiviral vector pseudotyped with Ross River Virus glycoproteins. *J Virol* **76**, 9378-88.
Kasahara, N., Dozy, A. M. & Kan, Y. W. (1994). Tissue-specific targeting of retroviral vectors through ligand-receptor interactions. *Science* **266**, 1373-6.
Kasono, K., Blackwell, J. L., Douglas, J. T., Dmitriev, I., Strong, T. V., Reynolds, P., Kropf, D. A., Carroll, W. R., Peters, G. E., Bucy, R. P., Curiel, D. T. & Krasnykh, V. (1999). Selective gene delivery to head and neck cancer cells via an integrin targeted adenoviral vector. *Clin Cancer Res* **5**, 2571-9.
Kayman, S. C., Park, H., Saxon, M. & Pinter, A. (1999). The hypervariable domain of the murine leukemia virus surface protein tolerates large insertions and deletions, enabling development of a retroviral particle display system. *J Virol* **73**, 1802-8.
Kieny, M. P., Lathe, R., Drillien, R., Spehner, D., Skory, S., Schmitt, D., Wiktor, T., Koprowski, H. & Lecocq, J. P. (1984). Expression of rabies virus glycoprotein from a recombinant vaccinia virus. *Nature* **312**, 163-6.
Kim, H. S., Goncalves, N. M. & Henson, J. W. (2000). Glial cell-specific regulation of the JC virus early promoter by large T antigen. *J Virol* **74**, 755-63.
Kirn, D., Niculescu-Duvaz, I., Hallden, G. & Springer, C. J. (2002). The emerging fields of suicide gene therapy and virotherapy. *Trends Mol Med* **8**, S68-73.
Kit, S., Otsuka, H. & Kit, M. (1992). Expression of porcine pseudorabies virus genes by a bovine herpesvirus-1 (infectious bovine rhinotracheitis virus) vector. *Arch Virol* **124**, 1-20.
Klenk, H.-D. & Garten, W. (1994). Activation cleavage of viral spike proteins by host proteases. In *Cell Receptors for Animal Viruses*, pp. 241-280. Edited by E. Wimmer. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
Kobasa, D., Kodihalli, S., Luo, M., Castrucci, M. R., Donatelli, I., Suzuki, Y., Suzuki, T. & Kawaoka, Y. (1999). Amino acid residues contributing to the substrate specificity of the influenza A virus neuraminidase. *J Virol* **73**, 6743-51.
Kobayashi, M., Iida, A., Ueda, Y. & Hasegawa, M. (2003). Pseudotyped lentivirus vectors derived from simian immunodeficiency virus SIVagm with envelope glycoproteins from paramyxovirus. *J Virol* **77**, 2607-14.
Koike, S., Taya, C., Kurata, T., Abe, S., Ise, I., Yonekawa, H. & Nomoto, A. (1991). Transgenic mice susceptible to poliovirus. *Proc Natl Acad Sci U S A* **88**, 951-5.

Komatsu, T., Takeuchi, K., Yokoo, J., Tanaka, Y. & Gotoh, B. (2000). Sendai virus blocks alpha interferon signaling to signal transducers and activators of transcription. *J Virol* **74**, 2477-80.

Krasnykh, V., Dmitriev, I., Mikheeva, G., Miller, C. R., Belousova, N. & Curiel, D. T. (1998). Characterization of an adenovirus vector containing a heterologous peptide epitope in the HI loop of the fiber knob. *J Virol* **72**, 1844-52.

Krasnykh, V. N., Mikheeva, G. V., Douglas, J. T. & Curiel, D. T. (1996). Generation of recombinant adenovirus vectors with modified fibers for altering viral tropism. *J Virol* **70**, 6839-46.

Krebs, C. J., McAvoy, M. T. & Kumar, G. (1995). The JC virus minimal core promoter is glial cell specific in vivo. *J Virol* **69**, 2434-42.

Kretzschmar, E., Buonocore, L., Schnell, M. J. & Rose, J. K. (1997). High-efficiency incorporation of functional influenza virus glycoproteins into recombinant vesicular stomatitis viruses. *J Virol* **71**, 5982-9.

Krisky, D. M., Wolfe, D., Goins, W. F., Marconi, P. C., Ramakrishnan, R., Mata, M., Rouse, R. J., Fink, D. J. & Glorioso, J. C. (1998). Deletion of multiple immediate-early genes from herpes simplex virus reduces cytotoxicity and permits long-term gene expression in neurons. *Gene Ther* **5**, 1593-603.

Kuhnle, G., Heinze, A., Schmitt, J., Giesow, K., Taylor, G., Morrison, I., Rijsewijk, F. A., van Oirschot, J. T. & Keil, G. M. (1998). The class II membrane glycoprotein G of bovine respiratory syncytial virus, expressed from a synthetic open reading frame, is incorporated into virions of recombinant bovine herpesvirus 1. *J Virol* **72**, 3804-11.

Kuo, L. L., Godeke, G. J., Raamsman, M. J. B., Masters, P. S. & Rottier, P. J. M. (2000). Retargeting of coronavirus by substitution of the spike glycoprotein ectodomain: Crossing the host cell species barrier. *J Virol* **74**, 1393-1406.

La Monica, N. & Racaniello, V. R. (1989). Differences in replication of attenuated and neurovirulent polioviruses in human neuroblastoma cell line SH-SY5Y. *J Virol* **63**, 2357-60.

Lai, C. J. & Monath, T. P. (2003). Chimeric flaviviruses: novel vaccines against dengue fever, tick-borne encephalitis, and Japanese encephalitis. *Adv Virus Res* **61**, 469-509.

Lai, C. M., Lai, Y. K. & Rakoczy, P. E. (2002). Adenovirus and adeno-associated virus vectors. *DNA Cell Biol* **21**, 895-913.

Landis, H., Simon-Jodicke, A., Kloti, A., Di Paolo, C., Schnorr, J. J., Schneider-Schaulies, S., Hefti, H. P. & Pavlovic, J. (1998). Human MxA protein confers resistance to Semliki Forest virus and inhibits the amplification of a Semliki Forest virus-based replicon in the absence of viral structural proteins. *J Virol* **72**, 1516-22.

Laquerre, S., Anderson, D. B., Stolz, D. B. & Glorioso, J. C. (1998). Recombinant herpes simplex virus type 1 engineered for targeted binding to erythropoietin receptor-bearing cells. *J Virol* **72**, 9683-97.

Lavi, E., Kuo, L., Haluskey, J. A. & Masters, P. S. (1998). Targeted recombination between MHV-2 and MHV-A59 to study neurotropic determinants of MHV. *Adv Exp Med Biol* **440**, 543-7.

Lavillette, D., Russell, S. J. & Cosset, F. L. (2001). Retargeting gene delivery using surface-engineered retroviral vector particles. *Curr Opin Biotechnol* **12**, 461-6.

Lee, L. E., Witter, R. L., Reddy, S. M., Wu, P., Yanagida, N. & Yoshida, S. (2003). Protection and synergism by recombinant fowl pox vaccines expressing multiple genes from Marek's disease virus. *Avian Dis* **47**, 549-58.

Lee, S. H. & Vidal, S. M. (2002). Functional diversity of Mx proteins: variations on a theme of host resistance to infection. *Genome Res* **12**, 527-30.

Letellier, C., Burny, A. & Meulemans, G. (1991). Construction of a pigeonpox virus recombinant: expression of the Newcastle disease virus (NDV) fusion glycoprotein and protection of chickens against NDV challenge. *Arch Virol* **118**, 43-56.

Levy, D. E. & Garcia-Sastre, A. (2001). The virus battles: IFN induction of the antiviral state and mechanisms of viral evasion. *Cytokine Growth Factor Rev* **12**, 143-56.

Liang, D., Sainz, I. F., Ansari, I. H., Gil, L. H., Vassilev, V. & Donis, R. O. (2003). The envelope glycoprotein E2 is a determinant of cell culture tropism in ruminant pestiviruses. *J Gen Virol* **84**, 1269-74.

Liljestrom, P. & Garoff, H. (1991). A new generation of animal cell expression vectors based on the Semliki Forest virus replicon. *Biotechnology (N Y)* **9**, 1356-61.

Lin, E. & Nemunaitis, J. (2004). Oncolytic viral therapies. *Cancer Gene Ther* **11**, 643-64.

Liu, X., Peng, D., Wu, X., Xing, L. & Zhang, R. (1999). A recombinant fowlpox virus vaccine expressing glycoprotein B gene from CVI988/Rispens strain of MDV: protection studies in different chickens. *Acta Virol* **43**, 201-4.

Logg, C. R., Logg, A., Matusik, R. J., Bochner, B. H. & Kasahara, N. (2002). Tissue-specific transcriptional targeting of a replication-competent retroviral vector. *J Virol* **76**, 12783-91.
Lorenzo, M. E., Ploegh, H. L. & Tirabassi, R. S. (2001). Viral immune evasion strategies and the underlying cell biology. *Semin Immunol* **13**, 1-9.
Lotti, F., Menguzzato, E., Rossi, C., Naldini, L., Ailles, L., Mavilio, F. & Ferrari, G. (2002). Transcriptional targeting of lentiviral vectors by long terminal repeat enhancer replacement. *J Virol* **76**, 3996-4007.
Louz, D., Bergmans, H., 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. *J Gene Med* **in press**.
Ludwig, B., Kraus, F. B., Allwinn, R., Doerr, H. W. & Preiser, W. (2003). Viral zoonoses - a threat under control? *Intervirology* **46**, 71-8.
Lundstrom, K. (2000). Alphavirus vectors: applications for DNA vaccine production and gene expression. *Intervirology* **43**, 247-57.
Lundstrom, K. (2003a). Alphavirus vectors for vaccine production and gene therapy. *Expert Rev Vaccines* **2**, 447-59.
Lundstrom, K. (2003b). Latest development in viral vectors for gene therapy. *Trends Biotechnol* **21**, 117-22.
Ma, M., Kersten, D. B., Kamrud, K. I., Wool-Lewis, R. J., Schmaljohn, C. & Gonzalez-Scarano, F. (1999). Murine leukemia virus pseudotypes of La Crosse and Hantaan Bunyaviruses: a system for analysis of cell tropism. *Virus Res* **64**, 23-32.
Mackett, M., Yilma, T., Rose, J. K. & Moss, B. (1985). Vaccinia virus recombinants: expression of VSV genes and protective immunization of mice and cattle. *Science* **227**, 433-5.
Mangeat, B., Turelli, P., Caron, G., Friedli, M., Perrin, L. & Trono, D. (2003). Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. *Nature* **424**, 99-103.
Mangeat, B., Turelli, P., Liao, S. & Trono, D. (2004). A single amino acid determinant governs the species-specific sensitivity of APOBEC3G to Vif action. *J Biol Chem* **279**, 14481-3.
Marconi, P., Krisky, D., Oligino, T., Poliani, P. L., Ramakrishnan, R., Goins, W. F., Fink, D. J. & Glorioso, J. C. (1996). Replication-defective herpes simplex virus vectors for gene transfer in vivo. *Proc Natl Acad Sci U S A* **93**, 11319-20.
Markert, J. M., Gillespie, G. Y., Weichselbaum, R. R., Roizman, B. & Whitley, R. J. (2000). Genetically engineered HSV in the treatment of glioma: a review. *Rev Med Virol* **10**, 17-30.
Marschall, M., Zach, A., Hechtfischer, A., Foerst, G., Meier-Ewert, H. & Haller, O. (2000). Inhibition of influenza C viruses by human MxA protein. *Virus Res* **67**, 179-88.
Marsh, M. & Bron, R. (1997). SFV infection in CHO cells: cell-type specific restrictions to productive virus entry at the cell surface. *J Cell Sci* **110**, 95-103.
Martin, F., Chowdhury, S., Neil, S., Phillipps, N. & Collins, M. K. (2002). Envelope-targeted retrovirus vectors transduce melanoma xenografts but not spleen or liver. *Mol Ther* **5**, 269-74.
Masson, E., Bruyere-Masson, V., Vuillaume, P., Lemoyne, S. & Aubert, M. (1999). Rabies oral vaccination of foxes during the summer with the VRG vaccine bait. *Vet Res* **30**, 595-605.
Masters, P. S. (1999). Reverse genetics of the largest RNA viruses. *Adv Virus Res* **53**, 245-64.
Matrosovich, M., Tuzikov, A., Bovin, N., Gambaryan, A., Klimov, A., Castrucci, M. R., Donatelli, I. & Kawaoka, Y. (2000). Early alterations of the receptor-binding properties of H1, H2, and H3 avian influenza virus hemagglutinins after their introduction into mammals. *J Virol* **74**, 8502-12.
Matsushita, T., Elliger, S., Elliger, C., Podsakoff, G., Villarreal, L., Kurtzman, G. J., Iwaki, Y. & Colosi, P. (1998). Adeno-associated virus vectors can be efficiently produced without helper virus. *Gene Ther* **5**, 938-45.
Maurice, M., Mazur, S., Bullough, F. J., Salvetti, A., Collins, M. K., Russell, S. J. & Cosset, F. L. (1999). Efficient gene delivery to quiescent interleukin-2 (IL-2)-dependent cells by murine leukemia virus-derived vectors harboring IL-2 chimeric envelope glycoproteins. *Blood* **94**, 401-10.
Maurice, M., Verhoeyen, E., Salmon, P., Trono, D., Russell, S. J. & Cosset, F. L. (2002). Efficient gene transfer into human primary blood lymphocytes by surface-engineered lentiviral vectors that display a T cell-activating polypeptide. *Blood* **99**, 2342-50.
Maury, W., Bradley, S., Wright, B. & Hines, R. (2000). Cell specificity of the transcription-factor repertoire used by a lentivirus: motifs important for expression of equine infectious anemia virus in nonmonocytic cells. *Virology* **267**, 267-78.
May, R. M., Gupta, S. & McLean, A. R. (2001). Infectious disease dynamics: What characterizes a successful invader? *Philos Trans R Soc Lond B Biol Sci* **356**, 901-10.

McCarty, D. M., Young Jr, S. M. & Samulski, R. J. (2004). Integration of Adeno-Associated Virus (AAV) and Recombinant AAV Vectors. *Annu Rev Genet* **38**, 819-45.
McKenna, P. M., Aye, P. P., Dietzschold, B., Montefiori, D. C., Martin, L. N., Marx, P. A., Pomerantz, R. J., Lackner, A. & Schnell, M. J. (2004). Immunogenicity study of glycoprotein-deficient rabies virus expressing simian/human immunodeficiency virus SHIV89.6P envelope in a rhesus macaque. *J Virol* **78**, 13455-9.
McKenna, P. M., Pomerantz, R. J., Dietzschold, B., McGettigan, J. P. & Schnell, M. J. (2003). Covalently linked human immunodeficiency virus type 1 gp120/gp41 is stably anchored in rhabdovirus particles and exposes critical neutralizing epitopes. *J Virol* **77**, 12782-94.
Mebatsion, T. & Conzelmann, K. K. (1996). Specific infection of CD4+ target cells by recombinant rabies virus pseudotypes carrying the HIV-1 envelope spike protein. *Proc Natl Acad Sci U S A* **93**, 11366-70.
Mebatsion, T., Finke, S., Weiland, F. & Conzelmann, K. K. (1997). A CXCR4/CD4 pseudotype rhabdovirus that selectively infects HIV-1 envelope protein-expressing cells. *Cell* **90**, 841-7.
Meyer, H., Sutter, G. & Mayr, A. (1991). Mapping of deletions in the genome of the highly attenuated vaccinia virus MVA and their influence on virulence. *J Gen Virol* **72**, 1031-8.
Michael, S. I., Hong, J. S., Curiel, D. T. & Engler, J. A. (1995). Addition of a short peptide ligand to the adenovirus fiber protein. *Gene Ther* **2**, 660-8.
Mizuguchi, H. & Hayakawa, T. (2002). Adenovirus vectors containing chimeric type 5 and type 35 fiber proteins exhibit altered and expanded tropism and increase the size limit of foreign genes. *Gene* **285**, 69-77.
Mizuguchi, H., Koizumi, N., Hosono, T., Utoguchi, N., Watanabe, Y., Kay, M. A. & Hayakawa, T. (2001). A simplified system for constructing recombinant adenoviral vectors containing heterologous peptides in the HI loop of their fiber knob. *Gene Ther* **8**, 730-5.
Molloy, S. S., Anderson, E. D., Jean, F. & Thomas, G. (1999). Bi-cycling the furin pathway: from TGN localization to pathogen activation and embryogenesis. *Trends Cell Biol* **9**, 28-35.
Monath, T. & Heinz, F. X. (1996). Flaviviruses. In *Fields virology*, pp. 961-1034. Edited by B. N. Fields, D. M. Knipe & P. M. Howley. Philadelphia: Lippincott-Raven.
Monath, T. P. (1999). Yellow Fever. In *Vaccines*, 3 edn, pp. 815-879. Edited by S. Plotkin & W. A. Orenstein. Philadelphia: WB Saunderrs.
Monath, T. P. (2001). Prospects for development of a vaccine against the West Nile virus. *Ann N Y Acad Sci* **951**, 1-12.
Monath, T. P., Soike, K., Levenbook, I., Zhang, Z. X., Arroyo, J., Delagrave, S., Myers, G., Barrett, A. D., Shope, R. E., Ratterree, M., Chambers, T. J. & Guirakhoo, F. (1999). Recombinant, chimaeric live, attenuated vaccine (ChimeriVax) incorporating the envelope genes of Japanese encephalitis (SA14-14-2) virus and the capsid and nonstructural genes of yellow fever (17D) virus is safe, immunogenic and protective in non-human primates. *Vaccine* **17**, 1869-82.
Moran, T. M., Isobe, H., Fernandez-Sesma, A. & Schulman, J. L. (1996). Interleukin-4 causes delayed virus clearance in influenza virus-infected mice. *J Virol* **70**, 5230-5.
Morgan, R. W., Gelb, J., Jr., Pope, C. R. & Sondermeijer, P. J. (1993). Efficacy in chickens of a herpesvirus of turkeys recombinant vaccine containing the fusion gene of Newcastle disease virus: onset of protection and effect of maternal antibodies. *Avian Dis* **37**, 1032-40.
Morgan, R. W., Gelb, J., Jr., Schreurs, C. S., Lutticken, D., Rosenberger, J. K. & Sondermeijer, P. J. (1992). Protection of chickens from Newcastle and Marek's diseases with a recombinant herpesvirus of turkeys vaccine expressing the Newcastle disease virus fusion protein. *Avian Dis* **36**, 858-70.
Morizono, K., Bristol, G., Xie, Y. M., Kung, S. K. & Chen, I. S. (2001). Antibody-directed targeting of retroviral vectors via cell surface antigens. *J Virol* **75**, 8016-20.
Moss, B. (1996). Genetically engineered poxviruses for recombinant gene expression, vaccination, and safety. *Proc Natl Acad Sci U S A* **93**, 11341-8.
Moss, B. & Shisler, J. L. (2001). Immunology 101 at poxvirus U: immune evasion genes. *Semin Immunol* **13**, 59-66.
Mossman, K., Upton, C., Buller, R. M. & McFadden, G. (1995). Species specificity of ectromelia virus and vaccinia virus interferon-gamma binding proteins. *Virology* **208**, 762-9.
Muller, O. J., Kaul, F., Weitzman, M. D., Pasqualini, R., Arap, W., Kleinschmidt, J. A. & Trepel, M. (2003). Random peptide libraries displayed on adeno-associated virus to select for targeted gene therapy vectors. *Nat Biotechnol* **21**, 1040-6.
Mulligan, R. C. (1993). The basic science of gene therapy. *Science* **260**, 926-32.

Murphy, B. R. & Collins, P. L. (2002). Live-attenuated virus vaccines for respiratory syncytial and parainfluenza viruses: applications of reverse genetics. *J Clin Invest* **110**, 21-7.
Nagai, Y. (1995). Virus activation by host proteinases. A pivotal role in the spread of infection, tissue tropism and pathogenicity. *Microbiol Immunol* **39**, 1-9.
Nagai, Y., Klenk, H. D. & Rott, R. (1976). Proteolytic cleavage of the viral glycoproteins and its significance for the virulence of Newcastle disease virus. *Virology* **72**, 494-508.
Nakamura, T., Peng, K. W., Vongpunsawad, S., Harvey, M., Mizuguchi, H., Hayakawa, T., Cattaneo, R. & Russell, S. J. (2004). Antibody-targeted cell fusion. *Nat Biotechnol* **22**, 331-6.
Nakayama, K. (1997). Furin: a mammalian subtilisin/Kex2p-like endoprotease involved in processing of a wide variety of precursor proteins. *Biochem J* **327**, 625-35.
Nettelbeck, D. M., Jerome, V. & Muller, R. (2000). Gene therapy: designer promoters for tumour targeting. *Trends Genet* **16**, 174-81.
Neumann, G. & Kawaoka, Y. (2001). Reverse genetics of influenza virus. *Virology* **287**, 243-50.
Neumann, G. & Kawaoka, Y. (2004). Reverse genetics systems for the generation of segmented negative-sense RNA viruses entirely from cloned cDNA. *Curr Top Microbiol Immunol* **283**, 43-60.
Nicklin, S. A., Buening, H., Dishart, K. L., de Alwis, M., Girod, A., Hacker, U., Thrasher, A. J., Ali, R. R., Hallek, M. & Baker, A. H. (2001). Efficient and selective AAV2-mediated gene transfer directed to human vascular endothelial cells. *Mol Ther* **4**, 174-81.
Nicklin, S. A., Dishart, K. L., Buening, H., Reynolds, P. N., Hallek, M., Nemerow, G. R., Von Seggern, D. J. & Baker, A. H. (2003). Transductional and transcriptional targeting of cancer cells using genetically engineered viral vectors. *Cancer Lett* **201**, 165-73.
Ohno, K., Sawai, K., Iijima, Y., Levin, B. & Meruelo, D. (1997). Cell-specific targeting of Sindbis virus vectors displaying IgG-binding domains of protein A. *Nature Biotechnology* **15**, 763-767.
Ori, A. & Shaul, Y. (1995). Hepatitis B virus enhancer binds and is activated by the Hepatocyte nuclear factor 3. *Virology* **207**, 98-106.
Palese, P. (2004). Influenza: old and new threats. *Nat Med* **10**, S82-7.
Palese, P., Zheng, H., Engelhardt, O. G., Pleschka, S. & Garcia-Sastre, A. (1996). Negative-strand RNA viruses: genetic engineering and applications. *Proc Natl Acad Sci U S A* **93**, 11354-8.
Pallier, C., Greco, A., Le Junter, J., Saib, A., Vassias, I. & Morinet, F. (1997). The 3' untranslated region of the B19 parvovirus capsid protein mRNAs inhibits its own mRNA translation in nonpermissive cells. *J Virol* **71**, 9482-9.
Paoletti, E. (1996). Applications of pox virus vectors to vaccination: an update. *Proc Natl Acad Sci U S A* **93**, 11349-53.
Paoletti, E., Taylor, J., Meignier, B., Meric, C. & Tartaglia, J. (1995). Highly attenuated poxvirus vectors: NYVAC, ALVAC and TROVAC. *Dev Biol Stand* **84**, 159-63.
Park, M. S., Garcia-Sastre, A., Cros, J. F., Basler, C. F. & Palese, P. (2003a). Newcastle disease virus V protein is a determinant of host range restriction. *J Virol* **77**, 9522-32.
Park, M. S., Shaw, M. L., Munoz-Jordan, J., Cros, J. F., Nakaya, T., Bouvier, N., Palese, P., Garcia-Sastre, A. & Basler, C. F. (2003b). Newcastle disease virus (NDV)-based assay demonstrates interferon-antagonist activity for the NDV V protein and the Nipah virus V, W, and C proteins. *J Virol* **77**, 1501-11.
Pastoret, P. P. & Brochier, B. (1996). The development and use of a vaccinia-rabies recombinant oral vaccine for the control of wildlife rabies; a link between Jenner and Pasteur. *Epidemiol Infect* **116**, 235-40.
Patterson, S. M., Swainsbury, R. & Routledge, E. G. (1999). Antigen-specific membrane fusion mediated by the haemagglutinin protein of influenza A virus: separation of attachment and fusion functions on different molecules. *Gene Ther* **6**, 694-702.
Pavlovic, J., Zurcher, T., Haller, O. & Staeheli, P. (1990). Resistance to influenza virus and vesicular stomatitis virus conferred by expression of human MxA protein. *J Virol* **64**, 3370-5.
Peeters, B., Bienkowska-Szewczyk, K., Hulst, M., Gielkens, A. & Kimman, T. (1997). Biologically safe, non-transmissible pseudorabies virus vector vaccine protects pigs against both Aujeszky's disease and classical swine fever. *J Gen Virol* **78**, 3311-5.
Peng, K. W., Donovan, K. A., Schneider, U., Cattaneo, R., Lust, J. A. & Russell, S. J. (2003). Oncolytic measles viruses displaying a single-chain antibody against CD38, a myeloma cell marker. *Blood* **101**, 2557-62.
Peng, K. W., Holler, P. D., Orr, B. A., Kranz, D. M. & Russell, S. J. (2004). Targeting virus entry and membrane fusion through specific peptide/MHC complexes using a high-affinity T-cell receptor. *Gene Ther* **11**, 1234-9.

Pennathur, S., Haller, A. A., MacPhail, M., Rizzi, T., Kaderi, S., Fernandes, F., Bicha, L., Schickli, J. H., Tang, R. S., Chen, W., Nguyen, N., Mathie, S., Mehta, H. & Coelingh, K. L. (2003). Evaluation of attenuation, immunogenicity and efficacy of a bovine parainfluenza virus type 3 (PIV-3) vaccine and a recombinant chimeric bovine/human PIV-3 vaccine vector in rhesus monkeys. *J Gen Virol* **84**, 3253-61.

Perabo, L., Buning, H., Kofler, D. M., Ried, M. U., Girod, A., Wendtner, C. M., Enssle, J. & Hallek, M. (2003). In vitro selection of viral vectors with modified tropism: the adeno-associated virus display. *Mol Ther* **8**, 151-7.

Pestka, S., Krause, C. D. & Walter, M. R. (2004). Interferons, interferon-like cytokines, and their receptors. *Immunol Rev* **202**, 8-32.

Pestova, T. V., Kolupaeva, V. G., Lomakin, I. B., Pilipenko, E. V., Shatsky, I. N., Agol, V. I. & Hellen, C. U. (2001). Molecular mechanisms of translation initiation in eukaryotes. *Proc Natl Acad Sci U S A* **98**, 7029-36.

Phillips, J. J., Chua, M., Seo, S. H. & Weiss, S. R. (2001). Multiple regions of the murine coronavirus spike glycoprotein influence neurovirulence. *J Neurovirol* **7**, 421-31.

Phillips, J. J., Chua, M. M., Lavi, E. & Weiss, S. R. (1999). Pathogenesis of chimeric MHV4/MHV-A59 recombinant viruses: the murine coronavirus spike protein is a major determinant of neurovirulence. *J Virol* **73**, 7752-60.

Phillips, J. J. & Weiss, S. R. (2001). MHV neuropathogenesis: the study of chimeric S genes and mutations in the hypervariable region. *Adv Exp Med Biol* **494**, 115-9.

Pilipenko, E. V., Viktorova, E. G., Guest, S. T., Agol, V. I. & Roos, R. P. (2001). Cell-specific proteins regulate viral RNA translation and virus-induced disease. *Embo J* **20**, 6899-908.

Pletnev, A. G., Bray, M., Huggins, J. & Lai, C. J. (1992). Construction and characterization of chimeric tick-borne encephalitis/dengue type 4 viruses. *Proc Natl Acad Sci U S A* **89**, 10532-6.

Pletnev, A. G. & Men, R. (1998). Attenuation of the Langat tick-borne flavivirus by chimerization with mosquito-borne flavivirus dengue type 4. *Proc Natl Acad Sci U S A* **95**, 1746-51.

Ploegh, H. L. (1998). Viral strategies of immune evasion. *Science* **280**, 248-53.

Ponnazhagan, S., Mahendra, G., Kumar, S., Thompson, J. A. & Castillas, M., Jr. (2002). Conjugate-based targeting of recombinant adeno-associated virus type 2 vectors by using avidin-linked ligands. *J Virol* **76**, 12900-7.

Qiao, C. L., Yu, K. Z., Jiang, Y. P., Jia, Y. Q., Tian, G. B., Liu, M., Deng, G. H., Wang, X. R., Meng, Q. W. & Tang, X. Y. (2003). Protection of chickens against highly lethal H5N1 and H7N1 avian influenza viruses with a recombinant fowlpox virus co-expressing H5 haemagglutinin and N1 neuraminidase genes. *Avian Pathol* **32**, 25-32.

Qing, K., Mah, C., Hansen, J., Zhou, S., Dwarki, V. & Srivastava, A. (1999). Human fibroblast growth factor receptor 1 is a co-receptor for infection by adeno-associated virus 2. *Nat Med* **5**, 71-7.

Rabinowitz, J. E., Rolling, F., Li, C., Conrath, H., Xiao, W., Xiao, X. & Samulski, R. J. (2002). Cross-packaging of a single adeno-associated virus (AAV) type 2 vector genome into multiple AAV serotypes enables transduction with broad specificity. *J Virol* **76**, 791-801.

Rabinowitz, J. E., Xiao, W. & Samulski, R. J. (1999). Insertional mutagenesis of AAV2 capsid and the production of recombinant virus. *Virology* **265**, 274-85.

Rainey, G. J., Natonson, A., Maxfield, L. F. & Coffin, J. M. (2003). Mechanisms of avian retroviral host range extension. *J Virol* **77**, 6709-19.

Ramshaw, I. A., Ramsay, A. J., Karupiah, G., Rolph, M. S., Mahalingam, S. & Ruby, J. C. (1997). Cytokines and immunity to viral infections. *Immunol Rev* **159**, 119-35.

Rapp, B., Pawellek, A., Kraetzer, F., Schaefer, M., May, C., Purdie, K., Grassmann, K. & Iftner, T. (1997). Cell-type-specific separate regulation of the E6 and E7 promoters of human papillomavirus type 6a by the viral transcription factor E2. *J Virol* **71**, 6956-66.

Rea, D., Havenga, M. J., van Den Assem, M., Sutmuller, R. P., Lemckert, A., Hoeben, R. C., Bout, A., Melief, C. J. & Offringa, R. (2001). Highly efficient transduction of human monocyte-derived dendritic cells with subgroup B fiber-modified adenovirus vectors enhances transgene-encoded antigen presentation to cytotoxic T cells. *J Immunol* **166**, 5236-44.

Recchia, A., Parks, R. J., Lamartina, S., Toniatti, C., Pieroni, L., Palombo, F., Ciliberto, G., Graham, F. L., Cortese, R., La Monica, N. & Colloca, S. (1999). Site-specific integration mediated by a hybrid adenovirus/adeno-associated virus vector. *Proc Natl Acad Sci U S A* **96**, 2615-20.

Recchia, A., Perani, L., Sartori, D., Olgiati, C. & Mavilio, F. (2004). Site-specific integration of functional transgenes into the human genome by adeno/AAV hybrid vectors. *Mol Ther* **10**, 660-70.

Reddy, S. K., Sharma, J. M., Ahmad, J., Reddy, D. N., McMillen, J. K., Cook, S. M., Wild, M. A. & Schwartz, R. D. (1996). Protective efficacy of a recombinant herpesvirus of turkeys as an in

ovo vaccine against Newcastle and Marek's diseases in specific-pathogen-free chickens. *Vaccine* **14**, 469-77.

Reeves, J. D., Miamidian, J. L., Biscone, M. J., Lee, F. H., Ahmad, N., Pierson, T. C. & Doms, R. W. (2004). Impact of mutations in the coreceptor binding site on human immunodeficiency virus type 1 fusion, infection, and entry inhibitor sensitivity. *J Virol* **78**, 5476-85.

Reimann, I., Depner, K., Trapp, S. & Beer, M. (2004). An avirulent chimeric Pestivirus with altered cell tropism protects pigs against lethal infection with classical swine fever virus. *Virology* **322**, 143-57.

Ren, R. B., Costantini, F., Gorgacz, E. J., Lee, J. J. & Racaniello, V. R. (1990). Transgenic mice expressing a human poliovirus receptor: a new model for poliomyelitis. *Cell* **63**, 353-62.

Renaut, L., Colin, M., Leite, J. P., Benko, M. & D'Halluin, J. C. (2004). Abolition of hCAR-dependent cell tropism using fiber knobs of Atadenovirus serotypes. *Virology* **321**, 189-204.

Reynolds, P. N., Nicklin, S. A., Kaliberova, L., Boatman, B. G., Grizzle, W. E., Balyasnikova, I. V., Baker, A. H., Danilov, S. M. & Curiel, D. T. (2001). Combined transductional and transcriptional targeting improves the specificity of transgene expression in vivo. *Nat Biotechnol* **19**, 838-42.

Rice, C. M., Grakoui, A., Galler, R. & Chambers, T. J. (1989). Transcription of infectious yellow fever RNA from full-length cDNA templates produced by in vitro ligation. *New Biol* **1**, 285-96.

Ried, M. U., Girod, A., Leike, K., Buning, H. & Hallek, M. (2002). Adeno-associated virus capsids displaying immunoglobulin-binding domains permit antibody-mediated vector retargeting to specific cell surface receptors. *J Virol* **76**, 4559-66.

Ries, S. J. & Brandts, C. H. (2004). Oncolytic viruses for the treatment of cancer: current strategies and clinical trials. *Drug Discov Today* **9**, 759-68.

Rivera, A. A., Davydova, J., Schierer, S., Wang, M., Krasnykh, V., Yamamoto, M., Curiel, D. T. & Nettelbeck, D. M. (2004). Combining high selectivity of replication with fiber chimerism for effective adenoviral oncolysis of CAR-negative melanoma cells. *Gene Ther* **11**, 1694-1702.

Robbins, P. D. & Ghivizzani, S. C. (1998). Viral vectors for gene therapy. *Pharmacol Ther* **80**, 35-47.

Roberts, A., Buonocore, L., Price, R., Forman, J. & Rose, J. K. (1999). Attenuated vesicular stomatitis viruses as vaccine vectors. *J Virol* **73**, 3723-32.

Roberts, A., Kretzschmar, E., Perkins, A. S., Forman, J., Price, R., Buonocore, L., Kawaoka, Y. & Rose, J. K. (1998). Vaccination with a recombinant vesicular stomatitis virus expressing an influenza virus hemagglutinin provides complete protection from influenza virus challenge. *J Virol* **72**, 4704-11.

Rodriguez, J. J. & Horvath, C. M. (2004). Host evasion by emerging paramyxoviruses: Hendra virus and Nipah virus v proteins inhibit interferon signaling. *Viral Immunol* **17**, 210-9.

Rolling, F. & Samulski, R. J. (1995). AAV as a viral vector for human gene therapy. Generation of recombinant virus. *Mol Biotechnol* **3**, 9-15.

Romero, C. H., Barrett, T., Kitching, R. P., Carn, V. M. & Black, D. N. (1994). Protection of cattle against rinderpest and lumpy skin disease with a recombinant capripoxvirus expressing the fusion protein gene of rinderpest virus. *Vet Rec* **135**, 152-4.

Ross, L. J., Binns, M. M., Tyers, P., Pastorek, J., Zelnik, V. & Scott, S. (1993). Construction and properties of a turkey herpesvirus recombinant expressing the Marek's disease virus homologue of glycoprotein B of herpes simplex virus. *J Gen Virol* **74**, 371-7.

Rots, M. G., Curiel, D. T., Gerritsen, W. R. & Haisma, H. J. (2003). Targeted cancer gene therapy: the flexibility of adenoviral gene therapy vectors. *J Control Release* **87**, 159-65.

Roulston, A., Marcellus, R. C. & Branton, P. E. (1999). Viruses and apoptosis. *Annu Rev Microbiol* **53**, 577-628.

Rubio, M. P., Guerra, S. & Almendral, J. M. (2001). Genome replication and postencapsidation functions mapping to the nonstructural gene restrict the host range of a murine parvovirus in human cells. *J Virol* **75**, 11573-82.

Rupprecht, C. E., Blass, L., Smith, K., Orciari, L. A., Niezgoda, M., Whitfield, S. G., Gibbons, R. V., Guerra, M. & Hanlon, C. A. (2001). Human infection due to recombinant vaccinia-rabies glycoprotein virus. *N Engl J Med* **345**, 582-6.

Rupprecht, C. E., Hamir, A. N., Johnston, D. H. & Koprowski, H. (1988). Efficacy of a vaccinia-rabies glycoprotein recombinant virus vaccine in raccoons (Procyon lotor). *Rev Infect Dis* **10**, S803-9.

Russell, S. J., Hawkins, R. E. & Winter, G. (1993). Retroviral vectors displaying functional antibody fragments. *Nucleic Acids Res* **21**, 1081-5.

Ryman, K. D., Klimstra, W. B., Nguyen, K. B., Biron, C. A. & Johnston, R. E. (2000). Alpha/beta interferon protects adult mice from fatal Sindbis virus infection and is an important determinant of cell and tissue tropism. *J Virol* **74**, 3366-78.

Sakaguchi, M., Nakamura, H., Sonoda, K., Okamura, H., Yokogawa, K., Matsuo, K. & Hira, K. (1998). Protection of chickens with or without maternal antibodies against both Marek's and Newcastle diseases by one-time vaccination with recombinant vaccine of Marek's disease virus type 1. *Vaccine* **16**, 472-9.

Sanchez, C. M., Izeta, A., Sanchez-Morgado, J. M., Alonso, S., Sola, I., Balasch, M., Plana-Duran, J. & Enjuanes, L. (1999). Targeted recombination demonstrates that the spike gene of transmissible gastroenteritis coronavirus is a determinant of its enteric tropism and virulence. *J Virol* **73**, 7607-18.

Sawai, K. & Meruelo, D. (1998). Cell-specific transfection of choriocarcinoma cells by using Sindbis virus hCG expressing chimeric vector. *Biochem Biophys Res Commun* **248**, 315-23.

Schauber, C. A., Tuerk, M. J., Pacheco, C. D., Escarpe, P. A. & Veres, G. (2004). Lentiviral vectors pseudotyped with baculovirus gp64 efficiently transduce mouse cells in vivo and show tropism restriction against hematopoietic cell types in vitro. *Gene Ther* **11**, 266-75.

Schlender, J., Bossert, B., Buchholz, U. & Conzelmann, K. K. (2000). Bovine respiratory syncytial virus nonstructural proteins NS1 and NS2 cooperatively antagonize alpha/beta interferon-induced antiviral response. *J Virol* **74**, 8234-42.

Schmidt, A. C., McAuliffe, J. M., Huang, A., Surman, S. R., Bailly, J. E., Elkins, W. R., Collins, P. L., Murphy, B. R. & Skiadopoulos, M. H. (2000). Bovine parainfluenza virus type 3 (BPIV3) fusion and hemagglutinin-neuraminidase glycoproteins make an important contribution to the restricted replication of BPIV3 in primates. *J Virol* **74**, 8922-9.

Schmidt, A. C., Wenzke, D. R., McAuliffe, J. M., St Claire, M., Elkins, W. R., Murphy, B. R. & Collins, P. L. (2002). Mucosal immunization of rhesus monkeys against respiratory syncytial virus subgroups A and B and human parainfluenza virus type 3 by using a live cDNA-derived vaccine based on a host range-attenuated bovine parainfluenza virus type 3 vector backbone. *J Virol* **76**, 1089-99.

Schmitt, J., Becher, P., Thiel, H. J. & Keil, G. M. (1999). Expression of bovine viral diarrhoea virus glycoprotein E2 by bovine herpesvirus-1 from a synthetic ORF and incorporation of E2 into recombinant virions. *J Gen Virol* **80**, 2839-48.

Schneider, U., Bullough, F., Vongpunsawad, S., Russell, S. J. & Cattaneo, R. (2000). Recombinant measles viruses efficiently entering cells through targeted receptors. *J Virol* **74**, 9928-36.

Schnell, M. J., Buonocore, L., Kretzschmar, E., Johnson, E. & Rose, J. K. (1996). Foreign glycoproteins expressed from recombinant vesicular stomatitis viruses are incorporated efficiently into virus particles. *Proc Natl Acad Sci U S A* **93**, 11359-65.

Schnell, M. J., Johnson, J. E., Buonocore, L. & Rose, J. K. (1997). Construction of a novel virus that targets HIV-1-infected cells and controls HIV-1 infection. *Cell* **90**, 849-57.

Schnierle, B. S., Stitz, J., Bosch, V., Nocken, F., Merget-Millitzer, H., Engelstadter, M., Kurth, R., Groner, B. & Cichutek, K. (1997). Pseudotyping of murine leukemia virus with the envelope glycoproteins of HIV generates a retroviral vector with specificity of infection for CD4-expressing cells. *Proc Natl Acad Sci U S A* **94**, 8640-5.

Schroder, A. R., Shinn, P., Chen, H., Berry, C., Ecker, J. R. & Bushman, F. (2002). HIV-1 integration in the human genome favors active genes and local hotspots. *Cell* **110**, 521-9.

Seder, R. A. & Paul, W. E. (1994). Acquisition of lymphokine-producing phenotype by CD4+ T cells. *Annu Rev Immunol* **12**, 635-73.

Seet, B. T., Johnston, J. B., Brunetti, C. R., Barrett, J. W., Everett, H., Cameron, C., Sypula, J., Nazarian, S. H., Lucas, A. & McFadden, G. (2003). Poxviruses and immune evasion. *Annu Rev Immunol* **21**, 377-423.

Sen, G. C. (2001). Viruses and interferons. *Annu Rev Microbiol* **55**, 255-81.

Sena-Esteves, M., Tebbets, J. C., Steffens, S., Crombleholme, T. & Flake, A. W. (2004). Optimized large-scale production of high titer lentivirus vector pseudotypes. *J Virol Methods* **122**, 131-9.

Sharma, D. P., Ramsay, A. J., Maguire, D. J., Rolph, M. S. & Ramshaw, I. A. (1996). Interleukin-4 mediates down regulation of antiviral cytokine expression and cytotoxic T-lymphocyte responses and exacerbates vaccinia virus infection in vivo. *J Virol* **70**, 7103-7.

Shayakhmetov, D. M., Carlson, C. A., Stecher, H., Li, Q., Stamatoyannopoulos, G. & Lieber, A. (2002). A high-capacity, capsid-modified hybrid adenovirus/adeno-associated virus vector for stable transduction of human hematopoietic cells. *J Virol* **76**, 1135-43.

Shayakhmetov, D. M., Papayannopoulou, T., Stamatoyannopoulos, G. & Lieber, A. (2000). Efficient gene transfer into human CD34(+) cells by a retargeted adenovirus vector. *J Virol* **74**, 2567-83.

Sheehy, A. M., Gaddis, N. C., Choi, J. D. & Malim, M. H. (2002). Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. *Nature* **418**, 646-50.

Shi, W., Arnold, G. S. & Bartlett, J. S. (2001). Insertional mutagenesis of the adeno-associated virus type 2 (AAV2) capsid gene and generation of AAV2 vectors targeted to alternative cell-surface receptors. *Hum Gene Ther* **12**, 1697-711.

Shi, W. & Bartlett, J. S. (2003). RGD inclusion in VP3 provides adeno-associated virus type 2 (AAV2)-based vectors with a heparan sulfate-independent cell entry mechanism. *Mol Ther* **7**, 515-25.

Shinya, K., Hamm, S., Hatta, M., Ito, H., Ito, T. & Kawaoka, Y. (2004). PB2 amino acid at position 627 affects replicative efficiency, but not cell tropism, of Hong Kong H5N1 influenza A viruses in mice. *Virology* **320**, 258-66.

Shiroki, K., Ishii, T., Aoki, T., Ota, Y., Yang, W. X., Komatsu, T., Ami, Y., Arita, M., Abe, S., Hashizume, S. & Nomoto, A. (1997). Host range phenotype induced by mutations in the internal ribosomal entry site of poliovirus RNA. *J Virol* **71**, 1-8.

Shisler, J. L. & Moss, B. (2001). Immunology 102 at poxvirus U: avoiding apoptosis. *Semin Immunol* **13**, 67-72.

Sieczkarski, S. B. & Whittaker, G. R. (2002). Dissecting virus entry via endocytosis. *J Gen Virol* **83**, 1535-45.

Skiadopoulos, M. H., Schmidt, A. C., Riggs, J. M., Surman, S. R., Elkins, W. R., St Claire, M., Collins, P. L. & Murphy, B. R. (2003). Determinants of the host range restriction of replication of bovine parainfluenza virus type 3 in rhesus monkeys are polygenic. *J Virol* **77**, 1141-8.

Skiadopoulos, M. H., Surman, S. R., Riggs, J. M., Collins, P. L. & Murphy, B. R. (2001). A chimeric human-bovine parainfluenza virus type 3 expressing measles virus hemagglutinin is attenuated for replication but is still immunogenic in rhesus monkeys. *J Virol* **75**, 10498-504.

Skiadopoulos, M. H., Surman, S. R., Riggs, J. M., Orvell, C., Collins, P. L. & Murphy, B. R. (2002a). Evaluation of the replication and immunogenicity of recombinant human parainfluenza virus type 3 vectors expressing up to three foreign glycoproteins. *Virology* **297**, 136-52.

Skiadopoulos, M. H., Tao, T., Surman, S. R., Collins, P. L. & Murphy, B. R. (1999). Generation of a parainfluenza virus type 1 vaccine candidate by replacing the HN and F glycoproteins of the live-attenuated PIV3 cp45 vaccine virus with their PIV1 counterparts. *Vaccine* **18**, 503-10.

Skiadopoulos, M. H., Tatem, J. M., Surman, S. R., Mitcho, Y., Wu, S. L., Elkins, W. R. & Murphy, B. R. (2002b). The recombinant chimeric human parainfluenza virus type 1 vaccine candidate, rHPIV3-1cp45, is attenuated, immunogenic, and protective in African green monkeys. *Vaccine* **20**, 1846-1852.

Slobod, K. S., Lockey, T. D., Howlett, N., Srinivas, R. V., Rencher, S. D., Freiden, P. J., Doherty, P. C. & Hurwitz, J. L. (2004). Subcutaneous administration of a recombinant vaccinia virus vaccine expressing multiple envelopes of HIV-1. *Eur J Clin Microbiol Infect Dis* **23**, 106-10.

Sodeik, B. (2000). Mechanisms of viral transport in the cytoplasm. *Trends Microbiol* **8**, 465-72.

Sola, I., Alonso, S., Zuniga, S., Balasch, M., Plana-Duran, J. & Enjuanes, L. (2003). Engineering the transmissible gastroenteritis virus genome as an expression vector inducing lactogenic immunity. *J Virol* **77**, 4357-69.

Sondermeijer, P. J., Claessens, J. A., Jenniskens, P. E., Mockett, A. P., Thijssen, R. A., Willemse, M. J. & Morgan, R. W. (1993). Avian herpesvirus as a live viral vector for the expression of heterologous antigens. *Vaccine* **11**, 349-58.

Sonoda, K., Sakaguchi, M., Okamura, H., Yokogawa, K., Tokunaga, E., Tokiyoshi, S., Kawaguchi, Y. & Hirai, K. (2000). Development of an effective polyvalent vaccine against both Marek's and Newcastle diseases based on recombinant Marek's disease virus type 1 in commercial chickens with maternal antibodies. *J Virol* **74**, 3217-26.

Spear, M. A., Schuback, D., Miyata, K., Grandi, P., Sun, F., Yoo, L., Nguyen, A., Brandt, C. R. & Breakefield, X. O. (2003). HSV-1 amplicon peptide display vector. *J Virol Methods* **107**, 71-9.

Spector, D. H., Wade, E., Wright, D. A., Koval, V., Clark, C., Jaquish, D. & Spector, S. A. (1990). Human immunodeficiency virus pseudotypes with expanded cellular and species tropism. *J Virol* **64**, 2298-308.

Spiegel, M., Bitzer, M., Schenk, A., Rossmann, H., Neubert, W. J., Seidler, U., Gregor, M. & Lauer, U. (1998). Pseudotype formation of Moloney murine leukemia virus with Sendai virus glycoprotein F. *J Virol* **72**, 5296-302.

Spielhofer, P., Bachi, T., Fehr, T., Christiansen, G., Cattaneo, R., Kaelin, K., Billeter, M. A. & Naim, H. Y. (1998). Chimeric measles viruses with a foreign envelope. *J Virol* **72**, 2150-9.

Staba, M. J., Wickham, T. J., Kovesdi, I. & Hallahan, D. E. (2000). Modifications of the fiber in adenovirus vectors increase tropism for malignant glioma models. *Cancer Gene Ther* **7**, 13-9.

Staeheli, P., Colonno, R. J. & Cheng, Y. S. (1983). Different mRNAs induced by interferon in cells from inbred mouse strains A/J and A2G. *J Virol* **47**, 563-7.

Steinhauer, D. A. (1999). Role of hemagglutinin cleavage for the pathogenicity of influenza virus. *Virology* **258**, 1-20.
Steinhauer, D. A. & Skehel, J. J. (2002). Genetics of influenza viruses. *Annu Rev Genet* **36**, 305-32.
Stevenson, S. C., Rollence, M., Marshall-Neff, J. & McClelland, A. (1997). Selective targeting of human cells by a chimeric adenovirus vector containing a modified fiber protein. *J Virol* **71**, 4782-90.
Stope, M. B., Karger, A., Schmidt, U. & Buchholz, U. J. (2001). Chimeric bovine respiratory syncytial virus with attachment and fusion glycoproteins replaced by bovine parainfluenza virus type 3 hemagglutinin-neuraminidase and fusion proteins. *J Virol* **75**, 9367-9377.
Stremlau, M., Owens, C. M., Perron, M. J., Kiessling, M., Autissier, P. & Sodroski, J. (2004). The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. *Nature* **427**, 848-53.
Subbarao, E. K., London, W. & Murphy, B. R. (1993). A single amino acid in the PB2 gene of influenza A virus is a determinant of host range. *J Virol* **67**, 1761-4.
Summerford, C., Bartlett, J. S. & Samulski, R. J. (1999). AlphaVbeta5 integrin: a co-receptor for adeno-associated virus type 2 infection. *Nat Med* **5**, 78-82.
Summerford, C. & Samulski, R. J. (1998). Membrane-associated heparan sulfate proteoglycan is a receptor for adeno-associated virus type 2 virions. *J Virol* **72**, 1438-45.
Sung, V. M. & Lai, M. M. (2002). Murine retroviral pseudotype virus containing hepatitis B virus large and small surface antigens confers specific tropism for primary human hepatocytes: a potential liver-specific targeting system. *J Virol* **76**, 912-7.
Sutter, G. & Moss, B. (1992). Nonreplicating vaccinia vector efficiently expresses recombinant genes. *Proc Natl Acad Sci U S A* **89**, 10847-51.
Sutter, G., Ramsey-Ewing, A., Rosales, R. & Moss, B. (1994a). Stable expression of the vaccinia virus K1L gene in rabbit cells complements the host range defect of a vaccinia virus mutant. *J Virol* **68**, 4109-16.
Sutter, G., Wyatt, L. S., Foley, P. L., Bennink, J. R. & Moss, B. (1994b). A recombinant vector derived from the host range-restricted and highly attenuated MVA strain of vaccinia virus stimulates protective immunity in mice to influenza virus. *Vaccine* **12**, 1032-40.
Svitkin, Y. V., Pestova, T. V., Maslova, S. V. & Agol, V. I. (1988). Point mutations modify the response of poliovirus RNA to a translation initiation factor: a comparison of neurovirulent and attenuated strains. *Virology* **166**, 394-404.
Swayne, D. E., Garcia, M., Beck, J. R., Kinney, N. & Suarez, D. L. (2000). Protection against diverse highly pathogenic H5 avian influenza viruses in chickens immunized with a recombinant fowlpox vaccine containing an H5 avian influenza hemagglutinin gene insert. *Vaccine* **18**, 1088-95.
Swayne, D. E., Suarez, D. L., Schultz-Cherry, S., Tumpey, T. M., King, D. J., Nakaya, T., Palese, P. & Garcia-Sastre, A. (2003). Recombinant paramyxovirus type 1-avian influenza-H7 virus as a vaccine for protection of chickens against influenza and Newcastle disease. *Avian Dis* **47**, 1047-50.
Tang, H. & McLachlan, A. (2001). Transcriptional regulation of hepatitis B virus by nuclear hormone receptors is a critical determinant of viral tropism. *Proc Natl Acad Sci U S A* **98**, 1841-6.
Tang, R. S., MacPhail, M., Schickli, J. H., Kaur, J., Robinson, C. L., Lawlor, H. A., Guzzetta, J. M., Spaete, R. R. & Haller, A. A. (2004). Parainfluenza virus type 3 expressing the native or soluble fusion (F) Protein of Respiratory Syncytial Virus (RSV) confers protection from RSV infection in African green monkeys. *J Virol* **78**, 11198-207.
Taniguchi, T. & Takaoka, A. (2002). The interferon-alpha/beta system in antiviral responses: a multimodal machinery of gene regulation by the IRF family of transcription factors. *Curr Opin Immunol* **14**, 111-6.
Tao, T., Durbin, A. P., Whitehead, S. S., Davoodi, F., Collins, P. L. & Murphy, B. R. (1998). Recovery of a fully viable chimeric human parainfluenza virus (PIV) type 3 in which the hemagglutinin-neuraminidase and fusion glycoproteins have been replaced by those of PIV type 1. *J Virol* **72**, 2955-61.
Tao, T., Skiadopoulos, M. H., Davoodi, F., Riggs, J. M., Collins, P. L. & Murphy, B. R. (2000). Replacement of the ectodomains of the hemagglutinin-neuraminidase and fusion glycoproteins of recombinant parainfluenza virus type 3 (PIV3) with their counterparts from PIV2 yields attenuated PIV2 vaccine candidates. *J Virol* **74**, 6448-58.
Tao, T., Skiadopoulos, M. H., Davoodi, F., Surman, S. R., Collins, P. L. & Murphy, B. R. (2001). Construction of a live-attenuated bivalent vaccine virus against human parainfluenza virus (PIV) types 1 and 2 using a recombinant PIV3 backbone. *Vaccine* **19**, 3620-3631.

Tao, T., Skiadopoulos, M. H., Durbin, A. P., Davoodi, F., Collins, P. L. & Murphy, B. R. (1999). A live attenuated chimeric recombinant parainfluenza virus (PIV) encoding the internal proteins of PIV type 3 and the surface glycoproteins of PIV type 1 induces complete resistance to PIV1 challenge and partial resistance to PIV3 challenge. *Vaccine* **17**, 1100-1108.
Tartaglia, J., Perkus, M. E., Taylor, J., Norton, E. K., Audonnet, J. C., Cox, W. I., Davis, S. W., van der Hoeven, J., Meignier, B., Riviere, M. & *et al.* (1992). NYVAC: a highly attenuated strain of vaccinia virus. *Virology* **188**, 217-32.
Tashiro, M., Yamakawa, M., Tobita, K., Klenk, H. D., Rott, R. & Seto, J. T. (1990). Organ tropism of Sendai virus in mice: proteolytic activation of the fusion glycoprotein in mouse organs and budding site at the bronchial epithelium. *J Virol* **64**, 3627-34.
Tatsuo, H., Okuma, K., Tanaka, K., Ono, N., Minagawa, H., Takade, A., Matsuura, Y. & Yanagi, Y. (2000). Virus entry is a major determinant of cell tropism of Edmonston and wild-type strains of measles virus as revealed by vesicular stomatitis virus pseudotypes bearing their envelope proteins. *J Virol* **74**, 4139-45.
Taylor, J., Edbauer, C., Rey-Senelonge, A., Bouquet, J. F., Norton, E., Goebel, S., Desmettre, P. & Paoletti, E. (1990). Newcastle disease virus fusion protein expressed in a fowlpox virus recombinant confers protection in chickens. *J Virol* **64**, 1441-50.
Taylor, J., Trimarchi, C., Weinberg, R., Languet, B., Guillemin, F., Desmettre, P. & Paoletti, E. (1991). Efficacy studies on a canarypox-rabies recombinant virus. *Vaccine* **9**, 190-3.
Taylor, J., Weinberg, R., Kawaoka, Y., Webster, R. G. & Paoletti, E. (1988). Protective immunity against avian influenza induced by a fowlpox virus recombinant. *Vaccine* **6**, 504-8.
Tijssen, P. (1999). Molecular and structural basis of the evolution of parvovirus tropism. *Acta Vet Hung* **47**, 379-94.
Towers, G. J. & Goff, S. P. (2003). Post-entry restriction of retroviral infections. *AIDS Rev* **5**, 156-64.
Towers, G. J., Hatziioannou, T., Cowan, S., Goff, S. P., Luban, J. & Bieniasz, P. D. (2003). Cyclophilin A modulates the sensitivity of HIV-1 to host restriction factors. *Nat Med* **9**, 1138-43.
Tsai, J. C., Zelus, B. D., Holmes, K. V. & Weiss, S. R. (2003). The N-terminal domain of the murine coronavirus spike glycoprotein determines the CEACAM1 receptor specificity of the virus strain. *J Virol* **77**, 841-50.
Turan, K., Mibayashi, M., Sugiyama, K., Saito, S., Numajiri, A. & Nagata, K. (2004). Nuclear MxA proteins form a complex with influenza virus NP and inhibit the transcription of the engineered influenza virus genome. *Nucleic Acids Res* **32**, 643-52.
Turelli, P., Mangeat, B., Jost, S., Vianin, S. & Trono, D. (2004). Inhibition of hepatitis B virus replication by APOBEC3G. *Science* **303**, 1829.
Valsesia-Wittmann, S., Drynda, A., Deleage, G., Aumailley, M., Heard, J. M., Danos, O., Verdier, G. & Cosset, F. L. (1994). Modifications in the binding domain of avian retrovirus envelope protein to redirect the host range of retroviral vectors. *J Virol* **68**, 4609-19.
Valsesia-Wittmann, S., Morling, F. J., Nilson, B. H., Takeuchi, Y., Russell, S. J. & Cosset, F. L. (1996). Improvement of retroviral retargeting by using amino acid spacers between an additional binding domain and the N terminus of Moloney murine leukemia virus SU. *J Virol* **70**, 2059-64.
van Den Broek, M., Bachmann, M. F., Kohler, G., Barner, M., Escher, R., Zinkernagel, R. & Kopf, M. (2000). IL-4 and IL-10 antagonize IL-12-mediated protection against acute vaccinia virus infection with a limited role of IFN-gamma and nitric oxide synthetase 2. *J Immunol* **164**, 371-8.
Van Der Most, R. G., Murali-Krishna, K., Ahmed, R. & Strauss, J. H. (2000). Chimeric yellow fever/dengue virus as a candidate dengue vaccine: Quantitation of the dengue virus-specific CD8 T-cell response. *J Virol* **74**, 8094-8101.
van Gennip, H. G., van Rijn, P. A., Widjojoatmodjo, M. N., de Smit, A. J. & Moormann, R. J. (2000). Chimeric classical swine fever viruses containing envelope protein E(RNS) or E2 of bovine viral diarrhoea virus protect pigs against challenge with CSFV and induce a distinguishable antibody response. *Vaccine* **19**, 447-59.
van Zijl, M., Wensvoort, G., de Kluyver, E., Hulst, M., van der Gulden, H., Gielkens, A., Berns, A. & Moormann, R. (1991). Live attenuated pseudorabies virus expressing envelope glycoprotein E1 of hog cholera virus protects swine against both pseudorabies and hog cholera. *J Virol* **65**, 2761-5.
Vanderkwaak, T. J., Wang, M., Gomez-Navarro, J., Rancourt, C., Dmitriev, I., Krasnykh, V., Barnes, M., Siegal, G. P., Alvarez, R. & Curiel, D. T. (1999). An advanced generation of adenoviral vectors selectively enhances gene transfer for ovarian cancer gene therapy approaches. *Gynecol Oncol* **74**, 227-34.

Vellinga, J., Rabelink, M. J., Cramer, S. J., van den Wollenberg, D. J., Van der Meulen, H., Leppard, K. N., Fallaux, F. J. & Hoeben, R. C. (2004). Spacers increase the accessibility of peptide ligands linked to the carboxyl terminus of adenovirus minor capsid protein IX. *J Virol* **78**, 3470-9.
Verardi, P. H., Aziz, F. H., Ahmad, S., Jones, L. A., Beyene, B., Ngotho, R. N., Wamwayi, H. M., Yesus, M. G., Egziabher, B. G. & Yilma, T. D. (2002). Long-term sterilizing immunity to rinderpest in cattle vaccinated with a recombinant vaccinia virus expressing high levels of the fusion and hemagglutinin glycoproteins. *J Virol* **76**, 484-91.
Verheije, M. H., Welting, T. J., Jansen, H. T., Rottier, P. J. & Meulenberg, J. J. (2002). Chimeric arteriviruses generated by swapping of the M protein ectodomain rule out a role of this domain in viral targeting. *Virology* **303**, 364-73.
Verhoeyen, E. & Cosset, F. L. (2004). Surface-engineering of lentiviral vectors. *J Gene Med* **6**, S83-94.
Vigne, E., Mahfouz, I., Dedieu, J. F., Brie, A., Perricaudet, M. & Yeh, P. (1999). RGD inclusion in the hexon monomer provides adenovirus type 5-based vectors with a fiber knob-independent pathway for infection. *J Virol* **73**, 5156-61.
Vincent, S., Tigaud, I., Schneider, H., Buchholz, C. J., Yanagi, Y. & Gerlier, D. (2002). Restriction of measles virus RNA synthesis by a mouse host cell line: trans-complementation by polymerase components or a human cellular factor(s). *J Virol* **76**, 6121-30.
Volpers, C. & Kochanek, S. (2004). Adenoviral vectors for gene transfer and therapy. *J Gene Med* **6**, S164-71.
Volpers, C., Thirion, C., Biermann, V., Hussmann, S., Kewes, H., Dunant, P., von der Mark, H., Herrmann, A., Kochanek, S. & Lochmuller, H. (2003). Antibody-mediated targeting of an adenovirus vector modified to contain a synthetic immunoglobulin g-binding domain in the capsid. *J Virol* **77**, 2093-104.
von Messling, V. & Cattaneo, R. (2004). Toward novel vaccines and therapies based on negative-strand RNA viruses. *Curr Top Microbiol Immunol* **283**, 281-312.
Von Seggern, D. J., Huang, S., Fleck, S. K., Stevenson, S. C. & Nemerow, G. R. (2000). Adenovirus vector pseudotyping in fiber-expressing cell lines: improved transduction of Epstein-Barr virus-transformed B cells. *J Virol* **74**, 354-62.
Vongpunsawad, S., Oezgun, N., Braun, W. & Cattaneo, R. (2004). Selectively receptor-blind measles viruses: Identification of residues necessary for SLAM- or CD46-induced fusion and their localization on a new hemagglutinin structural model. *J Virol* **78**, 302-13.
Wahlers, A., Kustikova, O., Zipfel, P. F., Itoh, K., Koester, M., Heberlein, C., Li, Z., Schiedlmeier, B., Skerka, C., Fehse, B. & Baum, C. (2002). Upstream conserved sequences of mouse leukemia viruses are important for high transgene expression in lymphoid and hematopoietic cells. *Mol Ther* **6**, 313-20.
Warrington, K. H., Jr., Gorbatyuk, O. S., Harrison, J. K., Opie, S. R., Zolotukhin, S. & Muzyczka, N. (2004). Adeno-associated virus type 2 VP2 capsid protein is nonessential and can tolerate large peptide insertions at its N terminus. *J Virol* **78**, 6595-609.
Whealy, M. E., Baumeister, K., Robbins, A. K. & Enquist, L. W. (1988). A herpesvirus vector for expression of glycosylated membrane antigens: fusion proteins of pseudorabies virus gIII and human immunodeficiency virus type 1 envelope glycoproteins. *J Virol* **62**, 4185-94.
White, S. J., Nicklin, S. A., Buning, H., Brosnan, M. J., Leike, K., Papadakis, E. D., Hallek, M. & Baker, A. H. (2004). Targeted gene delivery to vascular tissue in vivo by tropism-modified adeno-associated virus vectors. *Circulation* **109**, 513-9.
Whitley, R. J., Kern, E. R., Chatterjee, S., Chou, J. & Roizman, B. (1993). Replication, establishment of latency, and induced reactivation of herpes simplex virus gamma 1 34.5 deletion mutants in rodent models. *J Clin Invest* **91**, 2837-43.
Whittaker, G. R., Kann, M. & Helenius, A. (2000). Viral entry into the nucleus. *Annu Rev Cell Dev Biol* **16**, 627-51.
Wickham, T. J., Carrion, M. E. & Kovesdi, I. (1995). Targeting of adenovirus penton base to new receptors through replacement of its RGD motif with other receptor-specific peptide motifs. *Gene Ther* **2**, 750-6.
Wickham, T. J., Tzeng, E., Shears, L. L., 2nd, Roelvink, P. W., Li, Y., Lee, G. M., Brough, D. E., Lizonova, A. & Kovesdi, I. (1997). Increased in vitro and in vivo gene transfer by adenovirus vectors containing chimeric fiber proteins. *J Virol* **71**, 8221-9.
Wiktor, T. J., Macfarlan, R. I., Reagan, K. J., Dietzschold, B., Curtis, P. J., Wunner, W. H., Kieny, M. P., Lathe, R., Lecocq, J. P., Mackett, M. & *et al.* (1984). Protection from rabies by a vaccinia

virus recombinant containing the rabies virus glycoprotein gene. *Proc Natl Acad Sci U S A* **81**, 7194-8.
Willemse, M. J., van Schooneveld, S. H., Chalmers, W. S. & Sondermeijer, P. J. (1996). Vaccination against feline leukaemia using a new feline herpesvirus type 1 vector. *Vaccine* **14**, 1511-6.
Work, L. M., Nicklin, S. A., Brain, N. J., Dishart, K. L., Von Seggern, D. J., Hallek, M., Buning, H. & Baker, A. H. (2004a). Development of efficient viral vectors selective for vascular smooth muscle cells. *Mol Ther* **9**, 198-208.
Work, L. M., Nicklin, S. A., White, S. J. & Baker, A. H. (2002). Use of phage display to identify novel peptides for targeted gene therapy. *Methods Enzymol* **346**, 157-76.
Work, L. M., Ritchie, N., Nicklin, S. A., Reynolds, P. N. & Baker, A. H. (2004b). Dual targeting of gene delivery by genetic modification of adenovirus serotype 5 fibers and cell-selective transcriptional control. *Gene Ther* **11**, 1296-300.
Wu, B. W., Lu, J., Gallaher, T. K., Anderson, W. F. & Cannon, P. M. (2000a). Identification of regions in the Moloney murine leukemia virus SU protein that tolerate the insertion of an integrin-binding peptide. *Virology* **269**, 7-17.
Wu, N., Watkins, S. C., Schaffer, P. A. & DeLuca, N. A. (1996). Prolonged gene expression and cell survival after infection by a herpes simplex virus mutant defective in the immediate-early genes encoding ICP4, ICP27, and ICP22. *J Virol* **70**, 6358-69.
Wu, P., Xiao, W., Conlon, T., Hughes, J., Agbandje-McKenna, M., Ferkol, T., Flotte, T. & Muzyczka, N. (2000b). Mutational analysis of the adeno-associated virus type 2 (AAV2) capsid gene and construction of AAV2 vectors with altered tropism. *J Virol* **74**, 8635-47.
Wu, Q. & Tikoo, S. K. (2004). Altered tropism of recombinant bovine adenovirus type-3 expressing chimeric fiber. *Virus Res* **99**, 9-15.
Wu, X., Li, Y., Crise, B. & Burgess, S. M. (2003). Transcription start regions in the human genome are favored targets for MLV integration. *Science* **300**, 1749-51.
Xiang, Y., Condit, R. C., Vijaysri, S., Jacobs, B., Williams, B. R. & Silverman, R. H. (2002). Blockade of interferon induction and action by the E3L double-stranded RNA binding proteins of vaccinia virus. *J Virol* **76**, 5251-9.
Xiao, X., Li, J. & Samulski, R. J. (1998). Production of high-titer recombinant adeno-associated virus vectors in the absence of helper adenovirus. *J Virol* **72**, 2224-32.
Xuan, X., Nishikawa, Y., Takashima, Y., Tuchiya, K., Ueda, S., Yokoyama, N., Maeda, K., Mikami, T. & Otsuka, H. (1998a). Construction of canine herpesvirus vector expressing foreign genes using a lacZ-TK gene cassette as a double selectional marker. *Virus Genes* **17**, 25-32.
Xuan, X., Tuchiya, K., Sato, I., Nishikawa, Y., Onoderaz, Y., Takashima, Y., Yamamoto, A., Katsumata, A., Iwata, A., Ueda, S., Mikami, T. & Otsuka, H. (1998b). Biological and immunogenic properties of rabies virus glycoprotein expressed by canine herpesvirus vector. *Vaccine* **16**, 969-76.
Yanagiya, A., Ohka, S., Hashida, N., Okamura, M., Taya, C., Kamoshita, N., Iwasaki, K., Sasaki, Y., Yonekawa, H. & Nomoto, A. (2003). Tissue-specific replicating capacity of a chimeric poliovirus that carries the internal ribosome entry site of hepatitis C virus in a new mouse model transgenic for the human poliovirus receptor. *J Virol* **77**, 10479-87.
Yang, Q., Mamounas, M., Yu, G., Kennedy, S., Leaker, B., Merson, J., Wong-Staal, F., Yu, M. & Barber, J. R. (1998). Development of novel cell surface CD34-targeted recombinant adenoassociated virus vectors for gene therapy. *Hum Gene Ther* **9**, 1929-37.
Yewdell, J. W. & Bennink, J. R. (1999). Mechanisms of viral interference with MHC class I antigen processing and presentation. *Annu Rev Cell Dev Biol* **15**, 579-606.
Yilma, T., Hsu, D., Jones, L., Owens, S., Grubman, M., Mebus, C., Yamanaka, M. & Dale, B. (1988). Protection of cattle against rinderpest with vaccinia virus recombinants expressing the HA or F gene. *Science* **242**, 1058-61.
Young, D. F., Chatziandreou, N., He, B., Goodbourn, S., Lamb, R. A. & Randall, R. E. (2001). Single amino acid substitution in the V protein of simian virus 5 differentiates its ability to block interferon signaling in human and murine cells. *J Virol* **75**, 3363-70.
Young, D. F., Didcock, L., Goodbourn, S. & Randall, R. E. (2000). Paramyxoviridae use distinct virus-specific mechanisms to circumvent the interferon response. *Virology* **269**, 383-90.
Yun, S. I., Kim, S. Y., Rice, C. M. & Lee, Y. M. (2003). Development and application of a reverse genetics system for Japanese encephalitis virus. *J Virol* **77**, 6450-65.
Zabner, J., Chillon, M., Grunst, T., Moninger, T. O., Davidson, B. L., Gregory, R. & Armentano, D. (1999). A chimeric type 2 adenovirus vector with a type 17 fiber enhances gene transfer to human airway epithelia. *J Virol* **73**, 8689-8695.

Zerbini, L. F., Libermann, T. A. & Ventura, A. M. (2002). Insertion of an exogenous domain in the adenovirus type 2 fiber globular region. *Biochem Biophys Res Commun* **296**, 897-903.
Zhao, H., De, B. P., Das, T. & Banerjee, A. K. (1996). Inhibition of human parainfluenza virus-3 replication by interferon and human MxA. *Virology* **220**, 330-8.
Zhao, Y., Zhu, L., Lee, S., Li, L., Chang, E., Soong, N. W., Douer, D. & Anderson, W. F. (1999). Identification of the block in targeted retroviral-mediated gene transfer. *Proc Natl Acad Sci U S A* **96**, 4005-10.
Zhu, Z. B., Makhija, S. K., Lu, B., Wang, M., Kaliberova, L., Liu, B., Rivera, A. A., Nettelbeck, D. M., Mahasreshti, P. J., Leath, C. A., 3rd, Yamaoto, M., Alvarez, R. D. & Curiel, D. T. (2004a). Transcriptional targeting of adenoviral vector through the CXCR4 tumor-specific promoter. *Gene Ther* **11**, 645-8.
Zhu, Z. B., Makhija, S. K., Lu, B., Wang, M., Kaliberova, L., Liu, B., Rivera, A. A., Nettelbeck, D. M., Mahasreshti, P. J., Leath, C. A., Barker, S., Yamaoto, M., Li, F., Alvarez, R. D. & Curiel, D. T. (2004b). Transcriptional targeting of tumors with a novel tumor-specific survivin promoter. *Cancer Gene Ther* **11**, 256-62.

Recombinant en chimaere virussen:

Evaluatie van risico's ten gevolge van veranderingen in tropisme

OPDRACHT

In opdracht van de COGEM is in het kader van het onderzoeksprogramma 2004 een deskstudie verricht getiteld: “Gastheerbereik chimaere virussen” (Bijlage 1). Doel van het onderzoek was het in kaart brengen van de laatste wetenschappelijke ontwikkelingen op het gebied van recombinant virussen, chimaere virussen en virale vectoren, en van mogelijke risico’s ten gevolge van een mogelijk veranderend gastheerbereik van dergelijke virussen en vectoren. Tevens werd een inventarisatie uitgevoerd van de stand van zaken op het gebied van factoren die kunnen leiden tot veranderingen in het gastheerbereik van virussen.

De studie werd uitgevoerd door Dr. Ben Peeters, senior wetenschappelijk medewerker van de Animal Sciences Group van Wageningen Universiteit en Research Centrum.

De begeleidingscommissie had de volgende samenstelling:

Voorzitter: Prof. Dr. R.C. Hoeben, Leids Universitair Medisch Centrum

Leden: Dr. D. van Zaane, Wageningen Universiteit en Research Centrum
Dr. C. van Maanen, Gezondheidsdienst voor Dieren
Drs. D. Louz, Bureau GGO
Ing. P. van Beurden-Snoek, secretariaat COGEM

De begeleidingscommissie is in totaal drie keer bij elkaar gekomen; bij de start van het project, tijdens het project, en op het eind van het project.

Als externe deskundigen werden geraadpleegd:

Prof. Dr. Peter Rottier (Faculteit Diergeneeskunde, Universiteit van Utrecht)
Prof. Dr. Albert Osterhaus (Afdeling Virologie, Erasmus Universiteit Rotterdam)
Dr. Ron Fouchier (Afdeling Virologie, Erasmus Universiteit Rotterdam)

Het studierapport is - in overleg met de begeleidingscommissie - opgesteld in het Engels. Het rapport is daardoor toegankelijk voor buitenlanders en kan tevens gebruikt worden door buitenlandse zusterinstanties. Tevens kan het rapport eventueel als artikel worden gepubliceerd in een wetenschappelijk tijdschrift.

VERANTWOORDING

Dit rapport is samengesteld aan de hand van literatuurgegevens uit wetenschappelijke tijdschriften die toegankelijk zijn voor wetenschappelijke instellingen en individuele wetenschappers. Er is géén gebruik gemaakt van interne rapporten van bedrijven of instellingen die niet publiekelijk toegankelijk zijn. De inhoud van dit rapport is bedoeld als wetenschappelijke achtergrond informatie ten behoeve van de onderbouwing van wetenschappelijke adviezen van de COGEM. Dit rapport is niet bedoeld als opiniestuk. Interpretatie van de gegevens door de auteur is puur op persoonlijke titel vanuit een wetenschappelijke achtergrond en representeert niet de mening van de COGEM, noch die van de Animal Sciences Group.

LEESWIJZER

Dit rapport bestaat uit zeven hoofdstukken. Na een korte introductie over de genetische modificatie van virussen (hoofdstuk 1) en de definitie van recombinant en chimaere virussen (hoofdstuk 2), volgt in hoofdstuk 3 een korte beschrijving van de verschillende toepassingen van recombinant en chimaere virussen. Vervolgens wordt in hoofdstuk 4 een gedetailleerde beschrijving gegeven van recente ontwikkelingen op het gebied van de sturing van het tropisme van virussen en virale vectoren. In hoofdstuk 5 worden chimaere virussen beschreven die een heteroloog viraal oppervlakte eiwit tot expressie brengen dat wordt geïncorporeerd in chimaere virusdeeltjes, waardoor mogelijk veranderingen in tropisme optreden. Hoofdstuk 6 geeft een overzicht van onze huidige kennis van de virale en gastheer factoren die het tropisme van virussen bepalen. Tenslotte wordt in hoofdstuk 7 ingegaan op de eventuele risico's ten gevolge van een veranderd tropisme van recombinant en chimaere virussen.

MANAGEMENT SAMENVATTING

Door middel van moleculair biologische technieken is het mogelijk om het erfelijke materiaal van (micro-)organismen gericht te veranderen (‘genetische modificatie’). Dit heeft onder andere geleid tot de productie van genetisch gemodificeerde virussen. Tevens is het met deze technieken mogelijk om nieuwe virussen te maken die bestaan uit een combinatie van het erfelijke materiaal van twee verschillende virussen. Dergelijke recombinant virussen worden ook wel aangeduid met de term chimaere virussen. Recombinant virussen en chimaere virussen worden gebruikt voor tal van onderzoeksdoeleinden en voor toepassingen zoals experimentele gentherapie en vaccinontwikkeling.

Virusdeeltjes kunnen lichaamscellen herkennen en binnendringen door interacties met een receptor op het celoppervlak. Of het virus vervolgens in staat is om zich in deze cellen te vermeerderen, is afhankelijk van verschillende factoren die zowel door het virus als door de cel bepaald worden. Samen met de receptor bepalen deze factoren de cel-, weefsel-, en gastheerspecificiteit (gezamenlijk aangeduid met de term ‘tropisme’) van een virus. Veranderingen in tropisme kunnen spontaan optreden door natuurlijke veranderingen (mutaties) in het erfelijke materiaal. Soms leidt dit tot uitbreiding van het gastheerbereik, zoals bijvoorbeeld het geval is geweest bij virussen zoals HIV, influenza en het SARS geassocieerde coronavirus.

Tropisme veranderingen kunnen ook het gevolg zijn van gerichte genetische modificatie. Eén van de toepassingen waarbij vaak een gerichte verandering van het tropisme van virussen wordt beoogd is gentherapie. Hierbij worden virussen zodanig genetisch veranderd dat ze het vermogen krijgen om therapeutisch interessante cellen of weefsels te infecteren die normaalgesproken niet door het oudervirus worden geïnfecteerd. In andere gevallen, b.v. bij vaccin ontwikkeling, is verandering van het tropisme onbedoeld het gevolg van de inbouw van een oppervlakte eiwit van het ene virus in de virusmantel van een ander virus.

Gezien het infectieuze karakter van virussen en het feit dat ze ziekteverwekkend kunnen zijn, is de toepassing van virussen waarvan het tropisme – opzettelijk dan wel onopzettelijk - veranderd is, niet geheel zonder risico’s. Om een beter inzicht te krijgen in de eventuele risico’s ten gevolge van tropisme veranderingen van recombinant en chimaere virussen, heeft de COGEM een studierapport laten opstellen waarin een overzicht wordt gegeven van de wetenschappelijke stand van zaken betreffende de ontwikkeling en toepassing van dergelijke virussen. Tevens bevat het rapport een inventarisatie van de virale en gastheer factoren die het tropisme van een virus bepalen. De resultaten van dit rapport kunnen worden gebruikt als achtergrond informatie en als leidraad voor de COGEM ter beoordeling van mogelijke risico’s die gepaard gaan met de ontwikkeling en toepassing van recombinant en chimaere virussen.

De belangrijkste risicofactoren die werden geïdentificeerd kunnen worden ondergebracht in 4 categorieën, te weten (1) de eigenschappen van het dragervirus, (2) veranderingen in tropisme met

betrekking tot receptor interacties, (3) veranderingen in tropisme met betrekking tot antivirale afweer reacties van de gastheer, en (4) de gezondheidsstatus van de gastheer. Ofschoon niet altijd voorspelbaar is of een verandering in tropisme leidt tot een toename in het ziekteverwekkend vermogen van een virus, kan op basis van criteria die in dit rapport naar voren komen in veel gevallen een redelijke inschatting worden gemaakt van de kans op een dergelijke gebeurtenis en de daarmee gepaard gaande risico's.

Vanwege onze beperkte kennis van de relatie tussen tropisme en ziekteverwekkend vermogen van virussen, verdient het aanbeveling om onderzoek op dit gebied te stimuleren en wetenschappelijke ontwikkelingen op dit gebied nauwkeurig in de gaten te houden teneinde een reële inschatting te kunnen maken van eventuele risico's die werkzaamheden met recombinant en chimaere virussen met zich meebrengen.

NEDERLANDSE SAMENVATTING

Recombinant en chimaere virussen:

evaluatie van risico's ten gevolge van veranderingen in tropisme

Inleiding

Genetische modificatie kan worden gebruikt voor het maken van recombinant virussen of chimaere virussen die bestaan uit een combinatie van de genetische informatie (het 'genoom') van twee verschillende virussen. Door deze nieuwe combinatie kunnen de biologische eigenschappen van dergelijke virussen afwijken van die van de oorspronkelijke oudervirussen. Doordat onze kennis van de subtiele interacties tussen virussen en hun gastheer relatief beperkt is, is het niet altijd mogelijk om de biologische eigenschappen van dergelijke nieuwe virussen exact te voorspellen. Daarom zijn toepassingen van dergelijke virussen niet geheel vrij van risico's, met name wanneer het toepassingen in dieren of mensen betreft. In dit rapport wordt een overzicht gegeven van recente ontwikkelingen op het gebied van de ontwikkeling en toepassing van recombinant en chimaere virussen, met de nadruk op die virussen waarvan het cel-, weefsel- of gastheertropisme opzettelijk dan wel onopzettelijk is veranderd.

1. Genetische modificatie van virussen

Ontwikkelingen in de recombinant DNA technologie hebben geleid tot technieken die het mogelijk maken om het genetisch materiaal van organismen gericht te veranderen. Vanwege hun relatief kleine genoom zijn virussen in het verleden veelvuldig gebruikt als modelsysteem om allerlei moleculair biologische processen te bestuderen. Dit heeft onder andere geleid tot de productie van recombinant virussen en chimaere virussen. Omdat recombinant DNA technieken met name geschikt zijn voor de modificatie van DNA, werden aanvankelijk voornamelijk virussen gebruikt waarvan het genoom bestaat uit DNA. Later werden ook RNA virussen gebruikt dankzij technieken die het mogelijk maakten om RNA te vertalen in DNA en dit vervolgens (na eventuele genetische modificatie) weer terug te vertalen in RNA. Deze laatste technologie wordt ook wel aangeduid met de term 'reverse genetics'. Momenteel is genetische modificatie in principe mogelijk van vrijwel elk virus, ongeacht of het genetisch materiaal bestaat uit DNA of RNA.

2. Recombinant en chimaere virussen

De term 'recombinant virus' wordt over het algemeen gebruikt om aan te geven dat het genoom van een virus veranderd is, ofwel door herschikking, verwijdering, toevoeging, of vervanging van genetisch materiaal. Een voorbeeld van een recombinant virus is een virus dat een stukje genetische informatie (een 'gen') bevat afkomstig van een ander organisme en dat bijvoorbeeld codeert voor een therapeutisch eiwit. De term 'chimaer virus' wordt gebruikt om aan te geven dat het een recombinant virus betreft waarvan het genoom bestaat uit een combinatie van het genoom van twee verschillende virussen. Een voorbeeld van een chimaer virus is een virus waarvan het gen dat codeert voor een

oppervlakte eiwit is vervangen door een soortgelijk gen van een ander virus. In dit rapport zal de term chimaer virus worden gebruikt voor een recombinant virus dat tenminste één functioneel gen bevat van een ander virus. Dit nieuwe gen kan ofwel zijn toegevoegd aan het genoom van het dragervirus of het kan een vervanging zijn. Voor influenza A virus zullen we de gebruikelijke term 'reassortant' gebruiken indien het de uitwisseling van genoom segmenten betreft tussen verschillende influenza A stammen. Indien er genoom segmenten van een ander type influenza (B of C) betrokken zijn zullen we de term chimaer gebruiken.

3. **Toepassingen van recombinant en chimaere virussen**

De toepassingen van recombinant en chimaere virussen kunnen grofweg worden ingedeeld in 5 categorieën. De **eerste** toepassing betreft fundamentele studies naar virus-gastheer interacties. Hierbij valt te denken aan onderzoek naar interacties tussen virale oppervlakte eiwitten en cel receptoren, naar processen zoals endocytose en transport van virale componenten in de cel en naar de kern, en onderzoek naar nieuwe antivirale geneesmiddelen.

De **tweede** toepassing is gentherapie. Virussen zijn een uitstekend transportmiddel om therapeutische genen in een cel binnen te brengen. De meest gebruikte gentherapie vectoren zijn afgeleid van adenovirussen, adeno-associated virus (AAV), herpesvirussen, pokkenvirussen of retrovirussen (incl. lentivirussen). Gentherapie vectoren bestaan uit "kreupele" virussen die éénmalig in staat zijn om cellen te infecteren maar vervolgens niet meer in staat zijn om nakomelingen te produceren. Vaak bezitten gentherapie vectoren niet het gewenste tropisme voor bepaalde cel typen. Om hun toepassing specifieker en effectiever te maken wordt daarom vaak getracht het tropisme gericht te veranderen.

De **derde** toepassing betreft kankertherapie. Hiervoor worden verschillende soorten virussen gebruikt die, ofwel van nature, ofwel na genetische modificatie, in staat zijn tot efficiënte replicatie in met name kankercellen maar niet of nauwelijks in normale cellen. Deze virussen worden ook wel aangeduid met de term 'oncolytische virussen' en hun toepassing in kankerbestrijding met 'virotherapie'. Het betreft virussen die conditioneel replicatie-competent zijn en die in principe in staat zijn om nakomelingen te produceren. Om ervoor te zorgen dat deze virussen desondanks veilig kunnen worden gebruikt, wordt geprobeerd om hun tropisme gericht te veranderen en te beperken tot kankercellen.

De **vierde** toepassing betreft vaccinatie. Ofschoon er voor een aantal virale ziekten uitstekende vaccins bestaan (b.v. pokken, polio) is dat voor lang niet alle virale infectieziekten het geval. Daarom wordt getracht om veilige en goed gekarakteriseerde virale vaccinstammen als drager te gebruiken voor de expressie van immunogene eiwitten van andere virussen. Vaak zijn oppervlakte eiwitten die betrokken zijn bij herkenning van de gastheercel de meest immunogene eiwitten van een virus. Door deze eiwitten te incorporeren in een ander virus is het in principe mogelijk dat zo'n chimaer virus veranderde biologische eigenschappen krijgt doordat het bijvoorbeeld een uitgebreider tropisme heeft.

De **laatste** toepassing betreft het gebruik van recombinant virussen om het immuunsysteem te beïnvloeden. Bepaalde eiwitten zoals cytokines en chemokines kunnen het afweersysteem veranderen of stimuleren en zo als adjuvans functioneren waardoor de immuunrespons na vaccinatie sterk wordt verbeterd. Door genen die coderen voor dergelijke cytokines in te bouwen in virale vaccins

zou een effectievere vaccinatie bereikt kunnen worden. Door de vele verschillende effecten, zowel positieve als negatieve, van cytokines op het immuunsysteem is het echter niet altijd mogelijk om de effecten van tevoren te voorspellen.

4. Recente ontwikkelingen op het gebied van de sturing van het tropisme van virussen

Verschillende methoden zijn - en worden - gebruikt om het tropisme van virussen of virale vectoren gericht te veranderen. Eén van de methoden maakt gebruik van het tijdelijk incorporeren van een heteroloog oppervlakte eiwit in een virusdeeltje door dit eiwit tot expressie te brengen in dezelfde cel waarin dat virus zich vermenigvuldigt. Dit proces wordt 'pseudotypering' genoemd. Het betreft hier dus géén genetische modificatie van het virus. In het verleden werden cellen bijvoorbeeld met twee verschillende virussen geïnfecteerd waardoor het ene virus oppervlakte eiwitten van het ander virus in kan bouwen. Dit proces staat ook wel bekend onder de naam 'phenotypische menging'. Tegenwoordig wordt het in te bouwen eiwit meestal individueel via een andere methode tot expressie gebracht. Een ander methode maakt gebruik van zogenaamde bi-specifieke conjugaten. Dit zijn moleculen (meestal eiwitten) die bestaan uit twee componenten, nl. één die bindt aan het virusdeeltje, en een ander die bindt aan het celoppervlak. Op deze wijze worden virusdeeltjes min of meer gedwongen om contact te maken met de gewenste cellen.

De volgende stap in het veranderen van de oppervlakte eiwitten van virussen en virale vectoren is het gebruik van genetische modificatie van het virale genoom zelf om het gen dat codeert voor een oppervlakte eiwit te veranderen. Deze veranderingen kunnen bestaan uit het compleet vervangen van een gen door een ander gen, of uit het gedeeltelijke vervanging van bijvoorbeeld alleen de component die verantwoordelijk is voor de binding aan de receptor. In de meeste gevallen werkt dit alleen als de twee eiwitten vrij sterk op elkaar lijken. Vaak betreft het dan ook uitwisseling van tropisme-bepalende eiwitten of gedeelten van dergelijke eiwitten van virussen van dezelfde of een nauw verwante soort. Voorbeelden zijn het gebruik van capside eiwitten van verschillende subtypen van het adenovirus. Het adenovirus dat het meest gebruikt wordt voor gentherapie is het humane adenovirus type 2 (Ad2) en 5 (Ad5). Door het capside gen te vervangen door dat van bijvoorbeeld Ad35 kan het tropisme worden veranderd. Echter, omdat de diversiteit van de receptoren die door de capside eiwitten herkend worden beperkt is, is dit niet altijd de meest geschikte manier om het tropisme van een virus te veranderen. Bovendien is er niet altijd een ander adenovirus subtype beschikbaar met de gewenste weefsel specificiteit. De receptor voor adenovirus, CAR (coxsackie en adenovirus receptor), komt niet op alle cellen voor die therapeutisch interessant zijn. Daarom is getracht om de affiniteit van adenovirus voor intergrine, een eiwit dat vaak wél voorkomt op dergelijke cellen, te verhogen door gebruik te maken van een specifiek tripeptide bestaande uit de aminozuren arginine-glycine-aspartaatzuur (RGD) dat sterk bindt aan integrine. Dit RGD tripeptide is een voorbeeld van een bindingspartner (ligand) die op verschillende plaatsen kan worden ingebouwd in het adenovirus capside. Op deze wijze zijn ook andere liganden gebruikt zoals bijvoorbeeld poly-lysine dat bindt aan heparaan-sulfaat op het celoppervlak. De nieuwste ontwikkelingen maken gebruik van de inbouw van liganden die geselecteerd zijn uit een synthetische peptidenbank met behulp van bepaalde bindings- en selectie-technieken. Op deze wijze kunnen zeer specifieke interacties worden bewerkstelligd. Door

gebruik te maken van dergelijke technieken zijn bijvoorbeeld AAV vectoren verkregen waarvan de infectiviteit 100x was verhoogd in cellen met de geschikte receptor terwijl binding aan de normale AAV receptor compleet was geblokkeerd. De meeste liganden die worden ingebouwd in capside eiwitten zijn lineair en mogen niet groter zijn dan maximaal 10-30 aminozuren omdat ze anders de structuur van het capside eiwit teveel verstoren waardoor de infectiviteit compleet verloren gaat. Vaak is het echter wenselijk om een groter stuk eiwit te incorporeren zoals bijvoorbeeld een conformationeel domein van een eiwit of een antilichaam domein in de vorm van een scFv (*single-chain variable fragment*).
De inbouw van grotere conformationele liganden is over het algemeen een probleem voor niet-geënvelopeerde ('naakte') virussen zoals adenovirus en AAV, maar minder voor geënvelopeerde virussen zoals retrovirussen, herpesvirussen en enkelstrengs RNA virussen zoals paramyxovirussen en rhabdovirussen. Dat wil echter niet zeggen dat deze aanpak altijd succesvol is. Met name voor retrovirussen blijkt dat het wel mogelijk is om het tropisme van de virusdeeltjes te veranderen door de incorporatie van dergelijke liganden, maar vervolgens blijken dergelijke virusdeeltjes niet of nauwelijks in staat om de cel binnen te dringen. Kennelijk zijn de gemodificeerde envelop eiwitten niet meer in staat om een conformationele verandering te ondergaan die nodig is om het proces van membraanfusie te initiëren. Door gebruik te maken van specifieke proteolytische knipplaatsen tussen het virale envelop eiwit en de ligand is het soms tóch mogelijk om infectie te bewerkstelligen. In dit geval wordt het fusie eiwit na binding aan de cel gesplitst door celoppervlak-gebonden proteasen waardoor het virale envelop eiwit vrij komt en nu alsnog in staat is om fusie te induceren. Deze vorm van targeting wordt ook wel aangeduid met 'protease-targeting'.
De meeste geënvelopeerde virussen maken gebruik van één envelop eiwit dat verantwoordelijk is voor zowel binding aan de receptor en de inductie van membraanfusie. De paramyxovirussen maken echter gebruik van twee verschillende eiwitten, één voor receptorbinding en een ander voor fusie. Een voorbeeld is het mazelenvirus dat gebruik maakt van een hemagglutinine (H) eiwit voor binding en een fusie (F) eiwit voor membraanfusie. Dit betekent dat het H eiwit kan worden gebruikt om de bindings-specificiteit te veranderen terwijl de functie van het F eiwit niet wordt aangetast. Recombinant mazelenvirus waarin het H eiwit was gefuseerd met het epidermale groeifactor eiwit (EGF) of het insuline groeifactor eiwit (IGF1) bleken levensvatbaar en waren in staat om cellen te infecteren die de EGF-receptor of de IGF1-receptor tot expressie brachten maar niet de normale mazelenvirus receptor, CD46. Specifieke infectie van kankercellen die het carcino-embryonic antigen (CEA) tot expressie brengen kon worden aangetoond met een mazelenvirus recombinant waarin het H eiwit was gefuseerd met een scFv dat specifiek was voor CEA. Tevens konden in het H-scFv fusie eiwit mutaties worden aangebracht in het H eiwit gedeelte waardoor de binding met de normale receptor verloren ging.
Een generieke methode om specifiek cellen te infecteren met een recombinant virus berust op het incorporeren van een eiwitsequentie met een zeer hoge affiniteit voor de Fc-staart van antilichamen (b.v. proteïne A van *Staphylococcus aureus*). Door virusdeeltjes die dit eiwit in hun capside of envelop hebben geïncorporeerd te incuberen met een monoclonaal antilichamen tegen een specifiek

celoppervlakte eiwit, kunnen de virusdeeltjes specifiek binden aan dergelijke cellen en ze vervolgens infecteren.

5. **Chimaere virussen met heterologe oppervlakte eiwitten**

Met uitzondering van de hierboven beschreven gentherapie vectoren zoals adenovirus en AAV, zijn chimaere virussen waarbij een heteroloog eiwit op het oppervlak van het virusdeeltje aanwezig is, vrijwel uitsluitend gebaseerd op geënvelopeerde virussen. In de meeste gevallen betreft het chimaere virussen die bedoeld zijn voor vaccin toepassingen. De achterliggende gedachte hierbij is dat expressie en blootstelling aan het immuunsysteem van een heteroloog viraal eiwit door middel van een geschikt dragervirus (vector) resulteert in een immuunrespons tegen zowel het dragervirus als tegen het heterologe virus waarvan het geëxpresseerde eiwit afkomstig is. Eén van de bekendste voorbeelden is een vaccin gebaseerd op een pokkenvirus (vaccinia) dat het envelop glycoproteine (G) van het rabiesvirus tot expressie brengt. Dit vaccin is met succes in de natuur toegepast in lokaas om vossen te vaccineren tegen rabies (hondsdolheid) om op die manier de verspreiding van rabies te voorkomen. Verschillende verzwakte pokkenvirussen zijn sindsdien gebruikt als vector om vaccins te ontwikkelen tegen een veelvoud aan veterinaire ziekten. In vrijwel alle gevallen werd hierbij gebruik gemaakt van de expressie van immunogene oppervlakte eiwitten van verschillende virussen. Voor zover bekend zijn hierbij nooit problemen met de pathogeniteit van dergelijke recombinant virussen beschreven. Pokkenvirussen worden ook gebruikt als vector voor humane toepassingen. Het betreft sterk verzwakte humane stammen zoals MVA en NYVAC of aviaire stammen zoals TROVAC en ALVAC. Geen van deze pokkenvirussen is in staat om een productieve infectie te veroorzaken in zoogdiercellen en zijn daardoor zeer veilig.

Ook herpesvirussen worden gebruikt als vector voor vaccinatie doeleinden. Het betreft met name veterinaire toepassingen, bijvoorbeeld een herpesvirus van kippen dat wordt gebruik om te vaccineren tegen andere kippenvirussen zoals pseudovogelpest, of een herpesvirus van varkens dat kan worden gebruikt om te vaccineren tegen varkenspest. Een onverwachte verandering werd waargenomen nadat een envelop eiwit van een varkens herpesvirus werd vervangen door het overeenkomstige eiwit van een runder herpesvirus. Het chimaere virus bleek in varkens neurovirulenter te zijn dan het uitgangsvirus.

De klasse van de enkelstrengs negatief-strengs RNA virussen (SS(-)RNA) bevat een groot aantal virussen die ernstige ziekten kunnen veroorzaken bij zowel mensen als dieren. Voorbeelden zijn influenza virus, mazelen virus, Ebola virus, rinderpestvirus en rabies virus. Deze virussen bevatten ofwel één oppervlakte eiwit (G), ofwel twee oppervlakte eiwitten (afhankelijk van de subfamilie: H/HN + F, G + F, of HA + NA). Verschillende vaccinstammen van deze SS(-)RNA virussen zijn gebruikt om oppervlakte eiwitten van andere SS(-)RNA virussen toe te voegen of uit te wisselen. Een aantal van deze chimaere virussen beschikt over interessante vaccin eigenschappen, met name humane parainfluenza virus vaccins en respiratoir syncytium virus (RSV) vaccins. Dergelijke chimaere virussen vertoonden in sommige gevallen wel enige verandering in tropisme maar deze veranderingen bleken vaak beperkt te zijn omdat ook andere eiwitten, zoals replicatie eiwitten, betrokken waren bij het bepalen van het tropisme.

Bepaalde flavivirussen veroorzaken ernstige humane ziekten zoals gele koorts, Dengue koorts, Japanse encephalitis, Tick-borne encephalitis en West-Nijl koorts. Tegen gele koorts is een goed gekarakteriseerd en veilig levend verzwakt vaccin ontwikkeld, gebaseerd op de YFD stam. Deze YFD vaccinstam wordt momenteel gebruik voor het ontwikkelen van chimaere vaccins waarbij de genen die coderen voor de oppervlakte eiwitten prM en E worden vervangen door die van andere flavivirussen. Momenteel worden chimaere vaccins tegen een aantal van deze bovenstaande ziekten getest in preklinische en humane studies. De eerste resultaten zijn bemoedigend. Onlangs werd een chimaer flavivirus beschreven bestaande uit de YFD vaccinstam waarin de prM en E genen waren vervangen door die van een muizen flavivirus. Dit recombinant virus bleek neurovirulent in immuun-deficiënte SCID muizen, hetgeen suggereert dat het neurotropisme van het muizenvirus wordt bepaald door het prM en/of E eiwit.
Uitwisseling van het envelop glycoproteine (S ‘spike’ eiwit) tussen verschillende coronavirussen toonde aan dat neurovirulentie en het tropism voor maag-darm stelsel of het ademhalingsstelsel bepaald werd door het S eiwit. Reciproke uitwisseling van het S eiwit tussen een muizen coronavirus en een katten coronavirus leverde chimaere virussen op waarvan het gastheer tropisme in weefselkweek cellen was veranderd van muis naar en kat en *vice versa*. Deze chimaere virusen zijn tot nu toe nog niet gebruikt voor dierexperimenten in muizen of katten.

6. **Virale factoren en gastheer factoren die tropisme bepalen**

Om zich succesvol te kunnen vermenigvuldigen en verspreiden in een gastheer moet een virus een groot aantal obstakels overwinnen. Deze obstakels kunnen op twee niveaus liggen, nl. op cellulair niveau en op het niveau van het organisme. De voornaamste factoren die van invloed kunnen zijn op het tropisme van een virus zijn hieronder samengevat.
Gebruik van receptor en co-receptor. Herkenning en binding van een virus aan het celoppervlak berust op de interactie van een viraal oppervlakte eiwit met het celoppervlak. De aanwezigheid van de correcte receptor op de cel is dus een eerste vereiste voor succesvolle infectie. Er kunnen drie soorten cellulaire receptoren voor virussen worden onderscheiden, nl. (1) generieke receptoren met een relatief lage affiniteit (bv. siaalzuur or heparaansulfaat), (2) specifieke receptoren met een hoge affiniteit, en (3) co-receptoren. Specifieke receptoren en co-receptoren bestaan meestal uit oppervlakte eiwitten die betrokken zijn bij cel-cel herkenning en binding van extracellulaire factoren zoals hormonen, groeifactoren etc. In sommige gevallen binden virussen aan de primaire receptor maar zijn ze vervolgens afhankelijk van een co-receptor om daadwerkelijk in de cel binnen te dringen. Een voorbeeld is HIV-1 dat bindt aan de primaire receptor CD4 en vervolgens gebruik maakt van de co-receptoren CCR5 of CXCR4.
Penetratie en transport. Na de binding aan de cel moet het virusdeeltje de cel penetreren en vervolgens naar de juiste locatie worden getransporteerd. Penetratie en transport vindt meestal plaats door middel van membraan insnoeringen via endocytose. Door een pH verlaging in het endosoom wordt een conformatie-verandering geïnduceerd in een viraal oppervlakte eiwit dat vervolgens fusie induceert met het endosoom membraan waardoor het virusdeeltje in het cytoplasma terecht komt. Andere virussen, zoals herpesvirussen en paramyxovirussen, maken geen gebruik van endocytose

maar fuseren direct met het plasmamembraan aan het oppervlak van de cel en komen zo direct in het cytoplasma terecht.

Opname in de kern. De meeste DNA virussen en sommige RNA virussen repliceren in de kern. Om de kern te bereiken moet het virusdeeltje de kernporiën passeren. Mogelijk kunnen kleine virussen de kernporie rechtstreeks passeren terwijl voor grotere virussen, zoals adenovirussen en herpesvirussen, actief transport nodig is. Dit proces is nog niet goed opgehelderd maar het is duidelijk dat opname in de kern mede mogelijk wordt gemaakt door cellulaire import eiwitten die een interactie aangaan met virale componenten. Vreemd genoeg zijn lentivirussen wel in staat om de kern te bereiken terwijl andere retrovirussen dat niet kunnen. De meeste retrovirussen repliceren alleen in delende cellen waarbij de kernmembraan tijdens de celdeling verdwijnt.

Interferentie met de cellulaire interferon respons. Cellen reageren op virus infecties door het activeren van anti-virale responsen. Deze anti-virale responsen worden veroorzaakt door interferon. Interferon-α (IFNα) en interferon-β (IFNβ) behoren tot de Type I interferonen. IFNα wordt geproduceerd door leukocyten, terwijl IFNβ voornamelijk wordt geproduceerd door fibroblasten. Interferon productie resulteert in het aanschakelen van allerlei genen, hetgeen vervolgens leidt tot de productie van allerlei eiwitten die virus vermenigvuldiging tegen gaan. Virussen, op hun beurt, maken gebruik van virale factoren die interfereren met de werking van interferon en andere anti-virale eiwitten. Deze virale factoren zijn in de meeste gevallen soortspecifiek, d.w.z. ze werken alleen in cellen van de natuurlijke gastheer en niet in die van een andere gastheer. Dit betekent meestal dat infectie van een andere gastheer leidt tot een abortieve infectie omdat het virus niet in staat is om de interferon respons van de gastheer te ontregelen. De anti-virale interferon respons is dus een belangrijk factor die het tropisme van een virus kan bepalen.

Interferentie met de algemene immuunrespons. Om te overleven in de aanwezigheid van een actief immuunsysteem beschikken virussen over middelen om zich ofwel te onttrekken aan de zichtbaarheid voor het immuunsysteem, ofwel om actief te interfereren met het immuunsysteem van de gastheer. Virussen kunnen zich tijdelijk ‘verbergen’ door de expressie van virale genproducten op een laag pitje te zetten of helemaal uit te schakelen, bijvoorbeeld tijdens persistentie en latentie. Ook kunnen ze weefsels infecteren waar het immuunsysteem minder actief is, bijvoorbeeld in zenuwweefsel. Ze kunnen juist cellen van het immuunsysteem infecteren, bijvoorbeeld B- en T-cellen, om op die manier hun werking te remmen. Virussen beschikken ook over middelen om hun aanwezigheid in geïnfecteerde cellen te maskeren. Daartoe verhinderen ze de presentatie van peptiden door MHC-I moleculen op het oppervlak van de cel. Op die manier worden de geïnfecteerde cellen niet herkend door surveillerende lymphocyten. Remming van de antigeen presentatie kan, afhankelijk van het type virus, op een aantal manieren plaats vinden zoals (1) remmen van de afbraak van virale eiwitten door het proteasoom, (2) remmen van het transport van de peptiden naar het endoplasmatisch reticulum (ER), (3) remmen van het beladen van het MHC-I molecuul, (4) het induceren van afbraak van het MHC-I molecuul, (5) remmen van het transport van het MHC-I molecuul naar het Golgi-apparaat, (6) wijziging van de transport route van het MHC-I molecuul van het trans-Golgi netwerk naar lysosomen, en (7) verhinderen van transport van MHC-I moleculen van het trans-Golgi netwerk naar de celmembraan. Met name grote DNA virussen zoals herpesvirussen en pokkenvirussen maken vaak

gebruik van meerdere mechanismen tegelijk om het immuunsysteem te ontregelen. Pokkenvirussen bevatten bijvoorbeeld meerdere genen die coderen voor eiwitten die homoloog zijn aan cellulaire cytokines en hun receptoren. Deze eiwitten, virokines en viroceptors genaamd, zorgen ervoor dat de immuunrespons van de gastheer zodanig verandert dat de virussen er geen last van hebben of zelfs voordeel bij hebben.

Een ander klasse van virale eiwitten interfereert met het proces van apoptose (geprogrammeerde celdood). In sommige gevallen is er sprake van remming van apoptose om het virus voldoende tijd te geven om zich te vermenigvuldigen. In andere gevallen gaat het om de inductie van apoptose omdat dit leidt tot onderdrukking van ontstekingsreacties en tegelijkertijd de verspreiding van het virus kan bevorderen doordat omliggende cellen de apoptotische cellen opnemen.

Restrictiefactoren van de gastheer. Ofschoon het immuunsysteem primair verantwoordelijk is voor de bescherming tegen virus infecties, wordt het steeds duidelijker dat ook cellulaire factoren een actieve rol spelen bij het verhinderen van de replicatie van bepaalde virussen. Deze cellulaire factoren kenmerken zich door een dominante overerving en ze zijn werkzaam in een stadium na het binnendringen van het virusdeeltje in de cel. De bekendste en best bestudeerde restrictiefactoren zijn die welke interfereren met de replicatie van retrovirussen. Ofschoon de exacte werking van de meeste van deze factoren niet exact bekend is lijkt het erop dat ze de replicatie van retrovirussen remmen doordat ze interfereren met het proces van replicatie (reverse transcriptie), transport en import in de kern. Een van de bekendste restrictiefactoren die niet werkzaam is tegen retrovirussen maar tegen een breed scala aan negatief-strengs RNA virussen, is het Mx eiwit. Het prototype Mx eiwit, Mx1, werd voor het eerst geïdentificeerd in een muizen cellijn die resistent was tegen infectie met influenza A virus. Het Mx1 eiwit hoopt zich op in de kern en verhindert replicatie van influenza virus doordat het bindt aan het virale polymerase eiwit PB2 en het nucleoproteïne NP. In humane cellen hoopt het eiwit (MxA) zich op in het cytoplasma en verhindert daar de replicatie van verschillende klassen van virussen. Waarschijnlijk berust de werking op remming van het transport van virale componenten van en naar de kern.

Virale genen die het gastheerbereik bepalen. Studies aan mutanten van pokkenvirussen hebben genen aan het licht gebracht die betrokken zijn bij het bepalen van het gastheerbereik van deze virussen. Van de meeste van deze genen is het werkingsmechanisme nog niet opgehelderd. Slechts van enkelen is het werkingsmechanisme min of meer bekend, zoals bijvoorbeeld het E3L gen van vaccinia virus. Dit gen codeert voor een eiwit dat bindt aan dsRNA waardoor het interfereert met de werking van proteine kinase R (PKR) dat betrokken is bij de interferon respons en bij de inductie van apoptose. Een ander vaccinia virus product is K1L, dit eiwit is in bepaalde cellen nodig voor het activeren van een transcriptiefactor. Een derde voorbeeld is M11L van myxoma virus, dit eiwit voorkomt apoptose door remming van het vrijkomen van cytochroom C uit mitochondriën. Het gastheerbereik van een bepaalde stam van influenza A virus wordt bepaald door één enkele aminozuur mutatie op positie 627 in het PB2 gen. Het aminozuur op deze positie bepaalt of het virus wel of niet kan groeien in MDCK cellen. Onlangs werd aangetoond dat dezelfde mutatie in een ander influenza virus betrokken was bij virulentie voor muizen.

Gastheer proteasen. Een groot aantal cellulaire en virale eiwitten zijn voor hun werkzaamheid afhankelijk van activatie door middel van een proteolytische splitsing. Dit geldt met name voor een aantal virale oppervlakte eiwitten die betrokken zijn bij het proces van membraanfusie en penetratie. De meeste fusie-eiwitten van virussen worden geactiveerd door middel van proteolytische splitsing waarna de fusie activiteit wordt geïnduceerd door een lage pH (in het endosoom) of door eiwit-eiwit interacties met een fusiepartner. De cellulaire proteasen die verantwoordelijk zijn voor het activeren van fusie eiwitten behoren tot de familie van de '*subtilisin-like proprotein convertases*' (SPCs). De best bestudeerde vertegenwoordiger is furine (SPC1). Furine wordt door de meeste cellen geëxpresseerd en komt voor in het trans-Golgi netwerk, in het ER, en op de celmembraan. Furine splitst na een aantal basische aminozuren met het minimale motief R-X-R/K-R dat vaak voorkomt in virale fusie eiwitten. De activatie van fusie eiwitten kan een belangrijke factor zijn bij het bepalen van het weefseltropisme van bepaalde virussen. Een goed voorbeeld is Newcastle disease virus (NDV). Van dit virus zijn verschillende varianten bekend met een sterk uiteenlopende virulentie. De virulentie is afhankelijk van de aminozuur sequentie van de splitsingsplaats van het fusie (F) eiwit van het virus. De splitsingsplaats van het F eiwit van virulente stammen wordt herkend door een furine-achtig protease dat voorkomt in vrijwel alle cellen, terwijl de splitsingsplaats van het F eiwit van niet-virulente stammen wordt herkend door extra-cellulaire trypsine-achtige proteasen die alleen voorkomen in bepaalde cellen in het ademhalingsstelsel en het maagdarm stelsel. Infectie met een virulent virus resulteert dus in replicatie en verspreiding door het hele lichaam terwijl infectie met een niet-virulent virus beperkt blijft tot een locale infectie. Hetzelfde principe geldt ook voor het tropisme van Sendai virus in muizen en voor influenza A virus in kippen.
De eiwitten van virussen zoals picornavirussen of flavivirussen ontstaan door proteolytische splitsing van één precursor, het poly-proteïne, hetgeen uiteindelijk resulteert in individuele virale eiwitten. Voor andere virussen wordt tenminste een deel van de virale eiwitten op deze wijze geproduceerd (b.v. de polymerase eiwitten van coronavirussen of arterivirussen). Aangezien de proteolytische splitsing zowel door virale als door gastheer gecodeerde eiwitten plaatsvindt, zou men verwachten dat er ook op dit niveau sprake zou kunnen zijn van gastheer restrictie en verschillen in tropisme. Voorbeelden van dergelijke restricties zijn in de literatuur echter niet of nauwelijks te vinden, hetgeen suggereert dat dit type restrictie geen belangrijke rol speelt of over het hoofd is gezien. Een van de weinige uitzonderingen is een publicatie over een lentivirus van geiten waarbij een specifieke proteolytische splitsing van het envelop eiwit in een bepaalde cellijn verantwoordelijk was voor het feit dat het virus zich in die cellijn niet kon vermenigvuldigen.
Transcriptiefactoren. Virussen zijn voor hun vermenigvuldiging in hoge mate afhankelijk van de gastheer. Terwijl RNA virussen voornamelijk afhankelijk zijn van translatie processen, zijn DNA virussen en retrovirussen tevens afhankelijk van transcriptie processen aangezien deze virussen niet in staat zijn om hun eigen mRNAs te maken. Aangezien transcriptie van cellulaire genen sterk afhankelijk is van het cel-type en het ontwikkelingsstadium van de cel, is het voor de hand liggend dat ook de transcriptie – en daarmee de replicatie - van virussen sterk afhankelijk is van het cel-type. Dit blijkt voor een groot aantal DNA virussen en retrovirussen inderdaad het geval te zijn. Doordat bepaalde virussen helemaal aangepast zijn aan optimale replicatie in bepaalde cel-typen zijn ze vaak

niet meer in staat om in andere weefsels te repliceren. Voorbeelden van virussen die op deze wijze sterk gespecialiseerd zijn, zijn Hepatitis B virus dat een sterk tropisme heeft voor levercellen en humaan papillomavirus dat een sterk tropisme heeft voor epitheel cellen.
Andere factoren. Niet alleen transcriptie factoren maar ook translatie factoren kunnen een rol spelen bij het bepalen van het tropisme van een virus. Picornavirussen maken gebruik van een complexe RNA structuur aan het 5'-uiteinde van hun RNA genoom, de zgn. IRES (*internal ribosome binding site*), voor *cap*-onafhankelijke translatie. Mutaties of substituties in de IRES sequentie van bepaalde picornavirussen kunnen leiden tot virussen die zich niet meer kunnen vermenigvuldigen in bepaalde cellijnen. Dit suggereert dat bepaalde cel-type specifieke IRES-bindende eiwitten een rol spelen bij het bepalen van het tropisme van deze virussen.

7. **Evaluatie van risico's ten gevolge van veranderingen in tropisme**

Het voornaamste risico ten gevolge van het gebruik van virussen in het algemeen, en van recombinant virussen en chimaere virussen in het bijzonder, is hun vermogen om ziekte te veroorzaken. Het vermogen van een virus om ziekte (schade aan cellen, weefsels en organen) te veroorzaken wordt aangeduid met pathogeniteit. Dit vermogen is afhankelijk van een aantal factoren zoals, inoculatieroute, dosis, en virulentie van het virus. Virulentie is gedefinieerd als het relatieve vermogen om ziekte te veroorzaken. Virulentie van een virus wordt onder andere bepaald door het tropisme van een virus. Virulentie is dus een belangrijke risicofactor als we te maken hebben met virussen waarvan het tropisme is veranderd tengevolge van opzettelijke dan wel onopzettelijke veranderingen. Of het gebruik van dergelijke virussen ook inderdaad zal leiden tot een verandering in virulentie en daardoor in pathogeniteit, is niet altijd met zekerheid te voorspellen. Dit hangt af van een aantal factoren. Deze factoren zijn weergegeven in Tabel 1 en kunnen grofweg in 4 categorieën worden verdeeld, nl. (1) eigenschappen van de vector, (2) tropisme veranderingen met betrekking tot receptor interacties, (3) tropisme veranderingen met betrekking tot anti-virale responsen, en (4) gastheerfactoren.
Niet elke verandering in tropisme leidt automatisch tot een toename in virulentie en pathogeniteit. In veel gevallen is het tegenovergestelde het geval doordat de genetische veranderingen juist leiden tot een afname in 'fitness'. Fitness heeft betrekking op het vermogen van een virus om zich te vermenigvuldigen en te verspreiden in een levend wezen en wordt onder andere bepaald door factoren zoals tropisme, efficiëntie van virusreplicatie, stabiliteit, gevoeligheid voor afweerreacties van de gastheer, etc. Normaal gesproken heeft een virus in zijn natuurlijke gastheer een optimale fitness als gevolg van constante aanpassing aan de gastheer. Echter, wanneer het virus in een andere gastheer of gastheercel komt is het over het algemeen niet optimaal toegerust voor replicatie in deze nieuwe omgeving. Veranderingen in de genetische samenstelling van een virus en veranderingen in het tropisme van een virus leiden dan ook vaak tot een verminderde fitness. Dit is met name het geval voor chimaere virussen die zijn gebaseerd op kleine virussen zoals SS(-)RNA virussen, maar ook wel voor grotere virussen zoals coronavirussen.
De vraag of een verandering in tropisme resulteert in een toename van de pathogeniteit en/of virulentie kan over het algemeen niet gemakkelijk worden beantwoord. Echter, door een lijst vragen te

beantwoorden in de vorm van een checklist kan in individuele gevallen vaak een gedetailleerder beeld worden verkregen van de voornaamste risicofactoren waardoor een redelijk betrouwbare inschatting van de risico's mogelijk is. Terwijl een aantal van risicofactoren meestal bekend zijn of redelijk goed kunnen worden ingeschat, zijn andere factoren minder goed gedefinieerd. Met name de aanwezigheid van de virale receptor op verschillende cellen en in verschillende weefsels is niet altijd goed bekend. Mogelijk kunnen er ook andere receptoren worden gebruikt die nog niet bekend waren. Soms is de receptor-specificiteit van een heteroloog oppervlakte eiwit helemaal niet bekend. In dergelijke gevallen is niet te voorspellen of toepassing van een chimaer virus zal leiden tot pathogenese.
Veranderingen in het tropisme van virussen zijn niet altijd het gevolg van veranderingen in oppervlakte eiwitten. Zoals reeds vermeld kunnen ook veranderingen in factoren die de antivirale interferon respons van de gastheer beïnvloeden, resulteren in een veranderd cel tropisme. Dit suggereert dat dergelijke virussen ook virulenter zouden kunnen zijn dan de oudervirussen. Een aanwijzing hiervoor is de waarneming dat verzwakte virus stammen die zijn verkregen na veelvuldige passage in weefselkweek cellen, meestal genen hebben verloren die betrokken zijn bij de remming van de afweer respons van de gastheer.
Soms kan het verkrijgen van een nieuwe gastheerspecificiteit worden veroorzaakt door de aan- of afwezigheid van een specifieke receptor. Dit is bijvoorbeeld aangetoond voor poliovirus. Poliovirus infecteert normaal gesproken alleen mensen en géén muizen. Echter expressie van de poliovirus receptor in transgene muizen maakt deze muizen gevoelig voor poliovirus infectie en pathogenese. Het is echter de vraag of dit een algemeen principe is. Ofschoon van verschillende chimaere virussen is aangetoond dat de gastheerspecificiteit in weefselkweekcellen veranderd is, zijn er nauwelijks gegevens over testen met dergelijke virussen op het niveau van het organisme. Vaak is het zo dat waarnemingen betreffende veranderingen in tropisme in weefselkweekcellen niet altijd kunnen worden doorgetrokken tot op het niveau van het organisme.
Waarschijnlijk zijn factoren zoals de oorspronkelijke virulentie van het vectorvirus, en het feit of dit virus wél of niet replicatie competent is, de voornaamste factoren die bepalen of een recombinant of chimaer virus virulent is en ziekte kan veroorzaken. Daarnaast spelen ook gastheerfactoren een grote rol. Individuen met een verzwakt afweersysteem zullen vatbaarder zijn dan normale individuen. Dit is echter niet specifiek voor recombinant virussen maar geldt voor alle virussen. Een ander aspect dat van belang is, is of het recombinant virus in staat is om zich te handhaven in de populatie. Waarschijnlijk komt het in de natuur relatief vaak voor dat een virus een nieuwe gastheer infecteert, maar succesvolle handhaving in de nieuwe gastheer komt waarschijnlijk slechts sporadisch voor. Echter, áls het voorkomt kan dat vérstrekkende gevolgen hebben zoals bijvoorbeeld is gebleken bij HIV, influenza en SARS.
Een andere categorie recombinant virussen betreft virussen die een cytokine tot expressie brengen, met de bedoeling om daarmee de immuunrespons positief te beïnvloeden. Door middel van cytokines kan de immuunrespons min of meer gestuurd worden in de richting van een type 1 respons, die ook wel wordt gekarakteriseerd als een CTL response, of een type 2 response die grofweg kan worden gekarakteriseerd als een antilichaam response. Tegen sommige virus infecties is een type 1 respons het meest effectief terwijl tegen andere virussen een type 2 respons juist effectiever is. Vaccinia virus

recombinanten die een type 2 cytokine, zoals IL-4, tot expressie brachten bleken veel virulenter te zijn voor muizen dan vaccinia virus recombinanten die géén IL-4 produceerden. Doordat IL-4 de CTL respons remt, en de CTL respons juist belangrijk is om vaccinia virus infecties te bestrijden en op te ruimen, waren recombinanten die IL-4 produceerden juist veel virulenter geworden. Dit voorbeeld geeft aan dat co-expressie van cytokines door middel van virale vectoren niet altijd het gewenste resultaat oplevert. Het geeft ook aan dat we in veel gevallen nog te weinig kennis hebben van de verschillende typen regulatie functies en effector functies van het immuun systeem die betrokken zijn bij het bestrijden van virale infecties.
Vanwege onze beperkte kennis van de relatie tussen tropisme en ziekteverwekkend vermogen van virussen, verdient het aanbeveling om onderzoek op dit gebied te stimuleren en wetenschappelijke ontwikkelingen op dit gebied nauwkeurig in de gaten te houden teneinde een reële inschatting te kunnen maken van eventuele risico's die werkzaamheden met recombinant en chimaere virussen met zich meebrengen.

Tabel 1. Risicofactoren ten gevolge van veranderingen in tropisme van recombinant en chimaere virussen

Eigenschappen van de vector

1. Het virus is replicatie competent
2. De pathogeniteit en virulentie van het vector virus

Veranderingen in tropisme met betrekking tot receptor interacties

1. Het virus heeft een uitgebreid cel- en weefseltropisme verkregen
2. Het virus heeft een nieuw cel- en weefseltropisme verkregen
3. Het virus heeft een nieuwe gastheer specificiteit verkregen
4. Het virus heeft een specifiek tropisme voor kwetsbare cellen (b.v. cellen van het immuunsysteem, neuronen)
5. Het virus heeft een onbekend tropisme tengevolge van een onbekende receptor specificiteit
6. Het virus heeft een onbekend tropisme omdat niet bekend is op welke cellen de receptor voorkomt

Veranderingen in tropisme met betrekking tot anti-virale responsen

1. Het virus is beter in staat om de interferon respons te remmen
2. Het virus is ongevoelig geworden voor antivirale gastheer responsen
3. Het virus heeft het vermogen om de interferon respons van een andere species te remmen
4. het virus heeft het vermogen om te interfereren met de algemene immuun respons
5. Het virus produceert een cytokine dat aanleiding geeft tot een verkeerd type antivirale immuun respons waardoor de pathogeniteit van het virus wordt verhoogd

Gastheerfactoren

1. De gastheer is naïef voor de virusklasse waartoe het recombinant of chimaere virus behoort
2. De gastheer heeft een sterk verzwakt immuunsysteem

Bijlage 1. Beschrijving van het project volgens het COGEM onderzoeksprogramma 2004-2005

Titel: Gastheerbereik chimeare virussen

Onderwerp: Het inventariseren van mogelijk nieuwe ontwikkelingen op het gebied van genetisch gemodificeerde virussen en virale vectoren met een mogelijk veranderde 'host range' (tropie). Het gebruik van genetisch gemodificeerde of chimeare virussen en virale vectoren neemt steeds verder toe. Dit wordt onder meer in de hand gewerkt door de grotere beschikbaarheid van infectieuze klonen van tal van virussen. Bij het tot nu toe beperkte aantal voor vergunning aangevraagde werkzaamheden is geconcludeerd dat de ingebrachte sequenties vermoedelijk niet zouden resulteren in een veranderd gastheerbereik of verhoogde pathogeniteit van het recombinant virus. Echter met de toename in het gebruik van virussystemen en het breder beschikbaar komen van gegevens over het gebruik van recombinant virussen lijkt een inventarisatie van deze problematiek gewenst. Een overzicht van de internationale ontwikkelingen en de mogelijke consequenties op het gebied van bioveiligheid die hieruit getrokken kunnen worden is gewenst.

Doel: Het in kaart brengen van mogelijke risico's van recombinant virussen in samenhang met een mogelijk veranderend gastheerbereik.

Resultaat: Rapport met een inventarisatie van de laatste ontwikkelingen op het gebied van recombinante virussen en virale vectoren. Tevens een inventarisatie van de laatste kennis op het gebied van factoren die kunnen leiden tot veranderingen in het gastheerbereik.

Onderzoek: deskstudie