4.4 Genetic Engineering of Infectious Rabies Virus

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The genetic manipulation of animal viruses has led to extraordinary advances in the understanding of how viruses replicate, how they interact with the host cell, and what determines virulence. In addition, genetically altered viruses have been successfully used to express foreign genes and to generate efficient recombinant vaccines.

Viruses containing DNA, such as SV40- (1), Herpes-, Adeno-, and Poxviruses were the first to become amenable to genetic manipulation. After transfection into cells, the DNA of many of these viruses is infectious *per se* and gives rise to infectious particles. Alternatively, homologous recombination can be used to introduce defined DNA into the genomes of helper virus.

For some time, viruses with a positive stranded RNA genome (Picornaviruses, Alphaviruses) have been amenable to specific alteration (2). The genomic RNA of positive strand RNA viruses serves as mRNA and is infectious after introduction into a cell. Either RNAs transcribed in vitro from recombinant cDNA and then transfected into a cell or RNAs generated intracellularly from transfected cDNA may yield infectious virus.

The genetic engineering of negative stranded RNA viruses proved to be much more complicated. This group of viruses include many important human and animal pathogens, such as influenza, parainfluenza, respiratory syncytial, measles, and rabies viruses. Neither the naked genomic RNA nor the complementary (positive sense) RNA is infectious after transfection into cells. However, we recently showed for the first time that recombinant RNA corresponding to the entire genome of a negative stranded RNA virus, namely the rhabdovirus rabies virus, can be made infectious (3). This was achieved by simultaneous intracellular expression of the proteins constituting the viral polymerase complex and the viral RNA.

A full-length genomic cDNA copy of the rabies virus strain SAD B19, which is being used as a live vaccine for oral immunization of foxes, was cloned between a T7 RNA polymerase promoter and a hepatitis delta virus ribozyme sequence. After transfection of the plasmid into cells infected previously with a recombinant vaccinia virus providing T7 RNA polymerase, full-length 12 kb RNA with precise ends was produced. In addition, three other T7 RNA polymerase driven plasmids were co- transfected. They expressed the rabies virus N, P, and L proteins, which make up the viral polymerase complex. Assembly of the plasmid encoded RNA and the proteins into transcriptionally active rabies virus nucleocapsids and subsequently autonomous expression of the envelope proteins M and G resulted in the formation of infectious rabies virus.

Site specific alterations were then introduced into the genomic cDNA copy in order to probe the genome flexibility of rabies virus and to generate viruses which are distinguishable from standard SAD B19 virus. The first experiments concentrated on the variable pseudogene region between the

G and L gene, which is present in all natural rabies viruses and which is being used to discriminate closely related rabies virus isolates in molecular epidemiology studies. Recombinant infectious viruses possessing the introduced genetic tags, namely insertions or deletions of four nucleotides at various locations, were successfully recovered. Even a virus lacking the entire pseudogene sequence could be isolated. Growth characteristics and final titers were identical to those of the SAD B19 virus, demonstrating that the pseudogen is not essential for propagation of rabies virus, at least in cell culture. In addition, recombinant viruses with alterations within coding regions resulting in amino acid exchanges in the viral G and L proteins could be recovered. Most likely, recombinant rabies viruses mutated in all parts of the genome can be generated in the future. Since the vaccinia viruses, which are initially needed in the transfection experiments, can be removed easily from the culture supernatants by filtration, pure stocks of recombinant rabies viruses are obtained.

It is now possible to do structure/function studies of rabies virus genes and proteins and also to investigate virus-host interactions in detail. By using specifically designed mutant viruses, the mechanisms involved in rabies neurotropism, latency and pathogenesis may be revealed. It is also now feasible to identify virulence markers and to design safe attenuated, genetically marked viruses for use as live vaccines.

Moreover, it is likely that rabies virus possesses the capacity to express foreign genes. It was possible to introduce into the pseudogene of a recombinant virus a functional transcription signal copy from another part of the genome resulting in generation of an additional transcription unit. As bacterial and eukaryotic reporter genes have already been expressed from defective virus-like-particles (4), it appears probable that infectious rabies virus might find useful application as a vector for the expression of foreign genes.

Literature:

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Rabies Case Data from Europe
are tabulated on the following pages
of Section 5