

Position statement of the ZKBS on working with recombinant vaccinia viruses

1. Introduction

Vaccinia viruses are extensively used in biomedical research as vectors to express heterologous genes in eukaryotic cells. They are particularly suited for certain tasks, e.g. achieving high expression rates of heterologous genes or an immunization of animals. Their broad action spectrum permits replication in a great variety of cell types and a number of laboratory animals. The origin of the vaccinia virus is uncertain, perhaps it developed from the variola or cowpox virus: Before the formal extinction of smallpox in the late twentieth century vaccinia viruses were used to immunize against the human smallpox virus (*Variola*).

The vaccinia virus belongs to the genus *Orthopoxvirus*, family *Poxviridae*. Its viral genome consists of a double-stranded DNA molecule of approx. 187 kbp containing terminal hairpin-loop structures and inverted repetitions. The virus replicates in the cytoplasm of infected cells. After infection, early RNAs are formed in the cytoplasm by viral enzymes located in the viral core. These RNAs encode the virus's own DNA polymerase. After onset of DNA replication late RNAs are synthesized in time intervals coding for various enzymes of the nucleic acid metabolism and for viral structural proteins. So-called "intracellular, mature" virions comprising the nucleoprotein, the core particle, and one or two lateral bodies, are formed in the cytoplasm. They are released after cell lysis. One fraction of these intracellular, mature virions is enveloped by two further membranes which originate from the trans-Golgi network and into which virus-specific polypeptides are incorporated ("intracellular, enveloped virions"). These particles are released as "extracellular, enveloped" virions into the environment when the exterior membrane fuses with the cytoplasmic membrane.

The insertion of heterologous genes into the genome of the vaccinia virus proceeds by means of homologous recombination. Typical vaccinia integration plasmids are derived from pBR322 derivatives and contain a promoter specific of the vaccinia virus, downstream of which the foreign gene is inserted. The foreign gene is flanked by vaccinia virus DNA sequences, which enable a recombination in non-essential regions of the viral genome. The insertion into the thymidine kinase gene (Tk) allows for a selection for Tk-negative recombinant vaccinia viruses. It is possible to insert up to 25 kbp into the vaccinia virus genome. The inserted gene must not contain any introns, as splicing of vaccinia transcripts does not occur.

The ZKBS assigns genetic engineering operations with recombinant vaccinia viruses to containment level 2.

The Modified vaccinia virus Ankara (MVA) was attenuated by multiple passages and is avirulent in humans and in a great number of animals. The ZKBS assigned MVA to **risk group 1** (General position statement of the ZKBS, Vaccinia MVA, Ref. no. 6790-10-74, June 2002). Accordingly, genetic engineering operations with MVA containing heterologous genes not changing the host range and not increasing the hazard potential of MVA were assigned to **containment level 1**.

2. Identifying the problem

Although the probability of an accidental infection must be considered to be low when working with recombinant vaccinia viruses, provided that the basic principles of microbiological work procedures are observed, and should be ruled out almost completely under observance of level 2 safety measures and after sufficient instruction, occasional cases of infections are known to have occurred in genetic engineering laboratories working with recombinant vaccinia viruses since the German Genetic Engineering Act entered force in 1990. The clinical symptoms of

infection (local eye infection, encephalitis, generalized skin rash) were attributable to the pathogenic potential of vaccinia viruses.

In addition, the immunity which was created in the population by means of a nationwide vaccination campaign in Germany before 1976 has been steadily declining. Children born after 1976 are not immunized against vaccinia viruses and it is questionable whether vaccinated people will still possess a sufficient degree of vaccination protection as they grow older.

The ZKBS therefore gives out the following recommendation for people working with recombinant vaccinia viruses:

3. Recommendations

1. Genetic engineering operations should preferentially concentrate on vaccinia viruses with low virulence. The ZKBS exemplarily refers to the strains MVA and NYVAC which the ZKBS assigns to risk group 1, and to the strain ALVAC (strain of the canarypox virus, attenuated by passaging and plaque cloning), which was assigned to risk group 1 by the Centers for Disease Control and Prevention [CDC. Vaccinia (smallpox) vaccine: Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2001. MMWR 2001;50 (No. RR-10):1-25].
2. In order to rule out an infection risk when working with vaccinia viruses belonging to risk group 2, particularly the uncovered skin of the experimenter should be protected against splashes (e.g. safety workbench, protective clothing, protective gloves, if possible, protective shield or suitable face protector).
3. When inoculating experimental animals, or handling animals infected with the vaccinia virus, persons involved might be exposed to a higher risk of infection (due to bites, stings splashes during injection) than they would be when working with cell cultures. The same applies to the production of recombinant viruses in large volumes and for subsequent concentration steps, e.g. in the production of vaccines, during which the experimenter/worker might be exposed to considerably greater amounts of infectious viruses. For this reason, special safety measures must be observed in animal housing units and production areas:
 - application of special systems for the accommodation of animals (for example, individually ventilated cages, IVCs)
 - provision of fixation devices for the safe handling of the animals
 - use of a microbiological biosafety cabinet for operations during which aerosols might be produced.

If the technical measures mentioned are not sufficiently applicable, or not applicable at all, appropriate personal protection equipment (e.g. at least a particle-filtering half-mask FFP 2 and face protector) must be worn.

In this context, we refer to the fact that the inactivation of vaccinia viruses must be carried out at least by the application of disinfectants with "limited spectrum of virucidal activity" (cf. for example, "List of disinfectants and disinfection procedures tested and approved by the Robert Koch Institute", Federal Health Gazette. 2013: 56:1706–1728), as vaccinia viruses are very resistant and might retain their infectiousness after desiccation or treatment with organic solvents.

4. Note

A prophylactic vaccination with a replication-competent MVA strain is possible.