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Deres ref.:
Vår ref.: 14/11156-43
Saksbehandler: Ingrid Stavenes Andersen
Dato: 18.01.2022

KPM-Rikshospitalet - Godkjenning av laboratorier og anlegg for innesluttet bruk av genmodifiserte dyr og genmodifiserte mikroorganismer (GMM) i kombinasjon med dyr

Helsedirektoratet viser til melding datert 14. desember 2021 om endring av ansvarshavende for lokalene som benyttes til innesluttet bruk av GMO ved KPM-Rikshospitalet. Herved følger en oppdatert godkjenning hvor denne endringen er innarbeidet. Godkjenningen viderefører betingelser og godkjente rom som gitt i godkjenning fra 29.11.19 (vår ref. 14/11156-16). Det legges til grunn at det ikke er skjedd øvrige endringer siden tidligere godkjenninger.

Ny ansvarshavende for lokalene er Marianne Aannestad.

Godkjenningen til KMP-Rikshospitalet omfatter laboratorier for innesluttet bruk av genmodifiserte dyr og genmodifiserte mikroorganismer i kombinasjon med dyr.

Det er oppgitt at følgende Virale vektorer benyttes:
- Modified vaccinia Ankara (MVA)
- Replication deficient virus-like particles (VPL) basert på Semliki Forest virus (SFV)
- Lentivirus (HIV pro-virus)
- Rekombinert Adeno-assosiert virus (rAAV)
- Lentivirus, rAAV og VPL er tidligere risikovurdert og det foreligger ny risikovurdering av MVA.

Det er foreslått at vektorproduksjon, håndtering og testing av MVA i laboratoriet kan utføres på inneslutningsnivå 2 og at brukt av svekkede MVA i dyrestudier kan utføres på inneslutningsnivå 1. Vanligvis vil bruk av genmodifisert MVA i kombinasjon med dyr kreve inneslutningsnivå 2, selv om modifiseringen gjør at virus ikke kan replikere. Lavere inneslutningsnivå kan aksepteres der det foreligger en grundig risikovurdering.

Vedtak:
Med hjemmel i forskrift 21-12-2001 nr 1600 om innesluttet bruk av genmodifiserte mikroorganismer § 7 og forskrift 21-12-2001 nr 1602 om innesluttet bruk av genmodifiserte dyr § 8, godkjenner Helsedirektoratet oppstallingsrommene og prosedyrerommene oppsummert i tabell 1 og 2 under.

Ansvarlig for enhetene er seksjonsleder Marianne Aanestad. Ansvaret omfatter også å tilse at brukerne overholder meldeplikten og at aktiviteten skjer innenfor de meldinger som gis.

Helsedirektoratet
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De som bruker lokalene skal sørge for at det til enhver tid anvendes riktig inneslutningsnivå for de ulike genmodifiserte mikroorganismene, samt sende melding og forhåndsvurdering/søknad (jf. dyreforskriften § 3, GMM-forskriften § 5).

Direktoratet har myndighet til å føre tilsyn med virksomheten. Vi gjør oppmerksom på at godkjenningen kan trekkes helt eller delvis tilbake dersom virksomheten i anlegget er i strid med genteknologiloven, GMM-forskriften, dyreforskriften eller denne godkjenningen.

Tabell 1. Følgende lokaler ved Avdeling for komparativ medisin, bygning A3U ved Rikshospitalet, OUS er godkjent for innesluttet bruk av genmodifiserte mikroorganismer i kombinasjon med dyr på inneslutningsnivå 2:

Oppstallingsrom		Prosedyrerom	
A3.U032C	*A3.U053B*	*A3.U032B*	A3.U056
A3.U033B	*A3.U057*	*A3.U033A*	
A3.U034B	A3.U071B	*A3.U034A*	
A3.U039B	A3.U071C	*A3.U039A*	
A3.U038B		*A3.U038A*	
A3.U042		*A3.U049A*	
A3.U041		*A3.U051B*	
A3.U044		*A3.U052B*	
A3.U046		A3.U053A	
A3.U047		*A3.U024*	
A3.U049B		*A3.U022*	
A3.U051A		A3.U021	
A3.U052A		A3.U071D	

Prosedyrerommene vil benyttes til injeksjoner med virale vektorer mens oppstallingsrommene vil benyttes til oppstalling av dyr før og etter injeksjon.

Tabell 2. Følgende lokaler ved Avdeling for komparativ medisin, bygning A3U ved Rikshospitalet, OUS er godkjent for innesluttet bruk av genmodifiserte dyr:

Oppstallingsrom		Prosedyrerom	
A3.U028	A3.U047	A3.U032B	A3.U021
A3.U029	***A3.U048***	A3.U033A	***A3.U016***
A3.U031	A3.U049B	A3.U034A	***A3.U011***
A3.U032C	A3.U051A	A3.U039A	A3.U071D
A3.U033B	A3.U052A	A3.U038A	A3.U056
A3.U034B	A3.U053B	***A3.U043A***	
A3.U039B	***A3.U059B***	A3.U049A	
A3.U038B	A3.U057	A3.U051B	
A3.U043B	***A3.U59D***	A3.U052B	
A3.U042	***A3.U067B***	A3.U053A	
A3.U041	A3.U071B	***A3.U026B***	
A3.U044	A3.U071C	A3.U024	
A3.U046	A3.U062B	A3.U022	

*Rom markert med fet skrift er *ikke* godkjent for kombinasjonsbruk (GMM med dyr), jf. tabell 1.

Vennlig hilsen

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Dokumentet er godkjent elektronisk

28.06.19

RISK ASSESSMENT FOR USE OF ATTENUATED MODIFIED VACCINIA ANKARA (MVA) VIRAL VECTORS IN MICE

BACKGROUND

Project plans

We intend to vaccinate mice with MVA vector encoding various antigens (malaria, influenza and tumor antigens) alone, or coupled to a vaccibody construct, to examine humoral and cellular immune responses against encoded antigens. The vaccibody construct encode dimeric proteins where each chain consist of a targeting unit (antibody fragments or chemokines that bind molecules on immune cells) and a dimerization unit that is derived from the CH3 domain of IgG3 or based on a Fos and Jun leucine zipper motif. The targeting unit will target antigens to certain types of immune cells (antigen presenting cells), which will enhance the immune response. The dimerization unit will assemble the DNA encoded proteins into dimers. Injections of MVA vectors will be performed intramuscularly or intradermally. Concentration of attenuated MVA viral vectors applied during animal studies will not exceed 1*10^7 PFU per mouse, and the maximum volume injected will not exceed 100 µL per mouse.

Parent organism

MVA is derived from Chorioallantois Vaccine Ankara (CVA) strain of the vaccinia virus. The attenuated and genetically stabile form was renamed MVA after the 516th passage of CVA strain on primary chicken embryo fibroblasts (CEF). MVA is a large complex enveloped virion containing a linear double-stranded DNA genome of 178 kbp.

Biosafety aspects of using MVA as a vector

- Lack of persistence or genomic integration due to poxviruses cytoplasmatic replication (Im & Hanke 2004).
- Genomic studies has revealed that as a concequence of long-term passages, the MVA virus lost 15% of its genome compared to the parental CVA strain (Meyer et al. 1991). As a result of several deletions and disruptions, MVA no longer encodes many of the known poxviral immune evasion and virulence factors. The MVA virus is defective for replication in human cells and avirulent in test animals (Verheust et al. 2012).
- MVA has since the 1970s a history of safe use in humans for vaccine purposes. Only mild or moderate side effects has been associated with the use of MVA vaccines. It has been found to be safe and immunogenic without developing clinical disease (Parrino & Graham 2006). Moreover, MVA-based vaccines also proved safe in immunocompromised non-human primates (Stittelaar et al. 2001).

Production of attenuated MVA vectors

The most commonly used method for production of recombinant MVA involves homologous DNA recombination within infected cells using a shuttle plasmid that contains the immunogen gene expression cassette flanked by poxvirus sequences that direct the insert through homologous recombination to specific regions of the MVA genome. Since poxvirus transcription takes place in the

In line with the fact that MVA is rapidly cleared and unable to produce virus progeny, biodistribution studies have revealed that MVA does not persist more than 48 h inside the body (Gomez et al. 2008, Gomez et al. 2007).

The containment level of the transgenes intended for use in the MVA vectors is BSL1.

Viral particles can be found on the skin close to the site of administration (Verheust et al. 2012). To avoid spreading of viral vectors following intramuscular or intradermal vaccination, skin is disinfected using 70% alcohol or isopropanol upon injection of the vaccine. The risk of exposure of viral particles to the cage environment is unlikely. To prevent any leakage of viral suspensions from the injection site, an accurate injection technique during administration of the viral vector will be carried out to avoid negative pressure in the syringe and subsequent leak from the injection site. Another preventive measure to avoid exposure of viral particles to the cage environment will be a close follow-up of the injection site shortly after injection to observe and immediately disinfect any leaks of the infectious suspension from the injection site.

To decrease the risk of personnel exposure to the attenuated MVA vectors as well as to avoid risk of spreading the attenuated MVA to the environment, the MVA vaccines will be prepared in BSL2 outside animal facility. Activities in the animal facility include resuspension of the vaccine within tubes by pipetting and filling the syringe. In case of spills, 70% ethanol will be used for disinfection (Verheust et al. 2012). All vials, syringes, and other equipment containing the MVA vaccine will be decontaminated by autoclaving before disposal.

There is no risk of producing new infectious particles after administration in the animal because the MVA particles used are attenuated. After being administered in the animal, the attenuated MVA particles can infect the nearby cells only once and are not able to produce new functional particles after infection (Verheust et al. 2012).

To avoid spread of any attenuated MVA particles to the environment in the unlikely case of a leakage from the injection site, specific preventive measures will be carried out. Infected animals will be housed in the individually-ventilated cages (IVCs). Housing of animals in IVCs will ensure the containment of any leaked infectious suspensions within the cage and subsequently ensure fast desiccation of the viral suspensions in the case of any leaks from the injection site due to rapid air exchange (75 air change/h) in the IVC. To ensure desiccation of any leaks, only small volumes of the MVA injection suspension up to 100 µL that are able to dry out fast will be used. Vaccinated animals will be placed in cages with a disposable plastic container. The cages with injected animals will not be opened for at least 48 h after injection of viral suspension to allow viral inactivation via desiccation in the case of leakage. Cage exchange will be performed 7-14 days after the injection of viral suspension to allow viral inactivation via desiccation before subsequent washing of cages and disposal of bedding and plastic containers. As a safety measure, the old bedding after cage exchange will be emptied in the yellow risk waste containers that bear the appropriate labels. The risk waste containers will be stored temporarily in the animal facility until disposed by incineration. Emptied dry cages will be subjected to high-temperature washing. The carcasses of infected animals will be temporarily stored in carcass freezer until disposal by incineration. Both the cages and the room where the infected animals are housed will be marked with ABSL2.

20.11.2018.

RISK ASSESSMENT FOR USE OF REPLICATION-DEFICIENT SEMLIKI FOREST VIRUS IN MICE

BACKGROUND

Project plans

In current and future plans with Semliki Forest virus (SFV) vector it is intended to administer replication-deficient SFV particles intratumorally in subcutaneous syngeneic murine tumor models or subcutaneous patient-derived xenografts from lung tumors in mice. Intramuscular injections with replication-deficient SFV vector for cancer vaccination purposes may be carried out during another study. Due to the efficient expression capacity of SFV vector, the concentration of replication deficient SFV particles applied during animal studies won't exceed 1*10^8 IFU/injection and the maximum volume of liquid injected won't exceed 50 μL/ intratumoral injection and 100-200 μL/ intramuscular injection.

Parent organism

SFV belongs to the *Togaviridae* family, genus Alphavirus. SFV is an enveloped virus containing single-stranded positive RNA.

Biosafety aspects of using Semliki Forest virus as a vector

- Since SFV is an RNA virus and replicates in the cytoplasm the risk of chromosomal integration is removed.
- SFV induces apoptosis of infected cells (*Glasgow et al., 1998*); therefore, the virus genome does not persist in the tissue.
- No risk of person-to-person or animal-to-animal communication; naturally SFV is transmitted via mosquito bites (*PHAC, 2010*).

Production of replication-deficient SFV particles using a packaging system

Replication-deficiency of the SFV particles is provided by the recombinant pSFV1 vector with deleted viral structural proteins (*Liljestrom and Garoff, 1991*). A packaging system based on a helper vector 'pSFV-helper1' (*Liljestrom and Garoff, 1991*) is used. Replication-deficient SFV particles are produced after co-transfection of two mRNAs into packaging cells: an SFV1 mRNA with the packaging signal encoding the SFV polymerase and also the transgene, and the helper1 mRNA supplying the structural proteins - capsid and envelope proteins - in trans (*Liljestrom and Garoff, 1991*) for packaging of SFV1 RNA into nucleocapsids. The helper1 RNA lacks the region for RNA packaging (packaging signal) and therefore won't be packaged and hence, transfections with recombinant SFV1 and helper1 RNAs will produce viral particles that carry only recombinant SFV1 RNA encoding the transgene. Such viral particles produced will be able to infect cells once and produce the heterologous protein but won't be able to replicate.

Safety of the packaging system and particles produced

Liljestrom and Garoff produced SFV particles using Helper1 RNA for packaging, and were not able to observe expression of helper1-encoded structural proteins even while infecting cells with high concentrations (at m.o.i. 100, which corresponds to 100 infectious viral particles per one cell) of the replication-deficient viral particles (*Liljestrom and Garoff, 1991*). Their observation shows the safety of the helper1 packaging system because helper1 RNA is not packaged and therefore new virus particles can't be produced due to lack of structural proteins.

Decontamination

Decontamination of SFV may be carried out using steam sterilization, incineration or chemical disinfection with 70% ethanol (*PHAC, 2010*).

replication-deficient SFV vector stays localized specifically in the tumor (*Rodriguez-Madoz et al., 2005., Fig. 2B*); moreover, the infected tumor cells are located along the needle trajectory (*Rodriguez-Madoz et al., 2005., Fig. 2C*).

Therefore the risk of exposure of viral particles to the cage environment is negligible and is connected only to the leakage of viral suspension from the injection site which is unlikely. However, to prevent leakage of viral suspension from the injection site, an accurate injection technique during administration of the viral vector will be carried out to avoid negative pressure in the syringe and subsequent leak from the injection site. Another preventive measure to avoid exposure of viral particles to the cage environment in the unlikely case of leakage from the injection site will be a close follow-up of the injection site shortly after injection to observe and immediately disinfect any leaks of the infectious suspension from the injection site. 70% ethanol will be used as an efficient disinfectant for SFV (*PHAC, 2010*).

To avoid spread of any replication-deficient SFV particles to the environment in the unlikely case of a leakage from the injection site, specific preventive measures will be carried out. Infected animals will be housed in individually-ventilated cages (IVCs). Housing of animals in IVCs will ensure the containment of any leaked infectious suspension within the cage and subsequently ensure fast desiccation of the viral suspension in the case of any leaks from the injection site due to rapid air exchange (75 air changes/h) in the IVC. SFV has been shown to lose its infectivity rapidly when layered out in a thin film on the surface due to disruption by surface tensions and penetration into the air-water interface followed by desiccation (*de Jong et al., 1976*). To ensure desiccation of any leaks, only small volumes of the SFV injection suspension up to 200 µL that are able to dry out fast will be used. The cages with injected animals will not be opened for at least 48 h after injection of viral suspension to allow viral inactivation via desiccation in the case of leakage. Whereas cage exchange will be performed 7 to 14 days after the last injection of viral suspension to allow viral inactivation via desiccation before subsequent washing of cages and disposal of bedding. As a safety measure the old bedding after cage exchange will be emptied in the yellow risk waste containers that bear the appropriate labels. The risk waste containers will be stored temporarily in the animal facility until disposed by incineration. Emptied dry cages will be subjected to high-temperature washing. The carcasses of infected animals will be temporarily stored in a carcass freezer until disposed by incineration. Both the cages and the room where the infected animals are housed will be marked with ABSL-2 marking.

CONCLUSION

Work with replication-deficient SFV vectors in mice can be performed in an ABSL-1 setting, provided that specific work practices are followed during vector production and administration. Most importantly, the infectious suspension has to be produced and tested for replication deficiency at an approved BSL-2 facility outside the animal facility beforehand. Administration of replication-deficient SFV particles locally by needle injections in an ABSL-1 setting is recommended. Negligible risk is connected to accidental self-inoculation with a needle containing infectious material. Even then it is unlikely that sickness will be caused due to self-restrictive nature of infection by replication-deficient SFV particles. There is no risk of spreading the replication-deficient SFV particles to the environment due to replication-deficiency and specific containment measures used.