Objective: Verify the transmission of infectious hepatitis virus (MHV) from acutely-infected mice to normal mice through the use of Med-Jet (MIT, Inc.) injector (orifice of 0.006 thousand of an inch and 60 psi).

Animal model: To study the possibility of a viral transmission through the use of the air pressure injector, we have chosen the mouse hepatitis viral infection as a mouse model since this virus is highly infectious and induces a fulminant hepatitis leading to a rapid death in selected mouse strains.

The serotype 3 of MHV strain is the most virulent strain of this virus and induces hepatitis in 48hrs in C57BL/6 mice, the most susceptible mouse strain. The death is caused by a fulminant hepatitis and occurs within three days after infection. The viremia occurs after 48 hrs postinfection (p.i.). The MHV infection in mice is use as an animal model of human viral hepatitis since, the virus also induces a chronic hepatitis in some other mouse strains, such as C3H or SJL and F1(C57BL/6xAJ) mice. The chronic hepatitis follows the survival of the mice to the acute phase of the disease.

Hepatocytes are the most target cells to viral replication in the hepatitis process. However, blood cells can also be infected and support an efficient viral replication. The MHV3 infects macrophages, monocytes and B lymphocytes. An interesting point of this viral infection is the fact that the also induces a rapid immunodeficiency due to the viral replication in dendritic cells, macrophages and lymphocytes, increasing thus the gravity of the hepatitis.

The inoculation site has no effect on the outcome of the disease. The hepatitis can be induced following intraperitoneal, cutaneous, intramuscular, intravenous and also, intracranial injection. By natural ways, an intranasal or oral administration of the virus induces the hepatitis in C57BL/6 mice.

We have been working on the MHV model for over 20 years and our research is in continuity with the pioneer works of Dr. Jean-Marie Dupuy.
Methodology:

1. Viral infection: A C57BL/6 mouse was infected intraperitoneally with 1000 TCID<sub>50</sub> pathogenic L2-MHV3 strain (100 µl). At 48 hrs postinfection, the mouse was anesthetized with xylazin-ketamine (Rompun/Ketaset) (100 µl) intramuscularly.

2. Transmission test:
   a. A volume of 100 µl of phosphate buffer solution (PBS) was injected in the thigh of the MHV3-infected mouse using the Med-Jet (MIT. Inc) injector with an orifice of 0.006 thousand of an inch and 60 psi.
   b. A second non-anesthetized normal C57Bl/6 mouse received a similar volume of PBS with the Med-Jet (MIT. Inc) injector immediately after the injection of the MHV3-infected mouse.
   c. This protocol was performed eight times, re-injecting the same volume of PBS in the thighs of the MHV3-infected mouse (5 times on the left thigh and four times on the right thigh), and immediately after, injecting the same volume of PBS in the thigh of a normal mouse.

3. Detection of viruses at the surface of the injector:
   In order to verify the presence of infectious viral particles on the surface of the injector, the MHV3-infected mouse received PBS in the thigh using the injector and the injector was then washed with a 2 ml culture media (RPMI1640 supplemented with antibiotics). The presence of infectious viruses was assayed as described beneath.

4. Detection of viral infection in injected normal C57BL/6 mice:
   The transmission of the viral infection from MHV3-infected mouse to normal mice was verified as follows:
   a. The viral infection in MHV3-infected mouse was verified by macroscopic observation the liver immediately after the last injection of PBS.
   b. The injected normal mice were observed for a period of 72 hrs for the occurrence of clinical signs such as decrease of motor activity, decrease of reaction to external stimuli, dehydration, mucosal paleness, and a typical rounded posture. The mice were then sacrificed and the liver were collected for research of infectious viruses.

5. Infectious virus detection:
   The detection of infectious viral particles was done by culture of liver extracts from each mice in 10 ml RPMI1640 on L2 cells. The presence of infectious viruses was detected through the occurrence of a typical cytopathic effect characterised by a rounding of the cells and the presence of large syncitia. The lower threshold of virus detection of this test is 40 infectious viruses/ml.
Results

1. Observations:
   a. The injection of 100µl of PBS in the thigh of the MHV3-infected mice has provoked a small vesicle without any bleeding or detectable lesion to the skin. In addition, no bleeding or lesion were observed after 5 injections on the same thigh.
   b. The normal unanesthetized mice showed no discomfort or aggressive behavior during the injection. No bleeding or skin lesion were detected in the mice.

2. Clinical signs in normal injected C57BL/6 mice:
   No clinical signs of viral hepatitis were detected in the 9 normal injected mice up to 72 hrs p.i..

3. Macroscopic lesions:
   a. The liver from the MHV-3 infected mouse was lightly discoloured and more friable.
   b. Livers from injected normal mice were normal and no signs of hepatitis were detectable.

4. Detection of infectious viruses:
   a. No infectious viruses were detected in the washing media of the injector, as determined by the occurrence of typical cytopathic effects on cultured L2-cells after 72 hrs.
   b. Infectious viruses were detected in L2 cells inoculated with a liver extract from the MHV3-infected mice.
   c. No infectious viruses were isolated from the liver extract of injected-normal mice, as determined by culture onto L2 cells.

Conclusions

According to the clinical observations and virus detection assay, no transmission of infectious MHV3 virus was demonstrated in the experiments. No clinical signs, no macroscopic lesions of hepatitis and no infectious viruses in the liver were detected in normal mice injected with PBS immediately after the injection of MHV3-infected mice. In addition, no infectious viruses were detected on the injector.

These results are in accordance with the fact that no bleeding and no skin abrasion or lesion were observed in both MHV3-infected mice and normal injected mice. The transmission of MHV3 viral infection can take place not only by blood and blood cells contact, but also by skin contact since the virus can be excreted in feces and thus contaminate the skin surface of other mice in the same environment. To avoid this
contamination pathway, the skin of the mice was desinfected with alcool prior to the injection. In spite of this precaution, no abscess resulting from a bacterial infection, was observed in injected-normal mice. This observation was also supported by the fact that no bacterial contamination was detected in the injector tip’s washing media when put in culture onto L2 cells, in spite of the presence of low concentrations of antibiotics in the culture media.

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