

Biosafety research in medicine: Effects of endogenous retroviruses present in some well-established or experimental biomedical procedures

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Validation of a test to detect low concentrations of retroviruses

Retroviruses are known as the causative agents of numerous animal disorders. In humans, this virus family is responsible for AIDS, certain forms of leukemias, and some neurologic disorders. The development in our laboratory of an ultrasensitive test for the retroviral enzyme reverse transcriptase has made possible detection of retroviruses present at very low concentration [1]. This test, the so-called PERT assay (for Product-Enhanced Reverse Transcriptase), is meanwhile used worldwide for detection or, respectively, exclusion of unknown retroviruses in biomedical products. Negative results with the PERT assay are, for example, requested for FDA-approval of all newly developed live attenuated virus vaccines produced in mammalian cells. This test — whose validation was decisively supported by the *SPP Biotech Program of the SNF* — has thus resulted in a significant increase in the retroviral safety of biomedical products.

Use of this test in medical research

Investigations with the PERT assay during the past few years have demonstrated that certain medical procedures are indeed associated with a parenteral exposure of humans to animal retroviruses. These retroviruses include the endogenous avian retrovirus EAV-0 which is present in all live attenuated virus vaccines produced in chicken cells, namely chicken-cell derived measles, mumps, or yellow fever vaccines [2, 3], and the porcine endogenous retrovirus (PERV) which is contained in all porcine tissues considered for xenotransplantation [4].

Although there is currently no evidence for a transmission of these viruses to humans we think it necessary to investigate various aspects of infectiousness of these two viruses. In particular, we wish to address the possibility that the defective and non-infectious EAV-0 (it has a large deletion in the gene coding for the viral envelope and is thus by itself not infectious) may by means of interaction with the accompanying vaccine virus (measles, mumps, yellow fever) be enabled to enter human cells, to get integrated and be expressed, thereby also opening a possibility for recombination with human retroviruses both endogenous or exogenous.

Regarding PERVs we believe that it is necessary to investigate the possible role of a recently described new mechanism of horizontal gene transfer. This new mechanism involves uptake of DNA from apoptotic cells into phagocytes and transport of some of this DNA into the nucleus of the phagocyte, where it may even be expressed [5-7]. This newly described mechanism, by which entire retroviral genomes might be transmitted, is of particular relevance to xenotransplantation, since some of the mechanisms of xenograft rejection are known to involve apoptosis. Proviral PERV DNA originating from apoptotic cells of the xenotransplant might thus be transferred to human phagocytes without a need for a classical infectious cycle involving production and release of particles by porcine cells and infection of suitable, receptor-bearing human host cells by these particles.

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Investigating potential diagnostic problems

Finally, we would like to investigate a potential diagnostic problem that might arise when, as requested by the new law, the close contacts of xenotransplant recipients will be regularly tested by ultrasensitive PCR procedures for PERV sequences, in order to detect any possible transmission of the virus to humans at an early stage. Interestingly, a mouse model has demonstrated that short pieces of DNA taken up by food can persist in the gastrointestinal tract, may be transported across the intestinal wall and can be found integrated in the nuclei of the cells of various organs [8, 9]. There is thus a theoretical possibility that short pieces of PERV DNA taken up by eating pork may lead to false-positive results in PCR tests! This possibility should thus be investigated before further clinical trials of xenotransplantation are conducted.

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This topic was also the subject of the issue Nr 6 of the series „focus Bioécurité CH” published in French and German by the agency BICS. This publication is displayed at the Conference or can be sent by the Centre BATS.