Analysis of potential porcine endogenous retrovirus (PERV) transmission in a whole-organ xenotransplantation model without interfering microchimerism

Abstract The question whether porcine xenografts can lead to porcine endogenous retrovirus (PERV) infection of recipients is critical for the evaluation of the safety of pig-to-man xenotransplantation. Unfortunately, polymerase chain reaction (PCR)-based analysis of potential PERV infections in nonhuman-primate whole-organ xenotransplantation models is hampered by false positive results due to chimeric porcine cells. To avoid the inherent analytical problem of xenomicrochimerism, we developed a non-life-supporting pig-to-primate kidney xenotransplantation model: porcine kidneys were transplanted, whereas the functioning recipient kidneys remained in situ. Subsequent to rejection (after 2 hours to 15 days), xenografts were removed, and recipients remained alive for up to 287 days. Immunosuppressive therapy based on cyclophosphamide, cyclosporine, and steroids was maintained for 28 days after transplantation. Using appropriate PCR assays, xenochimerism was found in tissue samples and partly even in peripheral blood leukocytes (PBLs) while the porcine kidneys were in situ. After graft removal, xenochimerism was no longer detectable, thus allowing analysis for possible PERV transmission.

Keywords Xenotransplantation · Porcine endogenous retrovirus · Chimerism · Pig-to-primate · Kidney transplantation

Abbreviations PBL Peripheral blood leukocyte · PCR polymerase chain reaction · PERV porcine endogenous retrovirus · RT reverse transcriptase

Introduction Recent progress in the development of transgenic donor organs [7] has led to a renewed interest in xenotransplantation as a possible solution for the current donor organ shortage. Among the different species under consideration as suitable organ donors, pigs are the most likely donor animals [5, 6]. Currently, the potential for infection of xeno-organ recipients with xenozoonoses and, especially, the subsequent risk of transmission of any infective agent from the xenotransplant recipient to the general population are major concerns [1, 2, 4, 29]. In the xenotransplant setting, postoperative immunosuppression and immunomodulation [8] may lead to an enhanced susceptibility of recipients to any potential infective agent spread by the xeno-organ. Porcine en-
dogenous retroviruses (PERVs) are currently the main agents under investigation [21, 31]. This is due to the fact that PERV is carried in the porcine germline and cannot be eliminated from donor organs by classic means such as specific-pathogen-free animal housing. PERVs are detectable in all cell types and all pig strains analyzed so far [3, 14, 21, 34].

In vitro infection of various human cell lines with PERV during cocultivation with porcine cell lines or primary cell cultures has been demonstrated by several groups [16, 21, 28, 34]. In vivo, the available data on possible PERV infection in patients treated with living pig cells or tissues suggest that infection does not take place [11, 20, 22]. However, the viral load in the patients analyzed in these studies might have been low compared to solid-organ transplantation. In addition, only a small subset of patients had been treated with immunosuppressive therapy during xenotissue exposure. Therefore, although the available clinical data so far do not provide any evidence for cross-species transmission of PERV to humans, a definite conclusion concerning the risk of transmission of endogenous retroviruses to xenogeneic recipients under intense immunosuppression cannot be drawn from these data [32]. Consequently, additional data on this issue using solid-organ transplantation models are needed.

Unfortunately, classic animal models in solid-organ xenotransplantation (creating life-supporting situations) are associated with the inherent problem of xenomicrochimerism, leading to false positive PERV detection by polymerase chain reaction (PCR). It is well known from allotransplantation that the existence of microchimerism is dependent on the presence of the graft [13]. After graft removal (e.g., re-transplantation), chimeric cells (e.g., derived from the original graft) usually are no longer detectable in the recipient [12, 13]. We therefore developed a solid-organ discordant non-life-supporting kidney xenotransplantation model with special emphasis on long-term survival of the organ recipient after graft removal. In this setting, the recipients, which were under immunosuppression for 28 days after transplantation, were exposed to the porcine grafts for up to 2 weeks. After graft nephrectomy, the recipients were followed up for 28–272 days.

Selection of recipients

Twelve cynomolgus monkeys (Macaca fascicularis) weighing 3.8–9.0 kg and between 1.5 and 3.5 years of age were used. Animals were purchased from the German Primate Center at Göttingen. Preoperative sera from all monkeys were screened for anti-porcine antibody titer using flow-cytometric assay analysis (see below).

Surgical technique

Donor pigs were anesthetized with ketamine and intubated. Anesthesia was maintained with isoflurane and N₂O/O₂. Through a midline laparotomy and after anticoagulation with 300 IU/kg b.w. heparin, the abdominal aorta was cannulated, and the kidneys were perfused in situ with cold preservation solution (HTK, Köhler-Chemie GmbH, Alsbach-Hähnlein, Germany). After perfusion, the kidneys were dissected, removed, and stored in ice-cold perfusion solution until they were used. The cold ischemia time was between 75 and 330 min. Recipient cynomolgus monkeys were anesthetized using ketamine and propofol and intubated. Anesthesia was maintained with isoflurane and N₂O/O₂ and supplemented with boli of buphrenorphine. Through a midline abdominal incision, the inferior aorta and inferior vena cava were exposed. Transplantation was carried out by end-to-side anastomosis of the donor vessels to the aorta and inferior vena cava. The ureter was implanted into the bladder using a submucosal tunnel. In group A (n = 7) both native kidneys were left in place, thus creating a non-life-supporting model. In group B (n = 5), a life-supporting model was generated by ligation of the recipient ureters.

Immunosuppressive therapy

Immunosuppression was commenced on the day before the operation and continued until day 28 of the experiment. Immunosuppression consisted of cyclosporine, cyclophosphamide, and prednisolone (reducing dose). Cyclosporine was used to maintain the blood level from 400-600 ng/l. Cyclophosphamide was administered at a dose sufficient to suppress the leukocyte count down to 4–2 × 10³/l [9, 30].

Postoperative monitoring

Blood samples were taken every day for assessment of full blood count, urea, creatinine, and electrolytes. Cyclosporine blood levels were determined using a monoclonal-antibody-based method (EMIT 2000, Behringwerke, Liederbach, Germany). Serum was prepared by centrifugation at 3400 g for 10 min at 20 °C. For detection of antiportion xenoreactive antibodies, a flow-cytometric assay was utilized: frozen aliquots of porcine peripheral blood leukocytes (PBLS) obtained from an individual large white pig were thawed, and 0.5 × 10⁶ cells were stained by 20 μl of the respective cytomologus serum in different solutions. After 20 min of incubation at 4 °C, cells were washed twice with phosphate-buffered saline containing 1% bovine serum albumin and 0.1% sodiumazide. Bound cytomologus antibody was detected with goat antihuman fluorescein isothiocyanate secondary antibodies, detecting IgG and IgM (both by Diaova, Hamburg, Germany), respectively. These antibodies are known to cross-react with cytomologus immunoglobulins. The antibodies had been preabsorbed using porcine serum. After incubation for 20 min at 4 °C, cells were washed again.

Materials and methods

Selection of donors

Twelve nontransgenic large white Landrace pigs were used. Pigs were 3–12 weeks old and weighed 4.1–25.0 kg. Pigs were obtained from Schweinezuchtverband Weser-Ems, Oldenburg, Germany and were transferred to the central animal operation facility at Hannover Medical School on the day of operation.