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Xenotransplantation: Today and Tomorrow

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The Need

Xenotransplantation using pig cells, tissues or organs is at present thought to be the best approach to alleviate the increasing shortage of human tissues and organs for the treatment of tissue and organ failure. This shortage is the reason that approximately 25% of the patients on a waiting list die before they could be treated with an appropriate donor organ. In the US, 30,974 organ transplants were performed in 2015, however 118,066 people need a lifesaving organ transplant [1].

Xenotransplantation using pig islet cells may be also the most effective solution for the treatment of diabetes. In 2012, 29.1 million Americans, or 9.3% of the population, had diabetes, among them approximately 1.25 million children and adults with type 1 diabetes. Although the treatment of diabetes type 1 with insulin was quite successful in the past, nevertheless complications were observed mainly due to insufficient compliance of the patients. Among the complications described were amputations of the limbs and blindness. The total costs of diagnosed diabetes in the US in 2012 were 245 billion dollars, most of these costs were spend for the treatment of complications, the expenditure for insulin was relatively low [2].

Pigs are for numerous reasons (size, physiology, easily genetically modified, cloned, large number of progeny) the most preferred donor animal for xenotransplantation [3].

The Problems

As in the case of allotransplantation, the main problem in xenotransplantation is the rejection of the immunologically unrelated organ. As well-known, the relatedness of the donor and recipient are the main factor determining the survival of the transplant, then more genetic mismatches, than shorter the survival time.

A new problem not described for allotransplantation is the hyper acute rejection (HAR) [4]. Humans develop antibodies against certain sugar residues present on the cell surface of bacteria, among them galactose α1,3-galactose (α-gal)and 2 N-glycoylneuraminic acid-terminated gangliosides (Neu5Gc) [5]. These sugar residues are not present on human cells, but on cells from many animals including pigs. Transplanted pig tissues or organs carrying such sugar residues will be destroyed by these pre-existing antibodies and the human complement system in a few minutes.

Another potential risk associated with xenotransplantation is the possible transmission of porcine microorganisms (bacteria, fungi, protozoa, and viruses) which may lead to diseases, so-called zoonoses [6]. Transmission of microorganisms was also reported during allotransplantation, including human immunodeficiency virus-1 (HIV-1), human cytomegalovirus (HCMV) and rabies virus [7]. Whereas human pathogens are well adapted to humans, porcine microorganisms are not and it is unclear, whether they can infect human cells and replicate in humans. For some porcine viruses a zoonotic potential was described, for example for hepatitis E virus (HEV), genotype 3, which at least in immuno suppressed and patients with a pre-existing liver failure induces a chronic infection and disease [8,9]. Others, such as the porcine cytomegalovirus (PCMV) may be pathogenic without infecting cells of the host. In preclinical trials, transplanting pig kidneys in cynomolgus monkeys and baboons, the presence of PCMV led to an early transplant failure, possibly due to cytokines produced in response to viral antigens [10].

Finally, the porcine endogenous retroviruses (PERVs) are integrated in the pig genome, are produced as infectious virus particles and may infect certain types of human cells [11,12]. PERV-A and PERV-B are integrated in the genome of all pigs, whereas PERV-C was found in many, but not all pigs. In addition, recombinants between PERV-A and PERV-C were found in pigs which were highly replication-competent. PERVs like most other retroviruses may theoretically induce tumours and/or immuno deficiencies, but their zoonotic potential is yet unknown and in preclinical pig-to-non-human primate preclinical trials and in first clinical trials, no PERV transmission was observed [12-15].

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Another problem, the physiological incompatibility cannot be evaluated at present since the survival time of the transplanted organs is too short to analyse its long-term functionality.

The Solutions

To overcome the immunological rejection including HAR, genetically modified pigs were created. There are two types of genetic modifications, first knock-out animals in which genes encoding enzymes bringing the above mentioned sugar residues on the surface of pig cells, e.g., α1,3 galactosyltransferase (α1,3GT), were knocked out (GTKO), and second, transfection and expression of human genes responsible for complement activation and other processes of immune rejection and of coagulation [16,17].

To overcome the risk of transmission of porcine microorganisms, elimination programs were developed based on selection, treatment, vaccination, Cesarean delivery, early weaning and embryo transfer [6,9,10,18]. PERVs cannot be eliminated by the these mechanisms since the proviral DNA is integrated in the genome of all pig cells, therefore different strategies have been developed to prevent PERV transmission during xenotransplantation including a PERV-specific vaccine [19,20], antiretroviral drugs [21,22], transgenic pigs expressing a PERV-specific small-interfering (si)RNA [23-25] and genome editing using zinc finger nucleases (ZFN) [26] or CRISPR/Cas (clustered regularly interspaced short palindromic repeats, CRISPR-associated) [27].

The Achievements and the Future

First of all, multi transgenic pigs were successfully created in order to prevent rejection of pig cells and organs. Second, new pharmaceutical immuno suppression regimens were introduced, also in order to prevent rejection. Based on these achievements, longer survival times of transplanted pig hearts, kidneys, liver, and islet cells have been observed in preclinical trials [28-31]. Heterotopic heart transplants from GTKO, CD46 and thrombomodulin genetically modified pigs survived up to 945 days in baboons (median survival time 298 days) [32]. The longest survival time of orthotopic heart transplantation using GTKO/CD55 pigs was 57 days [33], the longest survival time of pig kidney transplants 310 days [34] and the survival time of pig islet cells was 950 days (median 303) [35]. Third, new and sensitive methods have been developed to screen the donor pigs for potential zoonotic microorganisms, making xenotransplantation eventually safer compared with allotransplantation, where in rare cases HIV-1, rabies virus, HCMV, and other pathogens have been transmitted [7]. Although PCMV has been transmitted in pig-to non-human primate kidney and heart transplantation [36-39], no transmission of porcine viruses was observed in first pig islet cell transplantation in humans [13-15]. The transmission of PCMV into cynomolgus monkeys and baboons however was associated with a significant reduction of the survival time of the pig transplant [36,37]. Fourth, the discussion on ethical aspects is on-going, an updated consensus document on how to perform safe and efficient xenotransplantation was prepared by the scientific community, and in several countries a national regulatory frame work was prepared [40]. All these achievements will allow clinical application of xenotransplantation in the near future.

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