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Introductory Chapter: The State of Xenotransplantation

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1. Basic research

Studies of discordant xenografts, such as guinea-pig to rat and pig to human, were started more than 30 years ago. The first subject to be addressed was the mechanism of discordant xenograft rejection [1], i.e., hyperacute rejection. After verification of the reaction of the complement system, xeno-specific glycoantigens in pig-to-human xenotransplantation, such as the α -gal epitope, were then studied [2, 3], followed by the other immune systems.

Therefore, the first gene modification on pigs was focused on issues related to complement regulatory proteins (CRPs) such as Membrane Cofactor Protein (MCP, CD46), Decay Accelerating Factor (DAF, CD55), and CD59 [4]. DAF (CD55)-transgenic pigs were then first produced in 1994 [5, 6], followed by other CRP-transgenic pigs [7, 8]. On the other hand, different from the mouse system, pig embryonic stem (ES) cells had not yet been established. Therefore, other methods for reducing the α -gal epitope, such as the overexpression of α 1,2 fucosyltransferase [9], End- β -GalC [10], and GnT-III [11, 12], were examined [13].

Fortunately, the gene targeting technique was combined with (fetus) fibroblasts and the nuclear transfer techniques, resulting in the successful development of α -gal knockout (KO) pigs in 2002 [14].

Many kinds of CRP and glycoantigens [15] are now being nominated for transgenic and knockout, respectively, based on improved genetic engineering (GE) techniques. The next obstacle to xenograft is cellular rejection by the innate immune system, which comprises natural killer (NK) cells and monocytes/macrophages.

Strategies for suppressing NK function on the pig cells have been extensively examined. HLA-class Ia molecules, such as HLA-C, but also class Ib, HLA-G1 [16, 17] and -E [18, 19], has been considered in the case of the transgenic pig. In addition, changing the pattern of glycosylation on the surfaces of pig cells is also a reasonable strategy [20–24].

The issue of how to regulate monocytes/macrophages, it is now known that only CD47 [25] binds to SIRP α on the surface of monocytes/macrophages that contains the immune receptor tyrosine-based inhibition motif (ITIM). Therefore, until quite recently, other routes to the downregulation of monocytes/macrophages have not been well studied.

However, especially in these past 5 years, additional key molecules for suppressing monocytes/macrophages have clearly been identified. Thus, for example, HLA class Ib, HLA-G1 [26], and -E [27] were identified as having a suppressive function not only for NK cells but also for monocytes/macrophages as well. Monocytes/macrophages actually have common receptors in common with NK cells. In addition, changes in glycoantigens, such as the overexpression of the α 2,6-sialic acids, as well as other methods [28], also function to downregulate monocytes/macrophages [29].

In addition, meanwhile, the many strategies for suppressing the movement of T cells have been proposed, such as class II dominant negative (CIIDN) [30, 31], HLA class I-KO [32], FasL, and tumor necrosis factor receptor I IgG-Fc (TNFRI-Fc) [33]. In addition to immunological studies, studies of coagulation systems, such as thrombomodulin (TM), the tissue factor pathway inhibitor (TFPI), the endothelial cell protein C receptor (EPCR), CD39 and CD73, and anti-apoptotic and anti-inflammatory genes, such as heme oxygenase 1 (HO-1) and A20, have also progressed.

2. Genetic engineering

The most progress during these past 5 years involves gene targeting technology. One involves zinc-finger nucleases (ZFN) [34] and is continued by the transcription activator-like effector nuclease (TALEN) [35] method, and finally the clustered regularly interspaced short palindromic repeats/CRISPR associated protein 9 (CRISPR/CAS) [36]. These methods had brought about a revolution in certain aspects of gene-targeting technology. Therefore, the KO of a special gene became extremely easier than in the past. Not only α -gal KO but also cytidine monophospho-*N*-acetylneuraminic acid hydroxylase (CMAH, the gene for producing the Hanganutziu-Deicher antigen; NeuGc)-KO, SLA class I-KO, β 4GalNT2-KO [37], etc. have been established in many institutes, combined with transgenic human genes. In addition, the 2A system is now popular in our field and also was a great help in producing transgenic pigs with multi-genes [38, 39].

In addition, as a new strategy, attempts are being made to retain the fixed expression of transgenes, because the transgenic expression of each gene was sometimes not stable over generations. Knockin (KI) human genes to the ROSA locus of the pig genome became of interest [40].

3. Preclinical study

During these 5 years since the first version, remarkable progress has been made in the area of preclinical xenotransplantation experiments [41–43].

Surprisingly, heterotopic hearts from the GE-pigs continued to beat for almost 2.5 years, when implanted in the monkey abdomen [44], and pig life-supporting kidney could function for nearly 1 year in monkeys [45].

Concerning islet cells, trials in which islet cells from GE-pigs are transplanted in monkeys have been reported. Several groups have reported survival periods of more than 1 year, using adult pig islets (APIs) [46, 47]. Generally speaking, results using neonatal porcine islet-like cell clusters (NPCCs) were worse than those using APIs. It is noteworthy that one group reported a survival of over 600 days using API from wild-type pigs [48], suggesting that the combination of API from GE-pigs and excellent drug therapy may permit islets to survive for more than 2–3 years.

4. Porcine endogenous retrovirus (PERV)

Concerning the problems associated with the porcine endogenous retrovirus (PERV) [49], new studies have appeared during these past 5 years, trials to knockout all PERV genes from the pig genome were done using the new techniques, ZFN and CRISPR [50, 51]. However, success has not yet been achieved.

However, in spite of hundreds of patients undergoing transplantation of pig organs or tissue, no reports have appeared of suffering [52]. The controversy associated with the risks of PERV has already been minimized.

5. For clinic

In Japan, in 2014, a law related to the pig cell (islets) transplants was passed. In 2016, the guidelines for xenotransplantation were revised. At this moment, clinical detection systems for identifying infectious diseases from pig tissue are being improved. Thus, it has already become possible to start clinical pig islets transplantation. In addition, in the USA, the councilors of the International Xenotransplantation Association (IXA) will be holding meetings with FDA-staff concerning the start of clinical trials in this September at IXA2017 in Baltimore. We are hoping for positive results from this meeting.

On the other hand, regarding clinical trials, many trials have completed and some are ongoing, such as in Sweden [53], China [54], Mexico [55], Argentina, Russia, the USA, and New Zealand [56].

In the near future, possibly within 1 or 2 years, in Japan, the USA, and Europe, some clinical trials involving the use of genetic-modified pigs or microencapsulation pancreatic islets in xenotransplantation will start.

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