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Chapter 1

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 α1,2 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 SIRPα 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 not 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 α2,6-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, β4GalNT2-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 multigenes [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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References

[1] Miyagawa S, Hirose H, Shirakura R, et al. The mechanism of discordant xenograft rejection. Transplantation. 1988;**46**:825-830

[2] Galili U, Swanson K. Gene sequences suggest inactivation of alpha-1,3-galactosyltransferase in catarrhines after the divergence of apes from monkeys. Proceedings of the National Academy of Sciences of the United States. 1991;**88**:7401-7404

[3] Cooper DKC. Depletion of natural antibodies in non-human primates – A step towards successful discordant xenografting in humans. Clinical Transplantation. 1992;**6**:178-183

[4] Miyagawa S, Yamamoto A, Matsunami K, et al. Complement regulation in the GalT KO era. Xenotransplantation. 2010;**17**:11-25

[5] Dalmasso AP, Vercellotti GM, Platt JL, et al. Inhibition of complement-mediated endothelial cell cytotoxicity by decay-accelerating factor. Potential for prevention of xenograft hyperacute rejection. Transplantation. 1991;**52**:530-533

[6] Cozzi E, White DJG. The generation of transgenic pigs as potential organ donors for humans. Nature Medicine. 1995;**1**:964-966

[7] Loveland BE, Milland J, Kyriakou P, et al. Characterization of a CD46 transgenic pig and protection of transgenic kidneys against hyperacute rejection in non-immunosuppressed baboons. Xenotransplantation. 2004;**11**:171-183

[8] Takahagi Y, Fujimura T, Miyagawa S, et al. Production of alpha-1,3-galactosyltransferase gene knockout pigs expressing both human decay-accelerating factor and N-acetylglucosaminyltransferase III. Molecular Reproduction and Development. 2005;**71**:331-338

[9] Sandrin MS, Fodor WL, Mouhtouris E, et al. Enzymatic remodelling of the carbohydrate surface of a xenogenic cell substantially reduces human antibody binding and complement-mediated cytolysis. Nature Medicine. 1995;**1**:1261-1267

[10] Ogawa H, Muramatsu H, Kobayashi T, et al. Molecular cloning of endo-beta-galactosidase C and its application in removing alpha-galactosyl xenoantigen from blood vessels in the pig kidney. Journal of Biological Chemistry. 2000;**275**:19368-19374

[11] Tanemura M, Miyagawa S, Ihara Y, et al. Significant downregulation of the major swine xenoantigen by N-acetylglucosaminyltransferase III gene transfection. Biochemical and Biophysical Research Communications. 1997; **235**:359-364

[12] Miyagawa S, Murakami H, Takahagi Y, et al. Remodeling of the major pig xenoantigen by N-acetylglucosaminyltransferase III in transgenic pig. Journal of Biological Chemistry. 2001;**276**:39310-39319

[13] Byrne GW, Du Z, Stalboerger P, et al. Cloning and expression of porcine β1,4 N-acetylgalactosaminyl transferase encoding a new xenoreactive antigen. Xenotransplantation. 2014;**21**:543-554

[14] Dai Y, Vaught TD, Boone J, et al. Targeted disruption of the alpha-1,3-galactosyltransferase gene in cloned pigs. Nature Biotechnology. 2002;**20**:251-255

[15] Miyagawa S. Xenotransplantation and glycomedicine. In: Kamerling JP, editor. Comprehensive Glycoscience. Elsevier Press; 2007. pp. 533-553.

[16] Sasaki H, Xu XC, Mohanakumar T. HLA-E and HLA-G expression on porcine endothelial cells inhibit xenoreactive human NK cells through CD94/NKG2-dependent and -independent pathways. Journal of Immunology. 1999;**163**:6301-6305

[17] Matsunami K, Miyagawa S, Nakai R, et al. The possible use of HLA-G1 and G3 in the inhibition of NK cell-mediated swine endothelial cell lysis. Clinical & Experimental Immunology. 2001;**126**:165-172

[18] Matsunami K, Miyagawa S, Nakai R, et al. Modulation of the leader peptide sequence of the HLA-E gene up-regulates its expression and down-regulates natural killer cell-mediated swine endothelial cell lysis. Transplantation. 2002;**73**:1582-1589

[19] Matsunami K, Kusama T, Okura E, et al. Involvement of position-147 for HLA-E expression. Biochemical and Biophysical Research Communications. 2006;**347**:692-697

[20] Miyagawa S, Nakai R, Yamada M, et al. Regulation of natural killer cell-mediated swine endothelial cell lysis through genetic remodeling of a glycoantigen. Journal of Biochemical. 1999;**126**:1067-1073

[21] Inverardi L, Clissi B, Stolzer AL, et al. Human natural killer lymphocytes directly recognize evolutionarily conserved oligosaccharide ligands expressed by xenogeneic tissues. Transplantation. 1997;**63**:1318-1330

[22] Artrip JH, Kwiatkowski P, Michler RE, et al. Target cell susceptibility to lysis by human natural killer cells is augmented by alpha-(1,3)-galactosyltransferase and reduced by alpha-(1,2)-fucosyltransferase. Journal of Biological Chemistry. 1999;**274**:10717-10722

[23] Baumann BC, Schneider MK, Lilienfeld BG, et al. Endothelial cells derived from pigs lacking Gal alpha-(1,3)-gal: No reduction of human leukocyte adhesion and natural killer cell cytotoxicity. Transplantation. 2005;**79**:1067-1072

[24] Christiansen D, Mouhtouris E, Milland J, et al. Recognition of a carbohydrate xenoepitope by human NKRP1A (CD161). Xenotransplantation. 2006;**13**:440-446

[25] Ide K, Wang H, Tahara H, et al. Role for CD47-SIRPα signaling in xenograft rejection by macrophages. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**:5062-5066

[26] Esquivel EL, Maeda A, Eguchi H, et al. Suppression of human macrophage-mediated cytotoxicity by transgenic swine endothelial cell expression of HLA-G. Transplant Immunology. 2015;**32**:109-115

[27] Maeda A, Kawamura T, Ueno T, et al. The suppression of inflammatory macrophage-mediated cytotoxicity and proinflammatory cytokine production by transgenic expression of HLA-E. Transplant Immunology. 2013;**29**:76-81

[28] Sakai R, Maeda A, Choi T-V, et al. Human CD200 suppresses macrophage-mediated xenogeneic cytotoxicity and phagocytosis. Surgery Today. 2017. DOI: 10.1007/s00595-017-1546-2

[29] Maeda A, Kawamura T, Nakahata K, et al. Regulation of macrophage-mediated xenocytotoxicity by overexpression of alpha-2,6-sialyltransferase in swine endothelial cells. Transplantation Proceedings. 2014;**46**:1256-1258

[30] Hara H, Witt W, Crossley T, et al. Human dominant-negative class II transactivator transgenic pigs – effect on the human anti-pig T-cell immune response and immune status. Immunology. 2013;**140**:39-46

[31] Yun S, Gustafsson K, Fabre JW. Suppression of human anti-porcine T-cell immune responses by major histocompati bility complex class II transactivator constructs lacking the amino terminal domain. Transplantation. 1998;**66**:103-111

[32] Reyes LM, Estrada JL, Wang ZY, et al. Creating class I MHC-null pigs using guide RNA and the Cas9 endonuclease. Journal of Immunology. 2014;**193**:5751-5757

[33] Park SJ, Cho B, Koo OJ, et al. Production and characterization of soluble human TNFRI-Fc and human HO-1(HMOX1) transgenic pigs by using the F2A peptide. Transgenic Research. 2014;**23**:407-419

[34] Hauschild J, Petersen B, Santiago Y, et al. Efficient generation of a biallelic knockout in pigs using zinc-finger nucleases. Proceedings of the National Academy of Sciences of the United States of America. 2011;**108**:12013-12017

[35] Miyagawa S, Matsunari H, Watanabe M, et al. Generation of α1,3-galactosyltransferase and cytidine monophospho-N-acetylneuraminic acid hydroxylase gene double-knockout pigs. Journal of Reproduction and Development. 2015;**61**:449-457

[36] Kwon DJ, Kim DH, Hwang IS, et al. Generation of α-1,3-galactosyltransferase knocked-out transgenic cloned pigs with knocked-in five human genes. Transgenic Research. 2017;**26**:153-163

[37] Martens GR, Reyes LM, Butler JR, et al. Humoral reactivity of renal transplant-wait-listed patients to cells from GGTA1/CMAH/B4GalNT2, and SLA class I knockout pigs. Transplantation. 2017;**101**:e86-e92

[38] Szymczak AL, Workman CJ, Wang Y, et al. Correction of multi-gene deficiency in vivo using a single 'self-cleaving' 2A peptide-based retroviral vector. Nature Biotechnology. 2004;**22**:589-594

[39] Fisicaro N, Londrigan SL, Brady JL, et al. Versatile co-expression of graft-protective proteins using 2A-linked cassettes. Xenotransplantation. 2011;**18**:121-1130

[40] Li S, Flisikowska T, Kurome M, et al. Dual fluorescent reporter pig for Cre recombination: Transgene placement at the ROSA26 locus. PLoS One. 2014;**9**:e102455

[41] Cooper DK, Satyananda V, Ekser B, et al. Progress in pig-to-non-human primate transplantation models (1998-2013): A comprehensive review of the literature. Xenotransplantation. 2014;**21**:397-419

[42] Cooper DK, Bottino R. Recent advances in understanding xenotransplantation: Implications for the clinic. Expert Review of Clinical Immunology. 2015;**11**:1379-1390

[43] Puga Yung GL, Rieben R, Bühler L, et al. Xenotransplantation: Where do we stand in 2016? Swiss Medical Weekly. 2017;**147**:w14403

[44] Mohiuddin MM, Singh AK, Corcoran PC, et al. Chimeric 2C10R4 anti-CD40 antibody therapy is critical for long-term survival of GTKO.hCD46.hTBM pig-to-primate cardiac xenograft. Nature Communications. 2016;**7**:11138

[45] Iwase H, Hara H, Ezzelarab M, et al. Immunological and physiological observations in baboons with life-supporting genetically engineered pig kidney grafts. Xenotransplantation. 2017. Mar;**24**(2). DOI: 10.1111/xen.12293. Epub 2017 Mar 17

[46] Bottino R, Wijkstrom M, van der Windt DJ, et al. Pig-to-monkey islet xenotransplantation using multi-transgenic pigs. American Journal of Transplantation. 2014;**14**:2275-2287

[47] Hawthorne WJ, Salvaris EJ, Phillips P, et al. Control of IBMIR in neonatal porcine islet xenotransplantation in baboons. American Journal of Transplantation. 2014;**14**: 1300-1309

[48] Shin JS, Kim JM, Kim JS, et al. Long-term control of diabetes in immunosuppressed non-human primates (NHP) by the transplantation of adult porcine islets. American Journal of Transplantation. 2015;**15**:2837-2850

[49] Patience C, Takeuchi Y, Weiss RA. Infection of human cells by an endogenous retrovirus of pigs. Nature Medicine 1997;**3**:282-286

[50] Semaan M, Ivanusic D, Denner J, et al. Cytotoxic effects during knock out of multiple porcine endogenous retrovirus (PERV) sequences in the pig genome by zinc finger nucleases (ZFN). PLoS One. 2015;**10**:e0122059

[51] Yang L, Güell M, Niu D, et al. Genome-wide inactivation of porcine endogenous retroviruses (PERVs). Science. 2015;**350**:1101-1104

[52] Morozov VA, Wynyard S, Matsumoto S, et al. No PERV transmission during a clinical trial of pig islet cell transplantation. Virus Research. 2017;**227**:34-40

[53] Groth CG, Korsgren O, Tibell A, et al. Transplantation of porcine fetal pancreas to diabetic patients. Lancet. 1994;**344**:1402-1404

[54] Wang W, Mo Z, Ye B, et al. A clinical trial of xenotransplantation of neonatal pig islets for diabetic patients. J Cent South Univ Med Sci. 2011;**36**:1134-1140

[55] Valdés-González RA, Dorantes LM, Garibay GN, et al. Xenotransplantation of porcine neonatal islets of Langerhans and Sertoli cells: A 4-year study. European Journal of Endocrinology. 2005;**153**:419-427

[56] Elliott RB, Escobar L, Tan PL, et al. Live encapsulated porcine islets from a type 1 diabetic patient 9.5 yr after xenotransplantation. Xenotransplantation. 2007;**14**:157-161