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The Past, Present, and Future of Xenotransplantation

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PAST

The history of xenotransplantation

A xenotransplantation is the transplantation of organs, tissues or cells from one species to another.[1,2] The first report of such a transplant was a bone zoograft in Russia, 1682. After that, an attempt was made in England to engraft frog's skin at the end of 19th century. Princeteau first attempted a kidney xenotransplantation to a human body.[3] A rabbit kidney was transplanted in a patient with end stage renal disease. Surprisingly, he observed that the uremic symptoms had reduced. Since then, the kidneys from pigs, goats, and monkeys have been transplanted into humans.[3] However, cases actually with a good clinical result are rare. Nevertheless, Reemtsma, who is known as the father of xenotransplantation, transplanted the kidney of a chimpanzee into the human body, and the human recipient survived 9 months.[3] At autopsy, there was no transplantation rejection reaction, and this patient was found to have died from a water-electrolyte imbalance.

Until the mid 1960s, the kidneys of primates such as chimpanzees, baboons, and rhesus monkeys were transplanted to human. In 1964, Hardy transplanted a heart of a chimpanzee for the first time.[4] Thereafter, heart transplants from primates were performed, but the survival rate was very low.[5] This was because of the ABO blood type incompatibility. The blood types in baboons mainly consist of A, B, and AB, with type O being quite rare. However, Bailey successfully transplanted a baboon's heart to baby Fae, who had a congenital anomaly of the development of the right side of the heart, and survived 20 days.[6,7]

Xenotransplantation has some benefits over allotransplantation. Many graft organs can become available for xenotransplantation, which means that there is no need to exclude some patients for health reasons, e.g. the age of the recipients. In addition, transplant operations are not emergencies, and the graft maintains a pathogen free condition. However, the degree of rejection is more severe than that of an allotransplantation.[8] Therefore, during 1970s, due to the successful allotransplantation, there has been less need for xenotransplantation.

Nevertheless, the demand for graft organs has increased by 15% every year in the United States only, where 50% of patients waiting for a compatible kidney donor can be treated, and 10 - 15% of patients die waiting for heart or liver donors. Consequently, there is still some need for developments in the field of xenotransplantation. Furthermore, there is an urgent need for developing in the field of xenotransplantation, because Koreans have a culture of Confucianism, where it is difficult to find acceptance for cadaveric donations.

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A source of xenogenic organ supply

Based on the patient survival rates, primates appear to be the most suitable for the source of the supply of xenografts. Above all, chimpanzees are known to be almost the same as human beings. However, chimpanzees, are threatened with extinction and are not easy to breed. They have a long gestation period, reproduce few offspring, can not meet the demand for organs, and the refusal of animal care groups are quite severe. Moreover, chimpanzees might be a source of infective pathogens, including viruses such as ebola, human immunodeficiency virus (HIV), JC virus (JCV), which exist in the chimpanzees in the wild. Baboons also are a source of infection e.g. Toxoplasma gondii, Mycoplasma tuberculosis, Encephalomyocarditis virus, Filovirus (Marburg, Ebola), Monkeypox, Simian hemorrhagic fever, lymphocytic choriomeningitis virus (LCMV), parasites and bacteria of the digestive system.[9,10] In addition, they have few individuals with blood type O, are difficult to breed, and can only produce infants less than 70 lb (about 32 kg) with a proper organ graft because the body size of a baboon is small. On the other hand, the size of body of pigs varies, according to the species, which make it is possible to provide organs for infants to adults. In addition, their breeding is economical and convenient, and they produce 6 - 12 offspring, after a short pregnancy of 114 days. However, there is a little chance that these animals contain pathogens that are harmful to the human body. Overall, a pig is the most suitable source for xenotransplantation.

PRESENT

Xenograft rejection

When an organ graft from a pig is transplanted to the human body, a serious multistep rejection process, as shown in Fig. 1 occurs, and this rejection process is the main problem to be solved.

Hyperacute Rejection

Xenotransplantation is divided into two types, concordant and discordant, according to discrepancy in species. Concordant xenotransplantation is the case where there is no crossreactive antibodies between the donor and recipient, e.g. the cases where the organs from primates are transplanted to humans or the organs from guinea pigs are transplanted to rats. Discordant xenotransplantation is where there are crossreactive antibodies between two species e.g. the case where the organ from a pig is transplanted into a human.[2] Humans, apes, and old-world monkeys have antibodies against galactose-α(1,3)-galactose (Gal-α(1,3)-Gal), which is widely distributed on the porcine vascular endothelial cells.[11] This antibody is probably against enterobacteria, which is presumed to have been obtained during the evolution process, and is called the xenoreactive natural antibody (XNA), because these animals have it from birth. If the organ of a pig is transplanted, human XNA binds to Gal-α(1,3)-Gal immediately, activates the serum complement, attracts platelets, induces thrombus formation, and interrupts the graft function.[11] This is known as the hyperacute rejection. The XNA is comprised mainly of IgM antibodies and is known to be the cause of the hyperacute rejection is XNA. It was also shown that if the antigen binding site is blocked by a pretreatment with Galactosyl-1-sugar, the hyperacute rejection diminishes,[11] and if the α(1,3) Gal transferase gene is expressed on the COS cell lines, the gene products bind with the human XNA and activate the complement.[11]

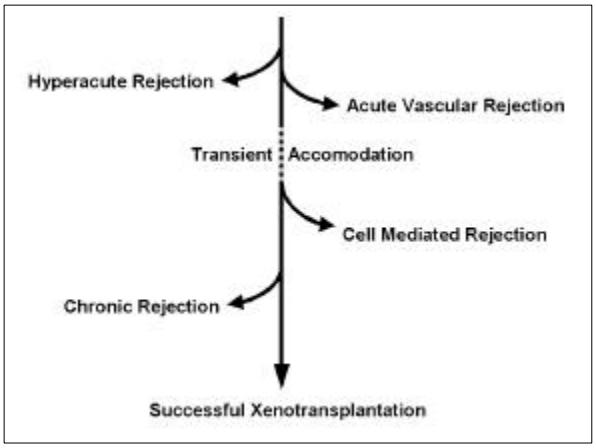

Fig. 1. Multistep rejection process in xenotransplantation.

The complement activation pathway on porcine endothelial cells is known as the 'classical path-

way', where the grade of injury of the vascular endothelial cells depends on the degree of complement activation. The terminal complement complex is procured as a result of the classical pathway and acquires vascular endothelial cells to have a tilt towards a procoagulant posture.[12]

In general conditions, the complement is activated, and the complement regulatory proteins (CRPs) are expressed on the vascular endothelial cells. The function of the CRPs is to inhibit cell or tissue injury subsequent to excessive complement activation. Decay accelerating factor (DAF/CD55), the membrane inhibitor of reactive lysis (CD59), the membrane cofactor protein (MCP/CD46), and the homologous restriction factor (HRF) belong to CRPs.[13,14] When the complement is activated, the CRPs are released from endothelial cells to protect its own tissue. In xenotransplantation, however, the CRPs from endothelial cells of the donor, can not inhibit the activated complement of humans due to the difference in the molecular structure.[15] Therefore, the graft is damaged more seriously as a result of the complement-mediated cytotoxicity. As a result, the heparan sulfates on the endothelial cells disappear and the platelets are activated to form a fibrin clot.[16] This leads to the hypercoagulate state, which finally interrupts the blood flow.

Two methods to control the hyperacute rejection have been reported, namely, (1) a method to reduce the XNA reaction, and (2) a method to block the complement activation.

(1) At first, there are two ways to reduce the XNA reaction, one is to remove the XNA from the human recipient and the other way is to inhibit the expression of the Gal-α(1,3)-Gal epitope. The most widely known method of removing XNA, as reported by Cooper and Galili, is that the Gal-α(1,3)-Gal specific antibody or IgM antibody from the blood can be eliminated using a extracorporeal circulation column.[17-19] In addition, XNA can be eliminated through an intravenous injection of certain carbohydrates of using anti-idiotope antibodies.[20,21] The effect increases if combined with a splenectomy and immunosuppressant therapy.

In order to inhibit the expression of the Gal-α(1,3)-Gal epitope, many researchers tried to produce Gal-α(1,3)-Gal knock out pigs. However, these works were not easy because it was too difficult to obtain porcine embryonic stem cells. Accordingly, a method for the competitive inhibition of Gal-α(1,3)-Gal expression by the overexpressing H-transferase was attempted. That is, a-1,2-fucosyltransferase is an enzyme that acts competitively with a-1,3-galactosyl 1 transferase.[22] If transgenic pigs for this enzyme can be produced, and Gal-α(1,3)-Gal can be inhibited, a good supply of these pigs with the H-transferase gene can be produced within one to two years.

(2) Second, there are two ways to block the complement activation, one way is to remove the complement in the serum, and the other is to reinforce the function of the CRPs. The serum complement can be removed with drugs, such as the cobra venom factor (CVF), the soluble complement type1 (sCR1), FUT175, K76-COOH, but side effects are inevitable because of the systemic administration.[23-26] An alternative plan is to produce genetically manipulated pigs, which overexpress human CRPs, DAF, CD59, etc. Through these techniques, the hyperacute rejection does not occur, and it is possible to survive for 5 days using no extra immunosuppressants, when the organ graft of the pig is transplanted to primates. The XNA is restored to the original state at the fifth day after extracorporeal hemofiltration. However, the graft survival increases to 17.5 days if all methods are used, such as extracorporeal circulation, immunosuppressants, removal of the complement.

Acute vascular rejection

After overcoming the hyperacute rejection, organ graft from pigs are expected to undergo acute vascular rejection. Acute vascular rejection takes several days, accompanies monocytes and NK cell infiltration, localized ischemic change, interstitial hemorrhage and intravascular coagulation of microcirculation due to hypercoagulation.[27] However, inflammatory cell infiltration is not so severe.

It is known that the mechanism of this rejection is via the graft endothelial cells, which are activated and cause fibrin deposition. At acute vascular rejection, vascular endothelial cells became activated by XNA, the complement, NK cells, macrophages, platelets and any other incompati-

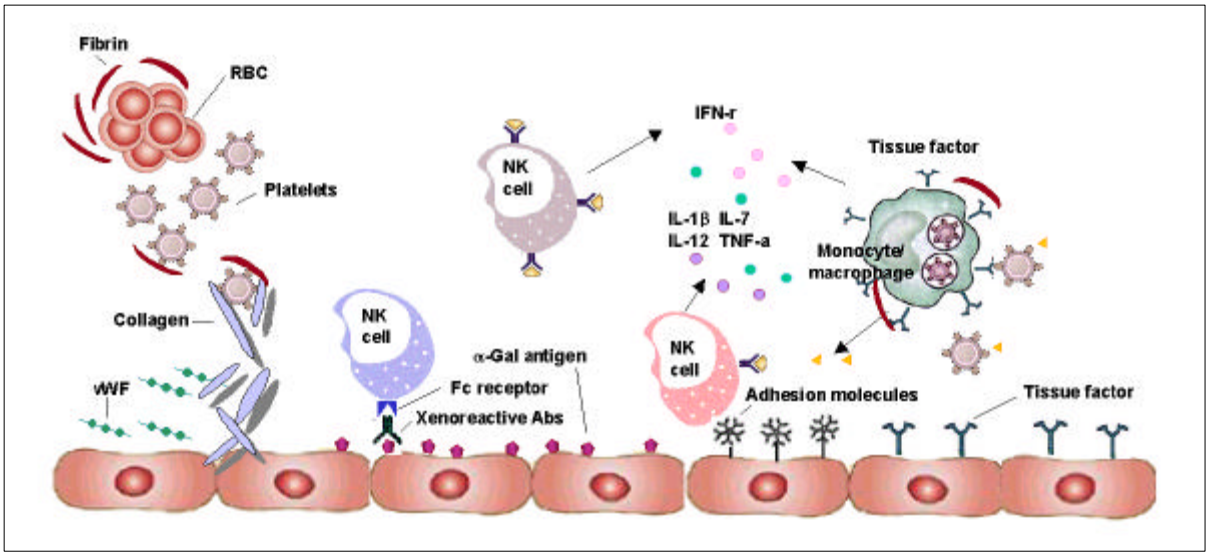

Fig. 2. Typical features of acute vascular rejection. Abnormal thrombomodulation, host NK cell and monocyte/macrophage infiltration and subsequent their activation and cytokine production and endothelial activation with up-regulation of adhesion molecules, tissue factor and production of cytokines are responsible for acute vascular xenograft rejection.

bilities of molecular structures between the host and the xenograft (Fig. 2). Therefore, XNA play the most significant role in activating the endothelial cells. When XNA binds to Gal-(1,3)-Gal on the endothelial cells, which are activated and increase the production of IL-1, induce vasoconstriction, causing inflammation and ischemic injury. In addition, activated endothelial cells induce platelet activation, vasodilatation, and release many cytokines.[27,28] In order to prevent acute vascular rejection, the following methods have been attempted: (1) the removal of XNA with extracorporeal circulation, (2) gene manipulation on the porcine vascular endothelial cells, (3) inhibition of the inflammatory mediators released from the porcine endothelial cells, (4) the reduction of platelet action using drug like cyclophosphamide. In the method which uses a transgenic pig production technique, the inhibition of NF-$_K$B with I_K and RelA, the transfection of anti-apoptotic genes such as A20, bcl-2, bcl-X_L,[29] and other genes including genes for hemoxygenase 1 (HO-1), thrombomodulin, ATPDase[30,31] have been used. These gene-manipulated animals can become available for transplant within two years.

Cell mediated rejection

After controlling hyperacute rejection and acute vascular rejection, the xenoorgan graft maintains its function. This phenomenon is called "Accommodation". Accommodation is a phenomenon, where vascular endothelial cells show resistance to an antibody mediated injury, even though the XNA level is restored to the original level within 1-3 weeks after removing the XNA prior to xenotransplantation.[32,33] If the mechanism of accommodation can be determined, it will be a great help in improving xenotransplantation. However, the mechanism is not well known, except that hemoxygenase-1 plays a role as a 'protective gene' based on animal experiments. After accommodation occurs once, the xenograft can undergo cell-mediated rejection. Both natural killer (NK) cells and T-lymphocyte participate in cell-mediated rejection. The NK cells exhibit cytotoxicity to vascular endothelial cells, while the T-lymphocyte are presented with xenoantigens directly or indirectly and induce a more severe cell mediated rejection than that observed with an allotransplantation.[34]

Unlike human beings, porcine vascular endothelial cells constitutively express CD86, which is one of the major costimulatory molecules.[35] In addition, human T-lymphocytes are presented with porcine endothelial cells directly or indirectly. The causes of the more severe cell medi-

ated rejection in xenotransplantation are explained by the following: (1) the type of xenoantigen varies, (2) substances such as heparan sulfate is released from the endothelial cells after the xenoantigen challenge, and stimulate the antigen presenting cells (APCs), (3) all types of substances and molecules released from the endothelium activate the T-lymphocytes and promote T-lymphocyte inflow into the xenoorgan graft, (4) a immune regulatory reaction does not occur between the host and the xenograft because of a discrepancy in the molecular structure, which functions to control of this immune reaction, is insufficient, (5) NK cell activation occurs because surface lectin of the NK cell can recognizes the Gal epitope of the xenograft, while the receptor that inhibits the recognition of MHC class I of NK cells can not inhibit the recognition of xenogeneic MHC class I, (6) cell mediated rejection in the xenotransplant after antibody mediated injury, occurs in the state where various inflammatory mediated cells have already flowed in. The mechanism or treatment of cell-mediated rejection is not well known. Some methods to remove SLA, which is the MHC of pigs, have been attempted.

Chronic rejection

Until now, chronic rejection in xenotransplantation is virtually unknown. It appears, however, that chronic rejection is likely to occur following pig organ transplantation in a primate unless every immune rejection has been overcome.

Non-immunologic problems of xenotransplantation

When xenotransplantation of the heart was accomplished, the mean survival of dogs with the xenotransplanted heart was 103 days, and the longest survival time was 250 days. The first human xenotransplant recipient survived 18 days. Currently, the longest survival rate of xenotransplantation using pigs is 78 days and the human recipient of xenotransplanted heart, baby Fae, survived for 20 days. Therefore, the success rate of xenotransplantation is much lower than that of allotransplantation. It is believed that xenotransplantation can be used as a bridge transplantation until an allotransplant can be found, and there needs to be a treatment for reversible graft failure. However, the xenoorgan supply using pigs itself is not available due to the requirement for expensive equipment, technologies such as gene manipulation, and sterile facilities.

Table 1. Incompatibilities between Human and Pig

Anatomical incompatibility
Physiological incompatibility
• Regulation of blood circulation
• Respiration
• Rheology
• Hormon system
Immunological incompatibility
Microbiology and Infection
• Zoonosis
• Porcine endogenous retroviruses
• Prions: transmissible spongeforn encephalopathy
Molecular incompatibility
• Protein metabolism
• Receptors
Pharmacological incompatibility

Besides, systems other than an immune system have a considerable difference between humans and pigs.[36] Compared with allotransplantation, it is assumed that there are considerable physiological differences, e.g. the blood flow direction, a hormone system, protein metabolism or drug response according to the discrepancy in the molecular structures such as enzymes and receptors, etc. as well as anatomical differences (Table 1).[36] Finally, a pig also has human pathogens, bacteria, fungi, parasites, virus, etc. In particular, viruses have a rapid self-modification capacity, for which is no adequate remedy. It was demonstrated that the porcine endogenous retrovirus (PERV) can also infect humans.[37]

The current status of studying in Korea

Koreans have a culture of Confucianism, which makes it difficult to Koreans to accept cadaveric donations. Consequently, there are few organ donors in Korea. Organ transplantation will become increasingly dependent on xenotransplantation, as the nuclear family becomes more common. In particular at the acute phase of life threatening serious organ function failure, the best way

is to replace a failed organ with a xenogenic organ transiently, until an allotransplantat becomes available.

In Korea, the scientific infrastructure, which can realize xenotransplantation in clinical fields, has advanced considerably. In 1996, the first born of a transformed cow at the Korea Research Institute of Bioscience and Biotechnology (KRIBB) initiated the study of xenotransplants in Korea. Since then, transgenic goats and pigs were born in 1998. A Korean research group succeeded in producing the somatic cloning animals in 1999, the fifth such result in the world. In 2002, world's second transformed pigs that express green fluorescent protein (GFP) were successfully produced.[38] It is expected that α-Gal knockout pigs will also be produced in the near future and will be able to supply organs for xenotransplantation in great numbers.[39]

FUTURE

Xenogeneic organ transplantation

In order to solve the immunological problems associated with xenotransplantation, (1) the establishment and application of "accommodation", and (2) insurance of the technology-producing knock out pigs, needs to be settled.[40] Regarding pigs, the technique for establishing embryonic cells with the germline transmission is possible. Therefore, there is the possibility of using stem cells to this field.

Xenogeneic cell transplantation

While there are problems with xenogeneic organ transplantation, xenogeneic cell transplantation is not invasive, induces immune reactions, which are less severe than the case of a xenogeneic organ, and there is no need to remove the graft cells because of cell death, even if rejection occurs. Moreover, it is easy to manipulate the required genes, and it is easy to amplify the cells. Therefore, they are easier to apply clinically than xenoorgan transplantation. Indeed, in 1995 an AIDS patient, J. Getty recovered his immune function after a transfusion of baboon's lymphocytes.[1,41] Since then, the xenotransplantation technologies using xenogenic cell lines such as myocardial cells for myocardial infarction patients, hepatocytes for familiar hypercholesterolemia patients, insulin secreting cells for diabetes, dopaminergic neuron cells for Parkinson's disease patients and retinal cells for retinal degeneration patients, are under development.[42-44] On the other hand, tissue engineering technology using xenogenic cells are also under development.

Xenogenic cells, tissue, organs have considerable differences from those of humans. In particular, cell mediated rejection or discordance of molecule/protein cannot be prevented completely, although Gal-(1,3)-Gal can be perfectly removed.[45,46] These problems leave room for an up-to-date-technology such as cloning, and the utilization of germ cells.[47] Aspects of bioethics will need to be considered before these technologies can be implemented.

REFERENCES

1. Taniguchi S, Cooper DKC. Clinical xenotransplantation: past, present and future. Ann R Coll Surg Engl 1997; 79:13-9.
2. Auchincloss H Jr. Xenogeneic transplantation. Transplantation 1988;46:1-20.
3. Reemtsma K, McCrackern BH, Schlegel JU, Pearl MA, Pearl CW, Dewitt CW, et al. Renal heterotransplantation in man. Ann Surg 1964;100:384-410.
4. Hardy JD, Kurrus FD, Chavez CM, Neely WA, Eraslan S, Turner MD, Fabian LW, Labecki TD. Heart transplantation in man. Developmental studies and report of a case. JAMA 1964:188:1132-40.
5. Hardy JD, Chavez CM. The first heart transplantation in man. Developmental animal investigations with analysis of the 1964 case in the light of current clinical experience. Am J Cardiol 1968;22:772-81.
6. Bailey LL, Nehlsen-Cannarella SL, Concepcion W, Jolley WB. Baboon-to-human cardiac xenotransplantation in a neonate. JAMA 1985;254:3321-9.
7. Walpoth BH, Nehlsen-Cannarella SL, Bailey LL. Xenotransplantation: extended indications for pediatric cardiac transplantation. Transplant Proc 1986;18:43.
8. Kissmeyer-Nielson F, Olsen S, Peterson VP, Fjeldborg O. Hyperacute rejection of kidney allografts, associated with pre-existing humoral antibodies against donor cells. Lancet 1966;2:662-5.
9. Douglas JD. Suitability and availability of chimpanzees as organ donors for man. Transplant Proc 1970;2:539.
10. Woodruff M. Summary. Transplant Proc 1970;2:557.

11. Kobayashi T, Cooper DKC. Anti-Gal, α-Gal epitopes and xenotransplantation. In: Galili U, Avila JL, editors. α-Gal and Anti-Gal: α-1,3-Galactosyltransferase. α-Gal Eiptopes and the Natural Anti-Gal antibody (Subcellular Biochemistry Series), vol. 32. New York, London: Kluwer Aacademic/Plenum; 1999. p.229-57.
12. Pruitt SK, Baldwin III WM, Barth RN, Sanfilippo F. The effect of xenoreactive antibody and B cell depletion on hyperacute rejection of guinea pig-to rat cardiac xenografts. Transplantaion 1993;56:1318-24.
13. Atkinson JP, Odlesby TJ, White OJG, Adams EA, Liszewski MK. Separation of self from non-self in the complement system. A role for membrane cofactor protein and decay accelerating factor. Clin Exp Immunol 1991;86:27-30.
14. Lachman P. The control of homologous lysis. Immunol Today 1991;12:312-5.
15. Fodor WL, Willians BL, Matis LA, Madri JA, Rollins SA, Knight JW, et al. Expression of a functional human complement inhibitor in a transgenic pig as a model for the prevention of xenogeneicn hyperacute organ rejection. Proc Natl Acad Sci USA 1994;91:11153-7.
16. McCurry KR, Kooyman DL, Alvarado CG, Cotterell AH, Martin MJ, Logan JS, et al. Human complement regulatory protein protect swine-to-primate cardiac xenografts from humoral injury. Nat Med 1995;1:423-7.
17. Leventhal JR, John R, Fryer JP, Witson JC, Derlich JM, Remiszewski J, et al. Removal of baboon and human antiporcine IgG and IgM natural antibodies by immunoadsorption: Results of *in vitro* and *in vivo* studies. Transplantation 1995;59:294-300.
18. Rydberg L, Hallberg E, Bjorck S, Cairns TDH, Groth C-G. Studies on the removal of anti-pig xenoantibodies in the human by plasmapheresis/immunoadsorption. Xenotransplantation 1995;2:253-63.
19. Taniguchi S, Neethling FA, Korchargina EY, Bovin N, Ye Y, Kobayashi T, et al. *In vivo* immunoadsorption of antipig antibodies in baboons using a specific Gal α1,3 Gal column. Transplantation 1996;62:1379-84.
20. Latinne D, Soares M, Havaux X, Cormont F, Lesnikoski B, Bach FH, et al. Depletion of IgM xenoreactive natural antibodies by injection of anti-mu monoclonal antibodies. Immunol Rev 1994;141:95-125.
21. Simon PM, Neethling FA, Taniguchi S, Goode PL, Zopf D, Hancock WW, et al. Intravenous infusion of Gal alpha 1-3 Gal oligosaccarides inbaboone delays hyperacute rejection of porcine heart xenograft. Transplantation 1998;65:346-53.
22. Sharma A, Okare J, Birch P, McClellan SB, Martin MJ, Platt JL, et al. Reduction in the level of Gal (alpha 1, 3) Gal in transgenic mice and pigs by the expression of an alpha (1,2) fucosyltransferase. Proc Natl Acad Sci USA 1996;93:7190-5.
23. Kobayashi T, Neethling FA, Koren E, Taniguchi S, Ye Y, Niekrasz M, et al. *In vitro* an *in vivo* investigation of anticomplement agents FUT-175 and K76COOH, in the prevention of hyperacute rejection following discordant xenotransplantion in a non-human primate model. Transplant Proc 1996;28:604.
24. Leventhal JR, Dalmasso AP, Cromwell JW, Platt JL, Manivel CJ, Bolman RM 3rd, et al. Prolongation of cardiac xenograft survival by depletion of complement. Transplantation 1993;55:857.
25. Pruitt SK, Bollinger RR, Colline BH, Marsh HC Jr, Levin JL, Rudolph AR, et al. Effect of continuous complement inhibition using soluble complement receptor type 1 on survival of pig-to-primate cardiac xenografts. Transplantation 1997;63:910-2.
26. Rollins SA, Matis LA, Springhorm JP, Setter E, Wolff DW. Monoclonal antibodies directed against human C5 and C8 flock complement-mediated damage of xenogeneic cells and organs. Transplantation 1995;60:1284-94.
27. Platt JL, Lin SS, McGregor CG. Acute vascular rejection. Xenotransplantation 1998;5:169-75.
28. Millan MT, Geczy C, Stuhlmeier KM, Goodman DJ, Feran C, Bach FH. Human monocytes activate porcine endothelial cells, resulting in increase E-selectin, interleukin-8, monocyte chemotactic protein-1 and plasminogen activator inhibitor-type I expression. Transplantation 1997;63:421-9.
29. Grimm S, Bauer MK, Baeuerle PA, Schulze-Osthoff K. Bcl-2 down-regulates the activity of transcription factor NF-κB induce upon apoptosis. J Cell Biol 1996;134:13-23.
30. Wrighton CJ, Kopp CW, McShea A, Vetr H, Bach FH. High-level expression of functional human thrombomodulin in cultured porcine aortic endothelial cells. Transplant Proc 1995;27:288-9.
31. Bach FH. Xenotransplantation: Problems and prospects. Annu Rev Med 1998;49:301-10.
32. Lin SS, Hanaway MJ, Gonzalez-Stawinski GV, Lau CL, Parker W, Davis RD, et al. The role of anti-Gal α1-3 Gal antibodies in acute vascular rejection and accommodation of xenografts. Transplantation 2000;70:1667-74.
33. Lin Y, Vandeputle M, Waer M. Accommodation and T-independent B cell tolerance in rats with long trem surviving hamster heart xenografts. J Immunol 1998; 160:369-75.
34. Sachs DH, Sablinski T. Tolerance across discordant xenogeneic barriers. Xenotransplantation 1995;2:234-9.
35. Maher SE, Karmann K, Min W, Hughes CC, Pober JS, Bathwell AL. Pocine endothelial CD86 is a major costimulator of xenogeneic cells human T cells: cloning, sequencing and functional expression in human endothelial cells. J Immunol 1996;157:3838.
36. Hammer C. Evolution: its complexity and inpact on xenotransplantation. In: Cooper DKC, Kemp E, Platt JL, White DJG, editors. 2nd ed. Heidelberg: Spinger; 1997. p.716-35.
37. Patience C, Takeuchi Y, Weiss RA. Infection of human cells by an endogenous retrovirus of pigs. Nat Med 1997;3:282-6.
38. Hyun S, Lee G, Kim D, Kim H, Lee S, Nam D, et al. Production of nuclear transfer-derived piglets using porcine fetal fibroblasts transfected with the enhanced

green fluorescent protein. Biol Reprod 2003;69:1060-8.
39. Phelps CJ, Koike C, Vaught TD, Boone J, Wells KD, Chen SH, et al. Production of alpha 1,3-galactosyltransferase-deficient pigs. Science 2003;299:411-4.
40. Bach FH. Genetic engineering as an approach to xenotransplantation. World J Surg 1997;913-6.
41. Platt JL. Immunobiology of xenotransplantation. Transplant Int 2000;13:7-10.
42. Cooper DKC. Xenotransplantation-will it happen? J Circ Support 1998;1:3-12.
43. Groth CG, Tibell A, Wennberg L, Bennet W, Lundgren T, Rydgard KJ, et al. Clinical aspect and perspectives in islet cell xenotransplantation. J Hepatobiliary Pancreat Surg 2000;7:364-9.
44. Hancock WW. The past, present and future of renal transplantation. Kidney Int 1997;51:932-44.
45. Lanza RP, Cooper DKC, Chick WL. Xenotransplantation. Scientific America; 1997. p.54-9.
46. Logan JS. Prospects for xenotransplantation. Curr Opin Immunol 2000;12:563-8.
47. Platt JL, Lin SS. The future promises of xenotransplantation. Ann NY Acad Sci 1998;862:5-18.