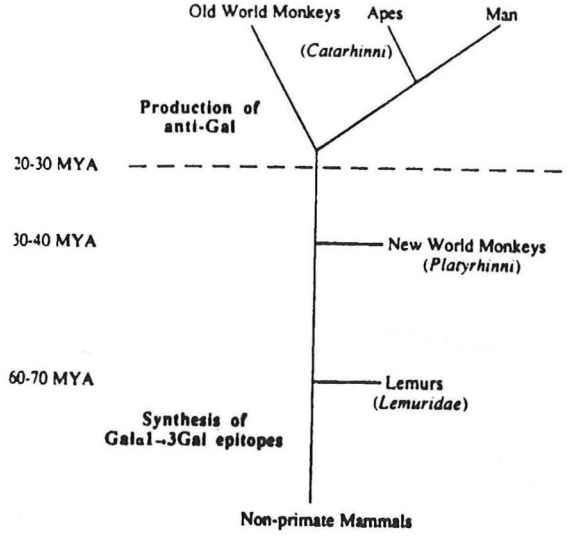

XENOTRANSPLANTATION

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INTRODUCTION

Humans currently receive organs from other humans, usually cadavers. Such transplants are "**allo**grafts" where donor and recipient are of the same species. Unfortunately, most accidents and illnesses causing death also damage kidneys, hearts, livers, and pancreases, making them unsuitable for transplantation. The number of donors is further diminished by the reluctance of relatives to allow donation, or the discovery of AIDS or hepatitis in the potential donor. Thus, the number of patients waiting for kidney transplantations in the U.S. far exceeds the number of available cadaveric organs. It is not uncommon to wait well over a year for a transplant. This wait may be fatal, particularly for patients awaiting a heart or liver transplant (1). Kidney transplantation is not a life-saving procedure.

Transplantation of organs from a different species would, at one stroke, alleviate the organ shortage. It would save the lives of the 2,000 plus patient who died waiting for a life-saving heart or liver transplant. It would decrease the morbidity and improve the quality of life for the 20,000 plus American patients waiting for a kidney transplant. Xenotransplantation is a real possibility because we can build upon the work of Pillemar and his discovery of the alternative pathway of complement, the work of Galili in defining the alpha-galactosyl residue as a major target of human natural antibodies, and the demonstration by Alexandre that transplantation can be performed across ABO incompatibilities.

The purpose of this manuscript is to review the distinctive features of rejection of discordant xenograft solid organs. I focus on the transplantation of swine organs into non-human primate recipients because the non-human primate models potential human patients. Other species combinations are discussed only to illustrate particular principles. I consider only solid organs such as kidney, heart, and liver where the vasculature is xenograft in origin, as opposed to the transplantation of skin, pancreatic islets, or bone marrow where the vasculature is provided by the host. In solid organ transplants, the xenograft endothelium is a major site of interaction between the host immune system and the xenograft. The number of references in this review has been limited by editorial policy. I apologize to any investigators who we have not been able to cite. The reader is referred to several excellent recent reviews throughout our text.

Major points will be the importance of complement and natural antibodies in "natural" immunity, the potential application of genetic engineering of swine donors to prevent complement mediated damage or to eliminate the target of natural antibodies, the active participation of xenograft endothelium in the rejection process, and unique features of acquired cellular immunity caused by interspecies signalling of cytokines and cell-surface molecules.

Transplantation of organs from non-human species ("**xeno**grafts") would eliminate the shortage of cadaveric organ. When chimpanzee or baboon organs are transplanted into humans, rejection may be controlled successfully using immunosuppressive therapy currently available to prevent rejection of allografts between humans (2,3). Transplants between such closely related species are "**concordant**"[1] xenografts (4,5). However, the widespread use of non-primate organs for human transplantation is not practical. The necessary number of organs are not available because non-human primates have single births and a long gestation period. There is also opposition to the use of non-human primates for ethical considerations.

TYPES OF TRANSPLANTS

ALLOGRAFT - TRANSPLANT BETWEEN INDIVIDUALS OF THE SAME SPECIES

XENOGRAFT - TRANSPLANT BETWEEN INDIVIDUALS OF DIFFERENT SPECIES

CONCORDANT - BETWEEN CLOSELY RELATED SPECIES - MECHANISMS OF IMMUNOLOGIC REJECTION ARE SIMILAR TO REJECTION OF ALLOGRAFTS

EXAMPLE = BABOON TO HUMAN

DISCORDANT - BETWEEN DISTANTLY RELATED SPECIES: SWINE TO HUMAN

ACUTE FULMINANT REJECTION DUE TO NATURAL IMMUNITY

COMPLEMENT ACTIVATION DUE TO FAILURE OF INHIBITORS OF C3B (ALTERNATIVE PATHWAY ACTIVATION)

COMPLEMENT ACTIVATION BY NATURAL ANTIBODIES

(SIMILAR TO HYPERACUTE REJECTIONS SEEN WHEN TRANSPLANTS MISTAKENLY OCCUR ACROSS ABO BARRIERS)

POTENTIALLY DIFFERENT MECHANISMS OF ACQUIRED IMMUNITY

Therefore, xenotransplantation will not be a practical solution to the current shortage of organs until the immunologic barriers preventing transplantation of organs from animals distantly related from humans. Swine, for example, may be ideal donors. They breed rapidly and may be amenable to genetic engineering to produce desired traits. Although there are no long term studies, there is reason to believe that swine organs would function appropriately in humans (6). Unfortunately, xenotransplantation of swine organs into untreated primates results in immediate fulminant rejection within minutes. Xenografts between species where such immediate fulminant rejection occurs are defined as "**discordant**" (4). "**Natural immunity**" involving "**natural antibodies**" and complement occurs. The term "natural immunity" is used because rejection occurs in the absence of prior exposure of the recipient to donor antigens. Such rejection cannot be satisfactorily controlled with currently available immunosuppressive agents.

I. Immediate rejection of discordant xenografts is mediated by activation of the complement system either via the alternative or via classical pathways depending upon the particular species combination of the xenograft.

The most distinctive feature of the rejection of discordant xenograft kidneys or hearts is their rapid destruction within minutes of transplantation. Such rapid rejection occurs in recipients not previously exposed or immunized against the donor species. Such immediate destruction of the xenograft results from activation of complement. Exactly how the complement is activated depends upon the particular species combination of the xenograft and recipient. Porcine xenografts placed into primates are destroyed by antibody-dependent complement activation. In contrast, activation via the alternative pathway occurs in other discordant species combinations: for example, guinea pig organs into rats. See Table 1.

[1]Leventhal and Matas (1994) have recently reclassified xenotransplantation according to the known mechanisms involved. We use the concordant versus discordant classification because it is currently the most widespread in the literature.

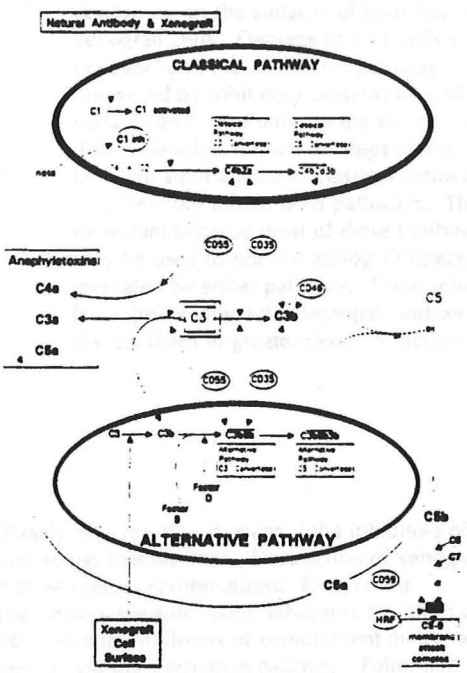
TABLE 1: Natural and Acquired Immunity against Allografts. Concordant vs. Discordant Xenografts:

			Natural Immunity		Acquired Immunity	
			Complement Activation			
host	donor	class	alternative	classical (natural antibody)	antibody	cellular
human	human (ABO compatible)	allograft	no	no	yes	yes
human	human (ABO incompatible)	allograft	no	yes	yes	yes
human	baboon, chimpanzee	concordant xenograft	no	no	yes	yes
human	pig	discordant xenograft	no	yes	yes	yes
rat	guinea pig	discordant xenograft	yes	yes	yes	yes
human	rabbit	discordant xenograft	yes	yes	yes	yes

I.A. Complement Overview.

SLIDE 4: A SIMPLIFIED OVERVIEW OF THE COMPLEMENT SYSTEM

Given the importance of complement in xenograft destruction, and the potential of genetic engineering of complement components to prevent immediate xenograft destruction, a brief discussion of complement is appropriate. For a detailed discussion, see review (7)



The critical regulatory component of complement is the serum protein C3. Ordinarily C3 exists in an dormant state. After proteolytic cleavage, C3 is activated and initiates the destructive effector mechanisms of complement. Thus, one may think of the complement system as C3 plus five groups of proteins: those which recognize the target and activate C3, those which are the effector mechanisms resulting from C3 activation, and those which regulate C3 activation to prevent excessive activation of C3 and damage to host tissues.

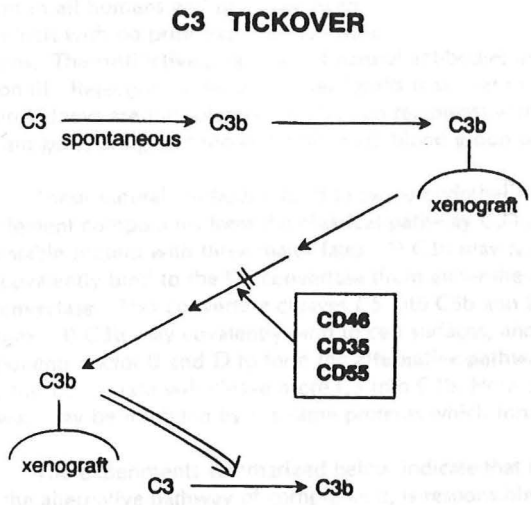
Targets to be attacked by complement are recognized by the **first** or **second** groups of proteins: antibody via the **classical** pathway or the **alternative** pathway (discussed in greated detail below). In either case C3 is converted to C3b. C3b then participates in the activation of the **third** group of proteins (C5-9). These form the membrane attack complex which lyses the target microbes, tumors, or xenograft cells. In the course of these chemical reactions, a **fourth** group of proteins are activated. These are the anaphylatoxins (C4a, C3a, and C5a). These are chemoattractants which recruit inflammatory cells into the xenograft. The anaphylatoxins also activate inflammatory cells and cause them to become adherent to endothelium, tumor and xenograft cells, to release reactive oxygen metabolites which damage microbes

and xenograft cells, and to secrete cytokines, and biologically active arachidonic acid metabolites (eg: prostaglandins, leukotrienes, and lipoxins).

These cytokines and arachidonic acid metabolites further activate inflammatory cells and endothelium thus amplifying the response. A **fifth** group of inhibitory proteins prevent excessive activation of complement from damaging host cells. These inhibitory proteins are particularly important because they may be used therapeutically to prevent xenograft rejection. They will be reviewed in greater detail below.

I.B. Failure of inhibitors of complement to act across species lines may result in immediate xenograft destruction via the alternative complement pathway.

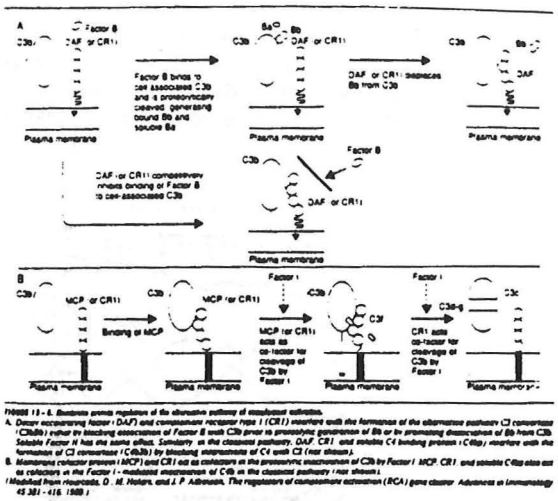
In some species combinations, destruction of the xenograft occurs via the alternative pathway in the absence of antibody (8,9). Complement activation via the alternative pathway occurs because C3 has an unstable thioester such that a small amount of C3 is continuously 'activated' to C3b (**"C3 tickover"**). This C3b binds to repetitive hydroxyl and amine groups on cell surfaces. The cell-surface C3b forms a C3 convertase (C3bBbP) by binding Factors B, D, and Properdin (P). The C3 convertase activates more C3 in a positive amplification loop, activates C5 to form C5a anaphylatoxin which recruits and activates inflammatory cells, and initiates construction of the membrane attack complex $(C5b678(9)_n$ which lyses the target cell.



"C3 tickover" results in C3b deposition on the surfaces of both host and xenograft cells. Damage to host cells via "C3 tickover" and the alternative pathway is prevented by inhibitory proteins on cell surfaces and in solution in the serum. Note that these inhibitors act on steps common to both the alternative and classical pathways, and therefore inhibit both pathways. This is important because most of these inhibitors may be used to prevent xenograft rejection mediated by either pathway. These inhibitors have great therapeutic potential, and we discuss them in greater detail in section I.D.

In contrast to host cells, deposition of C3b on xenograft cells may result in damage if the inhibitors of complement on the xenograft cell surface do not function across species lines. Destruction of xenografts via the alternative complement pathway then occurs in three species combinations: First, rabbit erythrocytes are lysed by human alternative pathway, presumably because rabbit inhibitors on rabbit cell surfaces do not work on human complement proteins (8). Second, inhibitors of complement on guinea pig endothelial cells do not prevent activation of rat complement via the alternative pathway. Fulminant rejection of guinea pig hearts occurs within minutes of transplantation into rats despite the removal of rat anti-guinea pig natural antibodies by a combination of plasmaphoresis, 15-deoxyspergualin, and splenectomy. Immunohistochemical analysis reveals the deposition of complement components but little immunoglobulin. See review (10). Third, the alternative pathway causes rejection of rabbit cardiac

xenografts in newborn piglets suckled on rabbit milk (11). There are no anti-rabbit natural antibodies in these piglets because all antibody in the circulation is rabbit antibody which was in the rabbit milk and absorbed by the piglet gastrointestinal tract. Maternal porcine antibody does not traverse the placenta, and the immature piglet B-cells do not make antibody.



I.C. Immediate destruction of swine-to-primate xenografts is caused by complement activation via the classical pathway by "natural antibodies."

There is a substantial body of evidence (see reviews (12,13) supporting the hypothesis that primate antibodies recognize the endothelium of the swine xenograft and trigger rapid fulminant rejection by activating complement by the classic pathway. Such antibodies are "natural" in that they are present in all humans and primates, even individuals with no prior exposure to swine antigens. The distinctive properties of natural antibodies and the B-cells making them are discussed in Section III. Rejection of discordant xenografts is similar to the "hyperacute" rejection occurring when human kidneys are transplanted into human recipients with pre-existing antibodies against either donor HLA antigens, or against incompatible ABO blood group oligosaccharides on allograft endothelium.

These natural antibodies bind to swine endothelium and activate C1, C4, and C2. These activated complement components form the **classical pathway C3 convertase** which "converts" C3 to C3b. C3b is an unstable protein with three major fates: 1) C3b may react with inhibitors and be inactivated. 2) C3b may covalently bind to the C3 convertase (from either the classical or alternative pathways) to form a **C5 convertase.** This convertase cleaves C5 into C5b and thus initiates formation of the membrane attack complex. 3) C3b may covalently bind to cell surfaces, and then interact with alternative pathway components Factor B and D to form the **alternative pathway C3 convertase.** In a positive amplification loop, this convertase will cleave more C3 into C3b. Note that complement activation via the classical pathway may be inhibited by the same proteins which inhibit the alternative pathway.

The experiments summarized below indicate that natural antibody via the classical pathway, rather than the alternative pathway of complement, is responsible for acute rejection of swine organs by primates (14,15).

1) Immunohistochemical data. The immunopathology of swine to primate heart transplants show the associated localization of primate immunoglobulin and classical, but not alternative pathway [factor B and properdin], complement components deposited along the endothelium of the swine heart xenograft (16-19).

2) The administration of "natural antibody" promotes the rejection of xenografts (20).

3) Human and Rhesus complement do not damage swine endothelial cells via the alternative pathway in vitro. Natural anti-porcine antibody must be present to activate complement via the classical pathway (13,21).

4) Removing natural anti-porcine antibodies by either plasmaphoresis or ex vivo perfusion of the blood through porcine heart, liver, and/or kidney prolongs swine-to-primate heart and kidney xenografts from minutes to many days (18,22-25). In the former treatment, the red and white blood cells and platelets are isolated and returned to the host. All other blood elements including the natural antibodies are discarded. In the latter treatment, anti-porcine antibodies are removed by pumping the entire blood volume of a primate through the vasculature of swine organs ex vivo and then returning the blood to the primate. The "natural antibodies" adhere to the endothelium of the ex vivo swine organs and are removed.

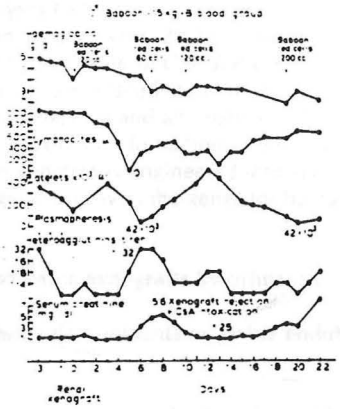


Fig. 3. Minipig-to-baboon renal xenograft. Normal renal function up to the 6th postoperative day at which time a rejection crisis occurred. Complete reversal of the rejection crisis. Occurrence of a second irreversible rejection and sacrifice of the animal on the 22nd day.

Both plasmaphoresis and ex vivo perfusion remove complement in addition to natural antibodies. However, serum immunoglobulin concentrations are depleted for a much longer period of time than complement components (26). Thus, after primates receive a series of plasmaphoresis treatments, serum complement levels will return to normal before natural antibody reappears. At this time there is no rejection, suggesting that complement via the alternative pathway does not cause xenograft rejection (21).

Plasmaphoresis and ex vivo perfusion have limited practical clinical application. Neither technique can be continued indefinitely, and rejection of the xenograft often occurs after "natural antibodies" return to the circulation. By removing all immunoglobulin and complement, these techniques cause profound immunosuppression and make the host susceptible to infection.

I.D. Immediate rejection of discordant xenografts. Therapeutic interventions via genetic engineering of inhibitors of complement.

As discussed above, activation of the complement pathway is important in the immediate destruction of discordant xenografts irrespective of whether complement is triggered by the classical or alternative pathways. Therapeutic use of naturally occurring inhibitors of complement, produced by genetic engineering, may eventually prevent immediate destruction of such xenografts. Such inhibitors may ordinarily not prevent immediate xenograft destruction by complement either because the local concentration of inhibitors in the xenograft is not sufficiently high, or because the inhibitors produced by the xenograft may not inhibit complement components of the recipient species.

One class of inhibitors are soluble and would be given by injection to xenograft recipients. One example is C1 inhibitor (C1 INH) which prevents the activation of C1 by natural antibody binding to porcine endothelium. C1 INH prevents damage to porcine endothelial cells by human natural antibody and complement in vitro (27). Another example is soluble complement receptor type 1 (sCR1 or CD35). This inhibitor causes the dissociation of the classical and alternative pathway C3 convertases. It prevents the activation of C3 to C3b (see Fig 3-5), and thus is a powerful inhibitor of both pathways of complement activation. It prolongs guinea pig xenografts in rats, where the alternative pathway is the major cause of rapid rejection (28). A major disadvantage of such soluble inhibitors is that they must be given

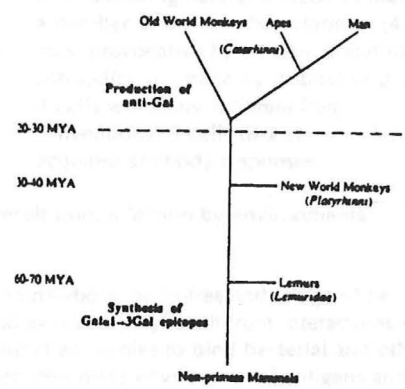
systemically, and, in addition to preventing complement-mediated xenograft destruction, would also inhibit appropriate destruction of infectious pathogens.

Another, even more powerful approach, is to genetically engineer pigs which have human inhibitors of complement on their cell surfaces. Such membrane bound inhibitors would only prevent complement-mediated distruction of the xenograft, and not affect complement activation elsewhere in the xenograft recipient. Three different membrane bound inhibitors may be considered: CD59, decay accelerating factor (DAF or CD55), and membrane cofactor protein (MCP or CD46). CD59 inhibits formation of the membrane attack complex (C5-9) which lyses target cells. Successful transfer of a functional human CD59 gene has been accomplished in in vitro systems (29,30). The disadvantage of using CD59 is that it does not prevent activation of C3 and production of C3a and C5a. These anaphylatoxins recruit leukocytes to the xenograft and activate them (see Figure 3). CD55 and CD46, on the otherhand, inhibit C3b, a central component in both the classical and alternative pathways. Inhibition of C3b would also prevent both the production of anaphylatoxins and formation of the membrane attack complex. See Figure 3. Both CD55 and CD46 have been genetically engineered into xenograft cells in vitro (31-33) and in transgenic mice (34). Recently, transgenic pigs with the genes for human inhibitors of complement have been produced (35).

III. Natural antibodies which mediate immediate rejection swine xenografts by primates.

III.A. "Natural antibodies" recognize oligosaccharide determinants on swine endothelial cell surfaces.

The most provocative hypothesis explaining the presence of natural anti-swine antibodies in humans, apes, and Old World monkeys is advanced by Galili (36). Approximately 28 million years ago, evolutionary pressure, possibly infections by pathogens containing α-galactosyl [Gal(α1-3)Gal(β1-4)GlcNAc-R] epitopes, caused the inactivation of the gene for α1,3 galactosyltransferase and prevented production of α-galactosyl epitopes. This allowed production of antibodies against pathogenic bacteria containing these epitopes. Several strains of E. coli, Salmonella, and Klebsiella do contain α-gal residues Incidentally, these anti-α-galactosyl antibodies also react against these sugars on swine cell surfaceS.

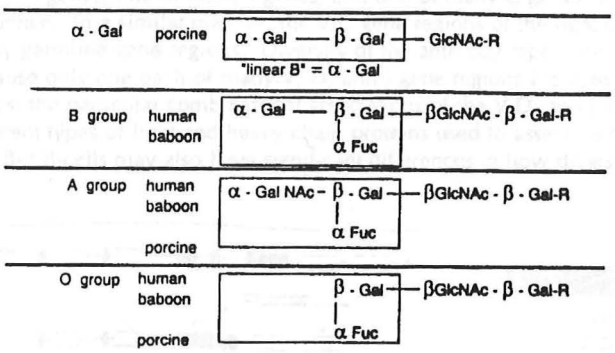


That "natural antibodies" from humans, apes, and Old World monkeys recognize oligosaccharide determinants, probably α-galactosyl residues, on swine endothelial cell surfaces has been confirmed by a number of different laboratories. There is, however, disagreement regarding the isotype of the "natural antibody" and the exact structure of lipid or protein component of the glycoconjugate. First, Galili et al (37) isolated "natural antibodies" from human sera by their binding to an immunoadsorbant column containing α-galactosyl residues. The purified IgG antibody bound to porcine endothelial and epithelial cells. Second, Platt et al (38) found that human and Rhesus sera contained "natural antibodies" which bound to 115 kd, 125 kd, and 135 kd glycoproteins (the gp 115/135 complex) isolated from porcine aortic endothelial cells. The antibodies bound oligosaccharides rather than the polypeptide cores of these glycoproteins. Enzymatic cleavage of N-linked oligosaccharides or subterminal β-D-gal residues abrogated antibody binding. The "natural antibodies" were of the IgM, instead of IgG, isotype. Their biologic

importance in xenograft rejection was supported by their removal when Rhesus serum was perfused through porcine kidneys. Third, Sandrin et al. (39) also found that natural antibodies bound α-galactosyl residues and were of the IgM isotype only. Fourth, Cooper et al. (40,41) eluted human antibodies bound to swine heart and kidney endothelia. These antibodies bound to α-galactosyl residues. However, the glycoproteins were 206 kd, 135 kd, and 55 kd instead of gp 115/135 (38). Furthermore, the isotypes of the "natural" antibodies included IgM, IgG, and IgA. The distribution of the α-galactosyl residues was localized, not only to the vascular endothelium, but also renal proximal tubules through the use of the anti-alpha-Gal isolectin B4 from Griffonia simplicifolia 1. Fourth, Sanfillipo (42) found both IgM and IgG2 natural antibodies with cross-reactivity between porcine endothelium and bacterial polysaccharides.

The identity of the antigen recognized by human "natural" anti-swine antibodies is of more than theoretical interest. Immunoabsorbant columns containing α-galactosyl oligosaccharides could be used to remove natural anti-swine antibodies (36,39-41) while allowing other antibodies to remain in the circulation and protect the patient from infection. α-galactosyl oligosaccharides could be injected intravenously so that natural antibody bound to the soluble oligosaccharides rather than the xenograft. As an example of this strategy in a different setting, injection of ABO blood group trisaccharides has been used to prevent rejection of kidney **allo**grafts across ABO blood group barriers (43-45). In the future, it may be possible to genetically engineer pigs without α-galactosyl epitopes on their cell surfaces. Organs from such pigs should not react with human natural antibodies (39).

A COMPARISON OF CARBOHYDRATE STRUCTURES



III.B. Special characteristics of the B-cells making natural antibodies.

Natural anti-swine antibodies belong to the large family of "natural" antibodies found in the sera of unimmunized individuals. These antibodies include anti-ABO blood group antibodies. A number of other natural antibodies have been discovered. Indeed, at any one time approximately 30% of B-cell clones in a normal individual may be making natural antibodies (46). The B-cells making natural antibodies may have a number of unique characteristics (47). The most provocative hypothesis is that natural antibodies are made by distinctive B-1 B cells which are different from conventional B-cells (B-2 cells) making acquired antibody responses.

One explanation for natural antibodies is that they result from infection by environmental pathogenic agents such as bowel flora.

However, some natural antibodies are present even in newborn, germ-free and antigen-free animals (46). A second hypothesis explaining natural antibodies is that they result from interactions of self-antigens with special fetal/neonatal B-cells. The ability of natural antibodies to bind bacterial and other environmental antigens is secondary to the cross-reactivity between these environmental antigens and self-antigens. According to this second hypothesis B-cells may be generated either from fetal tissues ("B-1" B-cells) or adult bone marrow ("B-2", or conventional, B-cells). B-1 precursors respond to antigens by becoming long-lived, self-replicating B-1 cells which make natural antibody and persist into adult life. B-2 precursors, in contrast, respond to antigen by becoming tolerant to the antigen - either by anergy or

apoptotic death (48).

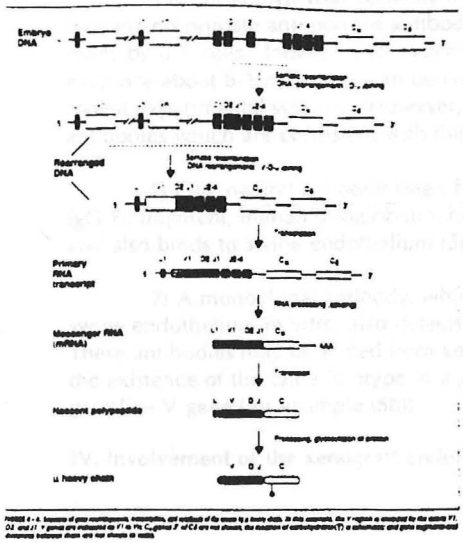
This difference may explain why hematopoietic chimerism between discordant species does not induce antibody tolerance while chimerism between concordant species does (49). In the former, B-1 cells interact with the discordant hematopoietic cells early in ontogeny and are stimulated to make natural antibody and become long-lived. In the latter case, B-2 cells are tolerized by the interactions. Whether B-1 and B-2 B-cells are separate lineages or the result of separate differentiation pathways is under debate (48,50,51).

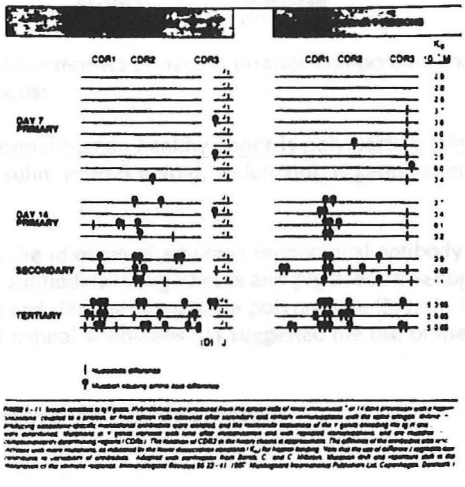
Table 1. Related ABO-Incompatible Living Donor Kidney Transplants From Mother With Splenectomy

Patient No	*Transplantation Date*	*Donor*	*Donor ABO*	*Recipient ABO*	*Prophylactic Immunosuppressive Therapy*	*Serum Creatinine and Follow-up (April 1991)*
1	06/30/82	Mother	A_1	0	ALG-Im-ster	1.35 mg%
2	11/11/82	Mother	A_1	0	ALG-Im-ster	1.00 mg%
3	11/17/82	Mother	A_1	0	ALG-Im-ster	1.26 mg%
4	12/08/82	Mother	B	0	ALG-Im-ster	RD 09/26/89
5*	11/16/83	Mother	B	0	CsA-ster	3rd graft: 1.48 mg%
6	05/09/84	Mother	A,B	0	ALG-CsA-ster	1.70 mg%
7	07/04/84	Mother	A_1	B	ALG-CsA-ster	Chronic rejection; received 2nd graft† ABO-incompatible
8	07/18/84	Mother	A_2	0	ALG-CsA-ster	2.06 mg%
9	12/05/84	Mother	A_1	0	ALG-Im-CsA-ster	Acute irreversible rejection 12/14/84
10	07/03/85	Mother	A_1	0	ALG-Im-CsA-ster	2 mg%
11	10/16/85	Mother	A,B	A_1	ALG-Im-CsA-ster	RD 12/31/90
12	07/16/86	Mother	B	0	ALG-Im-CsA-ster	1.37 mg%
13‡	5/13/87	Mother	A_2	0	ALG-Im-CsA-ster	0.8 mg%
14	07/08/87	Mother	A_1	0	ATG-Im-CsA-ster	1.06 mg%
15	12/23/87	Mother	B	0	ATG-Im-CsA-ster	1.36 mg%
16	05/18/88	Mother	A_2	0	ATG-Im-CsA-ster	1.05 mg%
17	06/03/88	Mother	B	A	ATG-Im-CsA-ster	1.09 mg%
18	06/16/88	Mother	B	0	ATG-Im-CsA-ster	1.51 mg%

Abbreviations: RD, return to dialysis; Im, Imuran; ster, steroids.
*Tertiary graft.
†Refer to Table 2, Patient No. 4.
‡Secondary graft.

The antigen-binding sites of natural antibodies made by B-1 cells is to a great extent determined by germline genes dictated by evolution, whereas the binding sites of conventional antibodies made by B-2 cells is, to a great extent, determined by somatic mutation. Antibody molecules are the products of two genes, one for the heavy chain and one for the light chain. In B-cells, the heavy chain gene consists of four gene regions in sequence - VDJC. A B-cell selects one of hundreds of V genes in the germline, one of many D genes, one of many J genes, and one of many C genes, and then joins them in the appropriate sequence. In a similar manner, the VJC gene regions of the light chain also result from rearrangements of many germline gene regions. Diversity of the antibody repertoire of both B-1 and B-2 B-cells occurs because only one each of many V, D, and J gene regions are used to construct the heavy or light chain genes; the particular combinatorial associations of the V,D, and J genes; and the combinations of the different types of light and heavy chain proteins used to assemble the antibody molecules. However, B-1 and B-2 B-cells may also have significant differences in how diversity in their antibody repertoire is





generated. Many somatic mutations occur in the V regions of B-2 cells during the course of an immune response. This allows for the selection of B-2 cells making antibody with greater and greater affinity for the antigen. Very few such somatic mutations occur in the V gene regions of B-1 cells. In B-2 cells, the enzyme terminal deoxyribonucleotidyl transferase (TdT) adds random nucleotides between the V and J, or V, D, and J regions before joining them together. This is another mechanism of creating diversity which does not often occur in B-1 cells. Finally, B-1 cells appear to preferentially use certain V gene regions. See reviews (48,50-53).

The low number of somatic mutations, the absence of TdT, and the preferential use of certain V gene regions have resulted in natural antibodies with properties determined to a large extent by the germline, and thus evolution. The natural antibodies are polyreactive by virtue of special properties of their variable regions (46). These regions of many natural antibodies contain large numbers of positively charged amino acids and amino acids with hydroxyl groups, thus allowing them to interact with antigens bearing repetitive negative charges such as DNA, and also carbohydrate structures of the cell membrane glycoproteins of discordant xenografts (48). Thus, one natural antibody may bind to a number of different antigens, including self-DNA and non-self-bacterial cell walls for example. Although most data from mice support the above hypothesis, antibodies made by CD5+ cells in normal humans may have the same number of somatic mutations as CD5- cells (54). The reconciliation of these results remains to be made.

There is controversy concerning the isotype of natural antibodies important in immediate rejection of porcine xenografts in primates. Although most natural antibodies are of the IgM isotype (15), B-1 cells are apparently influenced by T-cell cytokines. Some natural antibodies are of the IgA and IgG isotypes (46). This would be in line with studies showing that primate and human anti-porcine endothelial antibodies are of the IgG, IgM, and IgA isotypes (40,42).

NATURAL VS CONVENTIONAL ANTIBODIES: A CONTROVERSIAL AREA

	NATURAL ANTIBODY	CONVENTIONAL ANTIBODY
PRESENT W/O IMMUNIZATION	YES	NO
B CELL	B-1 CD5 SELF-REPLICATING	B-2
PREDOMINANT ISOTYPE	IGM	IGG AND OTHERS
LOW AFFINITY POLYREACTIVE	YES	NO
SOMATIC MUTATION	NO?	YES
N-REGION DIVERSITY	NO?	YES
SELF REACTIVE	YES	NO
EXAMPLES	ANTI-SWINE ABO	

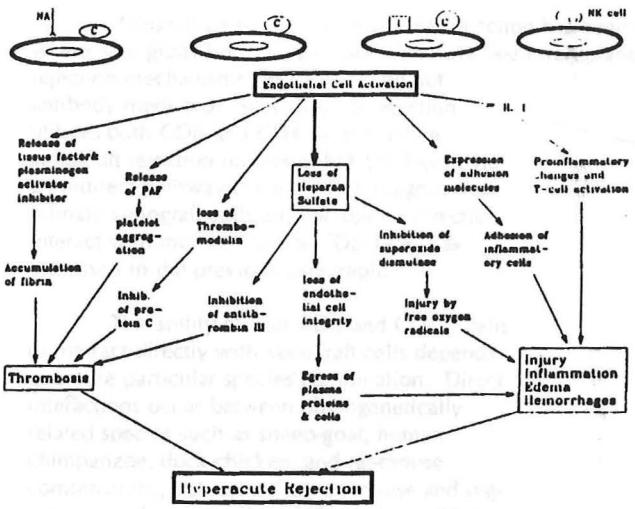
It is not known with certainty that natural human and primate anti-porcine antibodies are made by B-1 cells. Indeed, most experimental evidence about B-1 cells has been derived from rodent experimental systems. However, some characteristics of natural primate anti-porcine endothelial antibodies which are consistent with this hypothesis:

1) One natural antibody taken from a normal human healthy donor is polyreactive (binds ss DNA, IgG Fc fragment, human throglobulin, human insulin, tetanus toxoid, endotoxin), is germline encoded (55), and also binds to swine endothelium (56).

2) A monoclonal antibody, which binds the idiotype of a human monoclonal antibody recognizing swine endothelium in vitro, also detects natural antibodies in pig-Rhesus and pig-baboon xenografts. These antibodies may be eluted from xenografts and demonstrated to be polyreactive (57). In other studies the existence of the same idiotype in a group of natural antibodies has suggested the use of the same germline V gene (for example (58)).

IV. Involvement of the xenograft endothelium during the rejection process.

The porcine endothelium is not only a target of natural antibodies but is also an active participatant in and modulates the rejection process. The xenograft endothelium undergoes marked changes in its biology in response to the natural antibody and complement activation (59). Adhesion molecules appear on endothelial cell surfaces causing polymorphonuclear cells (PMN) and platelets to adhere to endothelium. PMN are further activated by activated complement components such as C5a, and then secrete superoxides and other products which damage endothelium. Heparan sulfates normally present on endothelial surfaces are lost. This has further consequences (60). The barrier function of endothelium to exudation of fluid and inflammatory cells is lost. Ordinarily some heparan sulfates bind and activate antithrombin III. Loss of heparan sulfate thus augments the deposition of fibrin associated with severe rejection. Heparan sulfate also binds extracellular superoxide dismutase to endothelial surfaces. Loss of heparan sulfate thus decreases anti-oxidant activity and increases the susceptibility of the tissues to oxidant-mediated injury. The released heparan sulfate may also augment antigen presenting activity and cellular rejection. The endothelial cells also release platelet activating factor, tumor necrosis factor alpha, and interleukin 1, which further augment the inflammatory process.



IV. Accommodation.

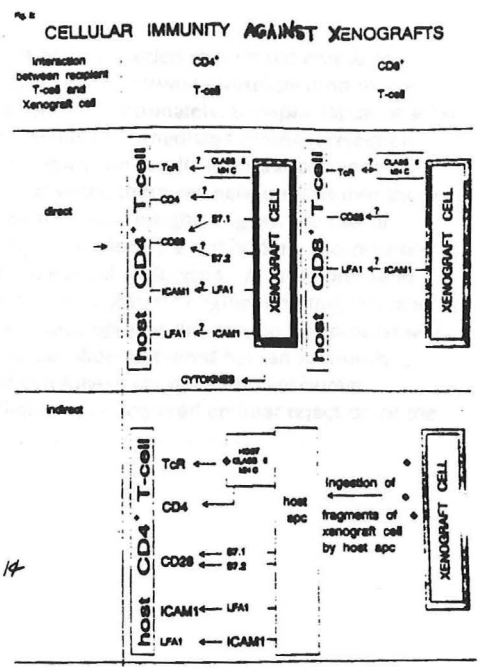
Bach and co-workers (59) have "accomodation" may allow a xenograft, or allograft, to survive if natural antibodies were temporary removed for several weeks immediately after transplantation. Graft survival would continue despite the return of anti-xenograft antibodies. "Accomodation" is not understood, but there is clear evidence that it occurs in certain specific instances. Fourteen of 18 human kidney allografts have survived across ABO incompatibility barriers (44). These were all parent to child transplants. The anti-blood group antibodies were removed by plasmaphoresis and neutralized by the infusion of blood group oligosaccharides. Although this therapy was stopped after three weeks, and the anti-AB blood group antibodies returned, there was excellent allograft survival. The natural antibodies against AB blood group carbohydrates on allograft endothelium in these patients may be similar to the natural antibodies against carbohydrates on xenograft endothelium. There is at least one report of a porcine heart xenograft surviving in a Rhesus monkey after plasmaphoresis, organ perfusion and quadruple immunosuppression; discontinuation of the therapy; and return of the natural antibody. The recipient was sacrificed with a functioning xenograft because of wound dehiscence. Pathology showed IgM deposition on the xenograft but no evidence of complement activation (24).

V. ACQUIRED IMMUNE RESPONSES AGAINST XENOGRAFTS

A. Special considerations in cellular immunity against xenografts.

Assuming that the immediate natural immunity against discordant xenografts are overcome, one would expect acquired immune responses to occur. However, cellular immunity against xenografts may be different than cellular immunity against allografts. As shown in Figure 2, cellular rejection of allografts requires that the host CD4 or CD8 T-cell interact with the allograft cell via a number of receptor - ligand pairs. This includes not only interactions between the T-cell receptor (TcR) and Class I or II MHC (Major Histocompatibility Complex antigen), but also interactions between CD28 on the T-cell and B7-1 and/or B7-2 on the allograft target cell; ICAM-1 and LFA-1; LFA-2 and LFA-3; and possibly other interactions as yet undescribed. In addition, the T-cell must interact with soluble cytokines, such as interleukin 1, released by the allograft target cell. These interactions may not occur across species. Thus, direct interactions between the xenograft target cell and the T-cell may not be possible, or may be impaired. Instead, there may be indirect interactions. Thus, host antigen-presenting cells may ingest molecules shed by the xenograft cell, and present these xenograft molecules to host CD4 T-cells via host Class II MHC.

Allograft versus xenograft cellular rejection has been most extensively studied in a model system where skin grafts from mice or primates have been transplanted onto mice. This is a system where the rejection mechanisms are cellular and not antibody mediated. Skin allograft rejection utilizes both CD8 and CD4 T-cells, while xenograft rejection utilizes only CD4 T-cells via an indirect pathway (61-63). This suggests that primate xenograft cells are not able to directly interact with murine CD4 or CD8 T-cells as discussed in the previous paragraph.



The ability of host CD8 and CD4 T-cells to interact directly with xenograft cells depends upon the particular species combination. Direct interactions occur between phylogenetically related species such as sheep-goat, human-chimpanzee, duck-chicken, and rat-mouse combinations, but not in human-mouse and pig-mouse combinations (64). Where direct CD4 T-cell to xenograft interactions do not occur, the site of the defect differs in different species combinations. For example, the inability of murine T-cells to interact directly with porcine xenograft cells cannot be restored by murine lymphokines (64). On the otherhand, human T-cell interactions with murine xenograft cells is restored by human lymphokines (65). There are few studies on the ability of human or primate T-cells to interact with porcine xenograft cells which is the clinically important xenograft combination (66,67). However, these studies do indicate that direct interactions are possible.

B. ACQUIRED (INDUCED) ANTIBODY RESPONSES.

Acquired antibody responses are characterized by switching of the immunoglobulin isotypes from IgM to IgG and by the production of large amounts of high affinity antibody. This occurs in rhesus monkeys previously sensitized to pig kidneys (16) indicating that such transplants are capable of inducing an acquired antibody response. Other investigations have been done in concordant xenograft combinations - hamster to rat, mouse to rat - to avoid confusion with natural antibody responses. The acquired antibody responses have been vigorous and not controllable with conventional immunosuppression. There is also evidence that, unlike natural antibodies, which are directed against oligosaccharides determinants of glycoproteins or glycolipid (see Section III), the acquired antibody responses are directed against MHC protein determinants - see review (63).

V. OTHER CONSIDERATIONS: NATURAL KILLER CELLS AND CHIMERISM.

Natural killer cells recognize and destroy target cells in the absence of prior sensitization. Their importance in xenograft rejection has not been extensively studied, but they may play a role (68).

Microchimerism may facilitate allograft survival (69-71). The mechanisms are not well understood. The effect of chimerism on discordant xenograft survival remains to be determined. The effect of chimerism on natural antibody production has not been extensively studied. In one model system, hematopoietic chimerism does not prevent rejection of xenografts (49).

VI. CONCLUSION.

Xenotransplantation would solve the critical shortage of organs needed to treat patients with endstage renal, cardiac, and hepatic disease. Practical and ethical issues prevent transplantation of non-human primate organs into humans. Swine might be ideal donors. Unfortunately, transplantation of solid organs between discordant species such as swine and humans results in immediate fulminant rejection within minutes to hours. Such rejection in the swine-human combination results from the presence of natural anti-swine antibodies in all humans. Over the last several years, there are new insights into these natural antibodies, their specificities, and the B-cells making them. These insights suggest the use of specific immunoabsorbant columns, injections of haptenic antigens to block the antibodies, and possibly genetic engineering of pigs to remove the antigenic targets of the natural antibodies. A more profound understanding of the regulation of complement has also suggested how genetic engineering might place inhibitors of human complement on the surfaces of pig cells and thus prevent destruction by complement activated by natural antibody. At present, there is little information about acquired human immunity against porcine xenografts. Xenograft adhesion molecules and cytokines may not stimulate human leukocytes. This would result in fundamentally different mechanisms of acquired cellular rejection of the xenograft as opposed to the allograft.

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