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Immunology

Anti-pig IgM antibodies in human serum react predominantly with Gal(α1-3)Gal epitopes

(xenoantigens/carbohydrate epitopes/transferase/transfection)

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ABSTRACT **A major problem with pig-to-human-tissue xenograft studies is that humans have natural antibodies to pig cells; these antibodies would cause hyperacute rejection if pig tissues were xenografted to humans. Here we show that most of human IgM antibodies present in the serum of healthy donors and reactive with pig cells react with galactose in an (α1-3) linkage with galactose—i.e., Gal(α1-3)Gal. Absorption studies demonstrated that the antibodies detected the same or similar epitopes on the surface of pig erythrocytes, blood and splenic lymphocytes, and aortic endothelial cells (EC). The antibodies were sensitive to 2-mercaptoethanol (2ME) treatment, did not bind to protein A or G, and were present in the high molecular weight fraction of serum; they are clearly IgM antibodies. Further, the antibodies did not react with human ABO blood group substances and are not related to human blood group A or B, which carry a terminal galactose. The reaction of human serum with pig erythrocytes was specifically inhibited by mono- and disaccharides: D-galactose, melibiose, stachyose, methyl-α-D-galactopyranoside, and D-galactosamine but not by D-glucose or methyl-β-D-galactopyranoside; demonstrating that the reaction is with galactose in an α and not a β linkage. A cDNA clone encoding the murine α-1,3-galactosyltransferase (which transfers a terminal galactose residue with an (α1-3) linkage to a subterminal galactose) was isolated by polymerase chain reaction (PCR), cloned, and transfected into COS cells, which are of Old World monkey origin and, like humans, do not express Gal(α1-3)Gal. After transfection, COS cells became strongly reactive with human serum and with IB4 lectin [which reacts only with Gal(α1-3)Gal]; this reactivity could be removed by absorption with pig erythrocytes. As most of the antibody reacting with pig cells can be removed by absorption with either melibiose or Gal(α1-3)Gal$^+$ COS cells, most of these react with Gal(α1-3)Gal. These findings provide the basis for genetic manipulation of the pig α-1,3-galactosyltransferase for future transplantation studies.**

The current shortage of tissues for transplantation has led to a reexamination of using xenografts (grafts from other species to human) as a possible source of organs (1, 2). However, when tissues from nonhuman species are grafted to humans, hyperacute rejection occurs within minutes because of the existence of natural antibodies in human serum that react with xenoantigens (1–4). If the target xenoantigens were identified, it should be possible to genetically manipulate the genes coding for the antigens to modify their structure and so avoid hyperacute rejection. In this light, a triad of glycoproteins of molecular masses 115, 125, and 135 kDa have been identified as the major targets on the surface of pig endothelial cells (EC) (5, 6). Other studies have shown that the binding of natural human IgM antibodies to endothelial xenoantigens was abrogated by digestion of the endothelial cell membranes with either N-glycosidases (which remove N-linked oligosaccharides or subterminal β-linked galactose), suggesting carbohydrate to be an important part of the xenoantigen (5). In contrast to the findings with IgM, normal human serum also contains IgG reactive with Gal(α1-3)Gal found on the erythrocytes (RBC) of all mammals except humans and Anthropoid apes (7–11). This epitope is carried on one of the major neutral glycosphingolipids of the RBC, ceramide pentahexoside, which is related to but distinct from human B blood group. Furthermore, it was shown that the Gal(α1-3)Gal epitope could also be found on the carbohydrate side chains of several serum proteins including mouse IgG and thyroglobulin (12).

The primary objective of our study was to examine the role of carbohydrates in the epitopes detected by natural IgM antibodies on pig cells and, in particular, the significance of the Gal(α1-3)Gal epitope. This was done by cloning the transferase DNA involved in the synthesis of the epitope and transfection experiments.

MATERIALS AND METHODS

Cells. Pig cells and tissues were obtained from the abattoirs; blood was centrifuged at 800 × *g*, and RBC were obtained and washed in phosphate-buffered saline (PBS); pig peripheral blood lymphocytes (PBL) were isolated using Isopaque–Ficoll (13); pig splenocytes were obtained by teasing tissue through a sieve. EC cultures were established after treatment of sterile pig aorta with collagenase type 4 (Worthington), and the isolated cells were grown in Dulbecco's modified Eagle's medium (DMEM) (ICN) on gelatin-coated plates at 37°C. The pig EC line PIEC was a gift from K. Welsh (Churchill, Oxford). COS cells were maintained in fully supplemented DMEM medium.

Serology. Samples were obtained from 10 normal volunteers and pooled. The monoclonal antibody (mAb) HuLy-m3 (CD48) was used as a negative control (13). Equal volumes of human serum and various concentrations of 2-mercaptoethanol (2ME) were incubated at 37°C for 1 hr to destroy IgM. Affinity-purified fluorescein isothiocyanate (FITC)-labeled sheep anti-human IgG and sheep anti-human IgM were obtained from Silenus Laboratories (Victoria, Australia). Pooled serum was absorbed with equal volumes of washed, packed pig cells for 15 min at 37°C and for 15 min at 4°C, serum was obtained, and the procedure was repeated twice. The human serum was absorbed with an equal volume of the following beads: (*i*) with melibiose-conjugated agarose (4% agarose; Sigma) or Sepharose 4B beads (4% agarose; Pharmacia LKB) at 37°C or (*ii*) with protein A-Sepharose or protein G-Sepharose beads (Pharmacia LKB) at 4°C. The following assays were used: (*i*) hemagglutination, in which 50

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Abbreviations: EC, endothelial cells; 2ME, 2-mercaptoethanol; PBL, peripheral blood lymphocytes; RBC, erythrocytes; FITC, fluorescein isothiocyanate; mAb, monoclonal antibody.

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μl of 1% pig RBC was added to 50 μl of human serum and incubated for a total of 2 hr at 37°C, room temperature, and on ice, after which hemagglutination was evaluated both macroscopically and microscopically; (*ii*) rosetting, in which sheep anti-human IgG was coupled to sheep RBC with chromic chloride (14); (*iii*) cytofluorographic analysis performed on FACScan (Becton Dickinson) (15); and (*iv*) indirect immunofluorescence, performed on cell monolayers in tissue culture plates with fluoresceinated immunopurified sheep anti-human IgM or IgG (15).

Sugar Inhibitions. Two types of sugar inhibition assays were performed. In one, 50 μl of different sugars (300 mM in PBS) was added to 50 μl of 1:1 dilutions of human serum in 96-well plates and incubated overnight at 4°C; then the hemagglutination assay was performed. In the other, human serum, diluted in PBS at one dilution less than that of the 50% hemagglutination titer, was added to 50 μl of 1:1 dilutions of sugars (starting at 300 mM) and incubated overnight at 4°C; then the hemagglutination assay performed.

Murine α-1,3-galactosyltransferase cDNA. A cDNA clone, encoding the mouse α-1,3-galactosyltransferase was produced by using the known sequence of this transferase (16) and the PCR technique. Two oligonucleotides were synthesized: αGT-1 (5′-GAATTCAAGCTTATGATCACTATGCTTCAAG-3′), which is the sense oligonucleotide encoding the first 6 amino acids of the mature α-1,3-galactosyltransferase and contains a *Hin*dIII restriction site; and αGT-2 (5′-GAATTCCTGCAGTCAGACATTATTCTAAC-3′), which is the antisense oligonucleotide encoding the last 5 amino acids of the mature α-1,3-galactosyltransferase and contains the in-phase termination codon and a *Pst* I restriction site. The oligonucleotides were used to amplify a 1185-bp fragment from a C57BL/6 spleen cell cDNA library (17), which was subsequently purified, digested with *Hin*dIII and *Pst* I (Pharmacia LKB) restriction endonucleases, and directionally cloned into *Hin*dIII/*Pst* I-digested CDM8 vector (18). A plasmid (pαGT-3) was selected for further studies, sequenced to confirm the correct DNA sequence, and used for COS cells transfection (15, 17, 18). The IB4 lectin of *Griffonia simplicifolia* was obtained from Sigma.

RESULTS

Human Anti-Pig Antibodies. Human serum contains anti-pig antibodies that are predominantly IgM (1–3, 5, 6, 19, 20). To provide a baseline for the studies described below, a pool of human serum was made (from 10 donors) and found to contain antibodies that reacted with pig RBC, lymphocytes, spleen cells, and EC (Table 1 and Fig. 1); absorption studies showed that the same xenoantigen(s) were present on all of these tissues (Table 1 and Fig. 1). Thus, common epitopes are present on pig RBC, PBL, spleen, and EC; it is not clear whether the epitope(s) are present on glycoproteins, glycolipids, or both in these cells. Most of the anti-pig antibody in the serum pool was IgM rather than IgG because little antibody was removed by protein A-Sepharose or protein-G agarose columns (Table 1) and the IgG antibodies eluted from the protein A-Sepharose column reacted only weakly with pig RBC. Furthermore, treatment of the serum with 2-ME (final concentration, 25 mM) led to a complete loss of antibody activity (2-ME used at this concentration destroyed IgM but not IgG) (Table 1). After fractionation by Sephacryl gel chromatography, the high molecular mass fractions (IgM) were reactive with RBC, whereas the low molecular mass fractions (IgG) were not (not shown). Thus, the different pig cells carry similar epitopes, all reacting with IgM antibodies, and in our assays there was little IgG activity found in the human serum for pig cells.

Human Anti-Porcine Antibodies Are Not Reactive to Human ABO Blood Groups. Human blood group B-like antigen, which reacts with natural antibodies in human blood, has been reported to be ubiquitous in animals (reviewed in ref. 10); to exclude this possibility, the serum of 10 individuals of blood groups O, A, B, and AB and of the pool was tested on pig cells. The individual serum samples contained antibodies reactive with pig RBC (median titer of 1/64, range 1/32–1/

Table 1. Serology on pig cells

Treatment of pooled NHS	Titer^{-1} RBC	PBL	Spleen
Untreated	64	32	128
Absorbtion with pig cells*			
RBC	<1	<1	<1
PBL	<1	<1	<1
Spleen cells	<1	<1	<1
EC	<1	<1	<1
Absorbtion with Sepharose conjugates			
Protein A	64	NT	NT
Protein G	64	NT	NT
Absorbtion with human RBC†			
Blood Group O	32	NT	NT
Blood Group A	32	NT	NT
Blood Group B	32	NT	NT
2ME (25 mM)	<1	<1	NT

NT, not tested; NHS, normal human serum.
*Pooled NHS was absorbed three times with equal values of packed pig cells.
†Pooled NHS was absorbed three times with equal volumes of packed RBC from A, B, or O individuals.

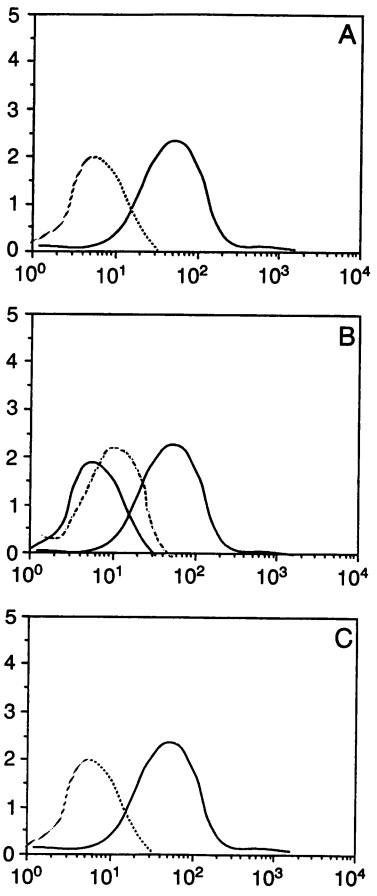

FIG. 1. Testing of pig EC with pooled human serum before absorption (—) and after absorption (. . . .) with pig EC (*A*), RBC (*B*), or spleen cells (*C*). The binding of human antibody was detected by using sheep anti-human IgM, and analysis was on the cytofluorograph. Abscissa, relative fluorescence; ordinate, cell number.

128); the differences in titer did not correlate with the blood group of the individual and thus did not correlate with the anti-ABO hemagglutinating antibodies present in serum of that individual. In particular, the serum of the A, AB, and O individuals, all of which have anti-B antibodies, gave the same reaction with pig RBC as did the serum of B-group individuals. In addition, absorption studies (absorbing pooled human serum three times with equal volumes of packed RBC from A, B, or O individuals) barely decreased the hemagglutination titer on pig RBC (from 1/64 to 1/32), compared with the absorption with pig RBC which completely cleared all reactive antibodies (Table 1). Thus there is no reactivity of anti-B antibodies with pig RBC.

Human Anti-Pig Antibodies React Predominantly with Terminal Galactose Residues. As natural antibodies have been shown to react with carbohydrate epitopes (21), we examined the ability of different carbohydrates to inhibit the hemagglutination reaction of pig RBC by human serum (Fig. 2*A*).

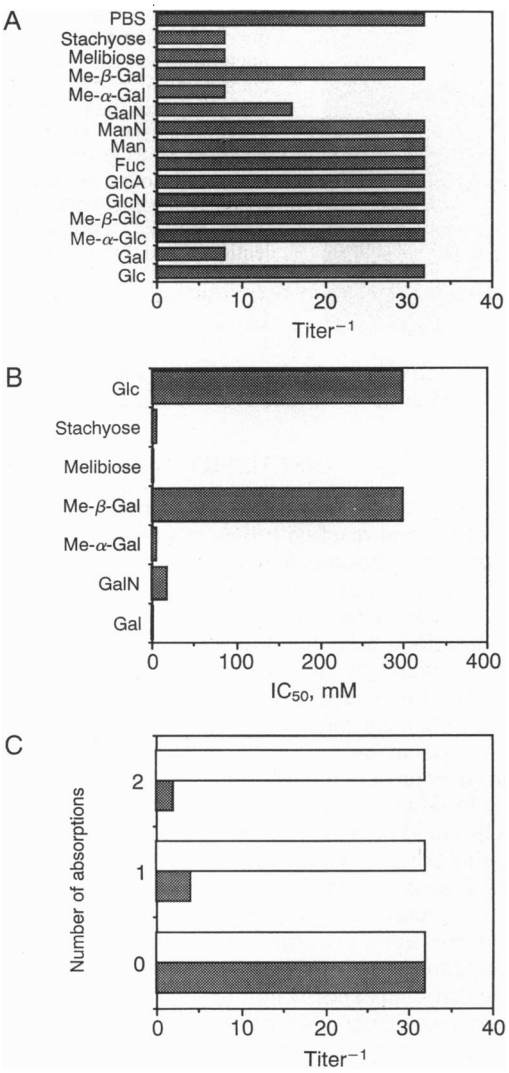

FIG. 2. (*A*) Carbohydrate inhibition of hemagglutination of normal human serum. Human serum was titered on pig RBC in the presence of carbohydrates at 300 mM. (*B*) Concentration of carbohydrate giving 50% inhibition of hemagglutination titer of normal human serum. Only carbohydrates inhibiting hemagglutination in *A* were used in this experiment, with glucose and methyl β-galactopyranoside as negative controls. (*C*) Hemagglutination titer of human serum on pig RBC before and after absorption on a melibiose-conjugated Sepharose column. Human serum was absorbed with equal volumes of melibiose-Sepharose (▨) or Sepharose (□) (the number of absorptions is indicated in the vertical figure axis).

Inhibition was observed with 300 mM galactose, methyl α-D-galactopyranoside, melibiose, and stachyose, all of which decreased the titer of the serum pool by 75% (Fig. 2*A*); and with 300 mM D-galactosamine, for which a 50% decrease in titer was observed (Fig. 2*A*). None of the other monosaccharides tested had any effect on the hemagglutination titer (Fig. 2*A*). These studies showed that galactose is the significant part of the epitope, as both melibiose and stachyose have terminal galactose residues. In particular, we noted the difference in the ability of galactose in the α configuration (methyl α-D-galactopyranoside, melibiose, and stachyose) but not the β configuration (methyl β-D-galactopyranoside) to inhibit the reactions. The relative avidity of the antibodies for the sugars that inhibited agglutination could be estimated from the concentration of sugar giving 50% inhibition of the agglutination titer (IC_{50}; Fig. 2*B*): both D-galactose and melibiose achieved this inhibition at <1.15 mM; stachyose and methyl α-D-galactopyranoside, at 4.7 mM; and D-galactosamine, at 18.7 mM (Fig. 2*B*). By contrast, D-glucose and methyl β-D-galactopyranoside had no effect, even at 300 mM concentration. Thus, D-galactose is an important part of the epitope, as it is a potent inhibitor of the xenoantibodies at low concentration (<1.15 mM); the ability of methyl α-D-galactopyranoside to inhibit agglutination at low concentrations (<1.15 mM), compared with the failure of methyl β-D-galactopyranoside (300 mM) to inhibit, demonstrates that the galactose residue (which is likely to be a terminal sugar) is in an α linkage rather than a β linkage with the subterminal residue. The results obtained with melibiose [Gal(α1-6)Glc] and stachyose [Gal(α1-6)Gal(α1-6)Glc(β1-2)Fru], which have α-linked terminal galactose residues, are in accord with this conclusion. The inhibition of hemagglutination observed with galactosamine is in keeping with these results. Identical inhibition results were obtained when individual serum samples from A, B, AB, and O individuals were used rather than the serum pool (not shown). To further examine the reaction with galactose, the serum pool was absorbed four times with equal volumes of packed melibiose [Gal(α1-6)Glc]-conjugated Sepharose or with Sepharose (Fig. 2*C*); one absorption with melibiose-Sepharose decreased the titer of the antibody from 1/32 to 1/4, and two sequential absorptions decreased the titer further to 1/2 (Fig. 2*C*). This absorption was specific for melibiose, as using Sepharose beads had no effect (Fig. 2*C*). Thus, the majority of the IgM antibody reactive with the xenoantigen(s) reacts with galactose in an α linkage.

Human Anti-Pig Antibodies React with COS Cells After Transfection with the α-1,3-Galactosyltransferase Gene. The cDNA coding for the α-1,3-galactosyltransferase has been cloned for both mouse (16) and ox (22); using this data, we isolated the murine cDNA for α-1,3-galactosyltransferase and used transfection experiments to determine if the newly expressed Gal(α1-3)Gal epitope reacts with the human antibodies. The mouse transferase gene was isolated from a cDNA library by PCR, and was directionally cloned into the CDM8 vector for expression studies in COS cells. The cDNA insert was sequenced in both directions (Fig. 3*A*) and shown to be identical to the published nucleotide sequence (16). COS cells, derived from Old World monkeys, were chosen as they do not react with human serum nor with the IB4 lectin [which is specific for Gal(α1-3)Gal] (Table 2). After transfection of COS cells with the α-1,3-galactosyltransferase gene, the Gal(α1-3)Gal epitope was detected on the cell surface by the binding of the IB4 lectin (Fig. 3*B* and Table 2); these cells were also strongly reactive with the serum pool (Fig. 3*C* and Table 2). Absorbing the human serum with pig RBC removed the reactivity for Gal(α1-3)Gal$^+$ COS cells (Table 2). Removal of IgG from the pool of human serum had no effect on its reactivity with COS cells by immunofluorescence; the eluted IgG had a weak reaction. Thus, human

A

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M I T M L Q D L H V N K I S M S R S K S E T S L P S S R S G S Q E K I M N V K G
ATGATCACTATGCTTCAAGATCTCCATGTCAACAAGATCTCCATGTCAAGATCCAAGTCAGAAACAAGTCTTCCATCCTCAAGATCTGGATCACAGGAGAAAATAATGAATGTCAAGGGA  120

K V I L L M L I V S T V V V V F W E Y V N R I P E V G E N R W Q K D W W F P S W
AAAGTAATCCTGTTGATGCTGATTGTCTCAACCGTGGTTGTCGTGTTTTGGGAATATGTCAACAGAATTCCAGAGGTTGGTGAGAACAGATGGCAGAAGGACTGGTGGTTCCCAAGCTGG  240

F K N G T H S Y Q E D N V E G R R E K G R N G D R I E E P Q L W D W F N P K N R
TTTAAAAATGGGACCCACAGTTATCAAGAAGACAACGTAGAAGGACGGAGAGAAAAGGGTAGAAATGGAGATCGCATTGAAGAGCCTCAGCTATGGGACTGGTTCAATCCAAAGAACCGC  360

P D V L T V T P W K A P I V W E G T Y D T A L L E K Y Y A T Q K L T V G L T V F
CCGGATGTTTTGACAGTGACCCCGTGGAAGGCGCCGATTGTGTGGGAAGGCACTTATGACACAGCTCTGCTGGAAAAGTACTACGCCACACAGAAACTCACTGTGGGGCTGACAGTGTTT  480

A V G K Y I E H Y L E D F L E S A D M Y F M V G H R V I F Y V M I D D T S R M P
GCTGTGGGAAAGTACATTGAGCATTACTTAGAAGACTTTCTGGAGTCTGCTGACATGTACTTCATGGTTGGCCATCGGGTCATATTTTACGTCATGATAGACGACACCTCCCGGATGCCT  600

V V H L N P L H S L Q V F E I R S E K R W Q D I S M M R M K T I G E H I L A H I
GTCGTGCACCTGAACCCTCTACATTCCTTACAAGTCTTTGAGATCAGGTCTGAGAAGAGGTGGCAGGATATCAGCATGATGCGCATGAAGACCATTGGGGAGCACATCCTGGCCCACATC  720

Q H E V D F L F C M D V D Q V F Q D N F G V E T L G Q L V A Q L Q A W W Y K A S
CAGCACGAGGTCGACTTCCTCTTCTGCATGGACGTGGATCAAGTCTTTCAAGACAACTTCGGGGTGGAAACTCTGGGCCAGCTGGTAGCACAGCTCCAGGCCTGGTGGTACAAGGCCAGT  840

P E K F T Y E R R E L S A A Y I P F G E G D F Y Y H A A I F G G T P T H I L N L
CCCGAGAAGTTCACCTATGAGAGGCGGGAACTGTCGGCCGCGTACATTCCATTCGGAGAGGGGGATTTTTACTACCACGCGGCCATTTTTGGAGGAACGCCTACTCACATTCTCAACCTC  960

T R E C F K G I L Q D K K H D I E A Q W H D E S H L N K Y F L F N K P T K I L S
ACCAGGGAGTGCTTTAAGGGGATCCTCCAGGACAAGAAACATGACATAGAAGCCCAGTGGCATGATGAGAGCCACCTCAACAAATACTTCCTTTTCAACAAACCCACTAAAATCCTATCT  1080

P E Y C W D Y Q I G L P S D I K S V K V A W Q T K E Y N L V R N N V ***
CCAGAGTATTGCTGGGACTATCAGATAGGCCTGCCTTCAGATATTAAAAGTGTCAAGGTAGCTTGGCAGACAAAAGAGTATAATTTGGTTAGAAATAATGTCTGA  1185
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B C

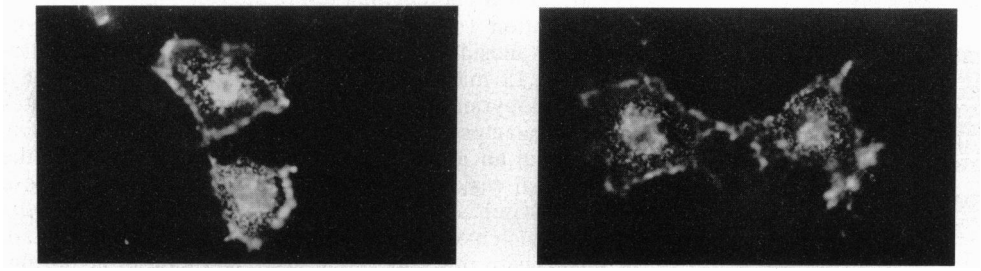

FIG. 3. Nucleotide sequence of the mouse α-1,3-galactosyltransferase. (*A*) Predicted amino acid sequence (single-letter code) is represented above the nucleotide sequence; numbers to the side of the nucleotide sequence represent nucleotide number, with nucleotide 1 being the A of the initiation codon; *** represents the stop codon. Fluorescence of COS cells transfected with the mouse α-1,3-galactosyltransferase gene was measured by using directly fluoresceinated IB4 lectin (*B*) and fluoresceinated sheep anti-human IgM (*C*) after treatment of COS cells with normal human serum.

serum has IgM antibodies to the Gal(α1-3)Gal epitope, which was expressed on Gal(α1-3)Gal$^+$ COS cells. The reaction of the serum with Gal(α1-3)Gal$^+$ COS cells is specific and not due to the transfection procedure, as control transfected CD48$^+$ COS cells were not reactive with human serum or with the IB4 lectin (Table 2). Finally, the reactivity of human serum for both pig RBC (as detected by hemagglutination) and EC (as detected by FACS analysis) could be completely removed by absorption with Gal(α1-3)Gal$^+$ COS cells but not nontransfected COS cells (not shown). Thus, human serum contains IgM antibodies reactive with Gal(α1-3)Gal.

Table 2. Serology on nontransfected and transfected COS cells

Agent	Target cell	Reaction*
NHS	GT$^+$ COS	+++
Absorbed with pig RBC		−
Absorbed with prot A-Sepharose		+++†
Eluted from prot A-Sepharose		+‡
CD48 mAb		−
IB4 lectin§		+++
NHS	CD48$^+$ COS	−
CD48 mAb		+++
IB4 lectin§		−
NHS	COS	−
CD48 mAb		−
IB4 lectin§		−

NHS, normal human serum; abs, absorbed; GT, gene for α-1,3-galactosyltransferase.

*Reactivity detected by indirect immunofluorescence with FITC-conjugated sheep anti-human immunoglobulin or FITC-conjugated sheep anti-mouse immunoglobulin unless otherwise stated. −, No reaction; +, weak reaction; +++, strong reaction.

†No difference in titer was observed when tested with FITC-conjugated sheep anti-human IgM.

‡Reaction detected on protein A-purified immunoglobulin with FITC-conjugated sheep anti-human immunoglobulin or FITC-conjugated sheep anti-human IgG but not with FITC-conjugated sheep anti-human IgM.

§Reactivity of cells detected with FITC-conjugated IB4 lectin specific for Gal(α1-3)Gal.

DISCUSSION

Naturally occurring antibodies that are found in the serum of all individuals and react with the cells of other species are a major barrier to xenotransplantation. In our previous study we reported that there are cytotoxic IgM antibodies in normal human sera that are reactive with sheep and pig cells (20). The studies described here demonstrate that the majority of the hemagglutinating IgM antibodies are directed against the carbohydrate epitope Gal(α1-3)Gal. Sugar inhibition experiments and absorption using melibiose coupled to agarose established that galactose in an α linkage is involved in the epitope recognized by most of the IgM antibody in normal human sera (Fig. 2). In addition, the Gal(α1-3)Gal antigen expressed on COS cells could be detected with normal human serum after transfecting the cells with a cDNA clone encoding the α-1,3-galactosyltransferase (Table 2). This clearly established that this gene is responsible for the major epitope on pig cells detected by human serum, particularly as the α-1,3-galactosyltransferase$^+$ COS cells reacted strongly with IB4 lectin [which binds specifically to the Gal(α1-3)Gal epitope] and could specifically and totally remove by absorption antibodies reactive with pig RBC and endothelial cells.

At present, it is not clear on which glycoprotein or glycolipid molecules the Gal(α1-3)Gal epitopes are found, and this important issue needs resolution. First, Platt *et al.* (5) demonstrated that IgM antibodies directed against three major glycoproteins of pig endothelial cells are potential targets for human serum antibodies, which could lead to hyperacute rejection after endothelial cell activation (5); glycolipids were not detected in their study. Second, Galili and coworkers (7–12) showed that normal human serum contains IgG antibodies reactive with the Gal(α1-3)Gal epitope found on the RBC of all mammals except humans and the anthropoid apes

(9, 10); in these important studies, Galili examined only RBC and IgG.

The preferred acceptor structure of the α-1,3-galactosyltransferase is Gal(β1-4)GlcNAc(β1-3)-R, which may be present at the nonreducing terminus of glycoproteins, glycolipids, and oligosaccharides (23). Based on these observations and the results described here that show serologically that α-1,3-galactosyltransferase produces the Gal(α1-3)Gal epitope detected by IgM, it is likely that the epitope will be found on both glycosphingolipids and glycoproteins. It is known that both RBC and epithelial cells can express the same carbohydrate determinant—present as either glycolipid (on RBC) or glycoprotein (on epithelial cells), human A and B blood groups being well-known examples (24). It is likely that Platt *et al.* (5) detected Gal(α1-3)Gal on glycoproteins on EC. Clearly we detect the same epitope on RBC, EC, and spleen cells, and it remains to be proven biochemically that glycoproteins in EC can carry the Gal(α1-3)Gal determinant.

Gal(α1-3)Gal is found on glycoconjugates of many mammalian species except Old World monkeys, apes, and man (10)—species that have naturally occurring high-titered antibodies to Gal(α1-3)Gal (10). Our studies show that the majority of human anti-pig IgM reactivity is to this epitope. The cDNA sequences for both the murine (16) and bovine (22) α-1,3-galactosyltransferase have been isolated, and the transferase encoded by these genes is functional in these species, as Gal(α1-3)Gal is present on their cells. Comparison of the predicted amino acid sequences of these transferases shows homology with the human B blood group transferase, thus suggesting that these genes shared a common ancestor. However, these transferase also differ in the substrate recognized: the B blood group transferase has an absolute requirement to recognize Gal(α1-2)Fuc for the addition of the terminal galactose, whereas the α-1,3-galactosyltransferase does not require the branched fucose. The homologous human genes for the α-1,3-galactosyltransferase have been isolated and shown to contain frameshift mutations that interrupt the coding sequence with stop codons (25, 26). Thus, humans do not encode a functional α-1,3-galactosyltransferase; since humans lack the enzyme, they do not have the Gal(α1-3)Gal epitope. Presumably natural antibodies are then made in the same way as to ABO blood group antigens, probably by exposure to bacteria in the gut; in turn, this leads to the production of natural antibodies to the enzymatic product, the Gal(α1-3)Gal epitope.

The finding that the majority of xenoreactive IgM is directed to the enzymatic product of a single transferase raises the possibility of producing transgenic pigs lacking the epitope by targeting destruction of the α-1,3-galactosyltransferase gene via homologous recombination technology. Toward this end we have isolated cDNA clones encoding the pig α-1,3-galactosyltransferase (unpublished data); it has high sequence homology with both the murine (16) and bovine (22) α-1,3-galactosyltransferase genes (59% and 71% identity, respectively). However, a less-radical therapeutic approach, which could have immediate use, would be the infusion of α-D-galactose-containing compounds into potential recipients of pig grafts. Such compounds such as galactose or melibiose could at least partially block the antibody reaction with the endothelium and delay hyperacute rejection, as has been done for ABO incompatible grafts in baboons (27, 28).

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