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Reduction in the level of Gal(α1,3)Gal in transgenic mice and pigs by the expression of an α(1,2)fucosyltransferase

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ABSTRACT **Hyperacute rejection of a porcine organ by higher primates is initiated by the binding of xenoreactive natural antibodies of the recipient to blood vessels in the graft leading to complement activation. The majority of these antibodies recognize the carbohydrate structure Gal(α1,3)Gal (gal epitope) present on cells of pigs. It is possible that the removal or lowering of the number of gal epitopes on the graft endothelium could prevent hyperacute rejection. The Gal(α1,3)Gal structure is formed by the enzyme Galβ1,4GlcNAc3-α-D-galactosyltransferase [α(1,3)GT; EC 2.4.1.51], which transfers a galactose molecule to terminal *N*-acetyllactosamine (N-lac) present on various glycoproteins and glycolipids. The N-lac structure might be utilized as an acceptor by other glycosyltransferases such as Galβ1,4GlcNAc 6-α-D-sialyltransferase [α(2,6)ST], Galβ1,4GlcNAc 3-α-D-sialyltransferase [α(2,3)ST], or Galβ 2-α-L-fucosyltransferase [α(1,2)FT; EC 2.4.1.69], etc. In this report we describe the competition between α(1,2)FT and α(1,3)GT in cells in culture and the generation of transgenic mice and transgenic pigs that express α(1,2)FT leading to synthesis of Fucα1,2Galβ- (H antigen) and a concomittant decrease in the level of Gal(α1,3)Gal. As predicted, this resulted in reduced binding of xenoreactive natural antibodies to endothelial cells of transgenic mice and protection from complement mediated lysis.**

Dramatic improvements in the success of organ transplantation have created a severe imbalance between organ supply and demand. This situation has stimulated the scientific and medical communities to look at the possibility of using animals as donors. Xenotransplantation using porcine organs is currently viewed as a possible approach to solving this problem. However, transplants from pigs to primates are rapidly rejected by a complement-mediated process termed hyperacute rejection (1). Hyperacute rejection is characterized by thrombosis, hemorrhage, and edema and results in an immediate decline in graft function and irreversible rejection within a few minutes to a few hours after transplant. This process is initiated by the binding of xenoreactive natural antibodies (XNAs) to carbohydrate structures present on the surface of endothelial cells leading to activation of the complement cascade (2).

The carbohydrate epitope recognized by XNA is predominantly Gal(α1,3)Gal (gal epitope), which is present in pigs, mice, and New World monkeys but is absent from Old World monkeys and humans (3, 4). The gal epitope is synthesized in the trans-Golgi by Galβ1,4GlcNAc3-α-D-galactosyltransferase [α(1,3)GT; EC 2.4.1.51], which catalyzes the addition of galactose to an *N*-acetyllactosamine (N-lac) core (5). Humans, apes, and other Old World monkeys do not have the gal epitope due to a lack of a functional α(1,3)GT gene (6, 7). Given the specificity of XNA and previous studies demonstrating that hyperacute rejection can be avoided by depletion of these antibodies, it might be proposed that the ideal xenograft donor would be designed in such a way as to lack that epitope. Here we describe the successful application of an approach to achieve that goal.

The Galβ 2-α-L-fucosyltransferase [α(1,2)FT; EC 2.4.1.69] enzyme is involved in the formation of the H antigen (Fucα1,2Galβ-), which is the precursor molecule in the human ABO blood group system. One of the substrate molecules for α(1,2)FT {or Galβ1,4GlcNAc 3-α-D-sialyltransferase [α(2,3)ST] and Galβ1,4GlcNAc 6-α-D-sialyltransferase [α(2,6)ST]} is N-lac, which is also utilized by α(1,3)GT as an acceptor of galactose (8, 9). *In vitro* substrate specificity studies have shown that fucosylated (or sialated) N-lac is a poor substrate for α(1,3)GT (5). In this report we show that in cells in culture expression of human α(1,2)FT can reduce the level of the gal epitope. In addition and more significantly, we demonstrate that *in vivo* in transgenic mice and pigs the expression of human α(1,2)FT results in the production of the H antigen in the endothelial cells of multiple organs and results in a dramatic decrease in the level of the gal epitope, and a reduction in XNA binding and subsequent complement activation.

MATERIALS AND METHODS

DNA Constructs. Porcine α(1,3)GT cDNA (1.1 kb) was obtained by reverse transcription (RT)-PCR using total RNA from porcine aortic endothelial cells and first strand cDNA synthesis kit (Pharmacia). The degenerate PCR primers were based on mouse and bovine GT sequence (7, 10). The cDNA was cloned into a eukaryotic expression vector pRex10 (pRex10/GT) containing enhancer and promoter sequences from Rous sarcoma virus long terminal repeat and the neomycin resistance gene. The human α(1,2)FT cDNA was cloned by RT-PCR using total RNA from the A431 cell line (11). The sense primer (TTTGGATCC**TCGGCCATGTGGCTCCG-GAGCCATCG**) flanked the ATG initiation codon and included a *Bam*HI site. The antisense primer (AAAGTCGAC**T-CAAGGCTTAGCCAATC**) flanked the TGA stop codon and included an *Sal*I site. The 1.1 kb cDNA was cloned into pREX10 (pRex10/FT) to express α(1,2)FT in tissue culture cells. For transgenic expression, the α(1,2)FT cDNA was cloned into vectors containing a 500-bp chicken β-actin promoter (876) or a 4.3-kb H2k^b promoter (881). The splice and polyadenylylation sequences were provided by a 900-bp *Hin*dIII/*Kpn*I fragment of human α globin gene containing sequences downstream from the second exon.

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Abbreviations: XNA, xenoreactive natural antibodies; GS-1B4 lectin, *Griffonia simplicifolia* 1 isolectin B4; UEA-I lectin, *Ulex europaeus* I lectin; α(1,3)GT, Galβ1,4GlcNac3-α-D-galactosyltransferase; α(1,2)FT, Galβ2-α-L-fucosyltransferase; N-lac, *N*-acetyllactosamine; CHO, Chinese hamster ovary; FITC, fluorescein isothiocyanate; EC, endothelial cells.

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Cell Culture and Transfections. The cell culture and transfection reagents were purchased from GIBCO/BRL. Chinese hamster ovary (CHO) K1 cells (ATCC, catalogue no. CCL-61) were maintained in F-12 (HAM's) with glutamax-1 supplemented with 10% fetal bovine serum. The transfections were done with lipofectamine using 1–2 μg of supercoiled plasmid DNAs (pRex10/GT or pRex10/FT) according to the manufacturer's instructions.

Lectin Binding Assay. Assay for cell surface antigen expression was performed as described (12). Briefly, the cells in 96-well plates were fixed with 1% glutaraldehyde and then incubated with different concentrations of lectin/biotin conjugates (EY Laboratories) for 1 hr at room temperature. The cells were washed with PBS and incubated with a 1:1000 dilution of streptavidin–horseradish peroxidase (HRP) (Pierce) for 1 hr. The plates were developed using 150 μl per well of HRP substrate solution in the dark for 5–10 min. The reaction was stopped with 50 μl of 2 M H_2SO_4. The plates were read at 505 nm on a Molecular Devices V_{max} microplate reader.

Cell Extracts. Total cellular protein extracts were prepared as described (13). Briefly, 5×10^6 cells were incubated for 10 min at 4°C in 0.8 ml of cell lysis buffer (0.16 M NaCl/1 mM EDTA/1% Nonidet P-40/2.5 mM deoxycholate/0.1% SDS/10 μg/ml aprotinin/10 μg/ml leupeptin/20 mM Tris·HCl, pH 8.0). Cells were scraped into Eppendorf tubes and spun at 12,000 × *g* for 5 min, supernatants were removed, and the protein concentration was determined.

Lectin Blotting. The lectin blotting was performed as described with minor modifications (13). Protein samples (10 μg) were resuspended in sample buffer (50 mM Tris·HCl, pH 6.8/100 mM 2-mercaptoethanol/2% SDS/0.2% bromophenyl/10% glycerol), heated for 3 min at 100°C, and loaded onto a 7.5% SDS gel. After electrophoresis the proteins were transferred to Immobilon-P membranes (Millipore) at 100 V for 2 hr. Following transfer the membranes were rinsed twice with Tris-buffered saline (TBS) (150 mM NaCl/1 mM $CaCl_2$/1 mM $MgCl_2$/1 mM $MnCl_2$/20 mM Tris·HCl, pH 7.5/0.1% Tween). After blocking for 1 hr with 1% ovalbumin/TBS the membranes were incubated for 1 hr at 4°C with 3 μg/ml of lectin/biotin conjugates in 1% ovalbumin/TBS/0.1% Tween. Membranes were washed 3 times with TBS/Tween and incubated for 1 hr with 1 μg/ml of streptavidin–HRP in 1% ovalbumin/TBS. After washing, the membranes were developed using a chemiluminescent detection kit (ECL Western Blotting Detection kit, Amersham) according to the manufacturer's instructions.

Northern Blot Analysis. Total RNA was prepared from tissue culture cells or animal tissues using RNA STAT-60 (Tel-Test, Friendswood, TX) according to the manufacturer's instructions. RNA (10 μg) was fractionated on 1% agarose gel containing formaldehyde and transferred to a nylon membrane (ICN). The probes were prepared by the Ready-To-Go kit (Pharmacia) and the hybridization was performed as described (14)

Production of Transgenic Animals. DNA was injected into male pronuclei of fertilized mouse (C57BL/6) or porcine oocytes (15). Transgenic animals were identified by Southern blot analysis of tail DNA.

Immunohistochemical Staining. After snap freezing in precooled isopentane, tissue samples were stored at −80°C until use. Immunohistochemical staining with fluorescein isothiocyanate (FITC)-labeled lectins was performed as described (16).

Flow Cytometric Analysis of Endothelial Cells. Freshly harvested organs (heart and liver) were minced and digested in dispase solution (Becton Dickinson) for 90 min at 37°C with gentle shaking. The resultant cell suspension was washed and counted using a Coulter Z1 Analyzer. Aliquots of 4×10^6 cells were used for immunofluorescent staining, utilizing a three-step procedure. First, the cells were incubated for 30 min on ice, with 10 μg of XNAs, isolated as described (17), with or without the addition of 5 mM Galactobiose (Vector Laboratories). Cells were washed twice and incubated 30 min on ice with 5 μg of biotinylated mouse anti-human IgM (PharMingen). Also, 10 μg of FITC-labeled sheep anti-human Factor VIII (Serotec) was added to all tubes to label endothelial cells within the suspension. Cells were washed twice and incubated for 15 min on ice with CyChrome conjugated streptavidin (PharMingen). Controls for all steps included matched species and isotype control antibodies (PharMingen), as well as second-step background staining. Finally, cells were washed twice, fixed in 2% paraformaldehyde/PBS, and analyzed on a Coulter EPICS Elite flow cytometer. Endothelial cells were identified and gated using a plot of FL1-FITC (Factor VIII)-vs-Side Scatter. This gate was used to generate plots of FL1-vs-FL3 (CyChrome) for the analysis of XNA binding.

Complement Mediated Lysis. Aliquots of 4×10^6 cells from the digested suspension described above, were pre-sensitized with 20 μg of human XNA, and simultaneously stained for Factor VIII, as described earlier. The cells were washed and incubated for 45 min at 37°C with decreasing concentrations of rabbit serum (Sigma). All tubes contained a final concentration of 10 μg/ml propidium iodide, to serve as a reporter for cell lysis. After incubation flow cytometric analysis, as described above, was performed to gate endothelial cells, and then construct plots of FL1-FITC (Factor VIII)-vs-FL3-propidium iodide to assess the percentage of lysed cells staining positive in the FL3 channel. Controls consisted of aliquots containing no rabbit serum plus propidium iodide, and those without PNA pre-sensitization, to assess the ability of the rabbit serum and Factor VIII antibody to lyse cells.

RESULTS

Competition Between α(1,3)GT and α(1,2)FT in CHO Cells. Prior to the generation of transgenic animals we used CHO cells as a model system to determine whether α(1,2)FT would compete with α(1,3)GT for modification of glycoproteins and glycolipids. These cells are deficient in α(1,3)GT and α(1,2)FT but do express N-lac, which can act as an acceptor for both of these enzymes (11, 18). CHO cells were transfected with the porcine α(1,3)GT cDNA (pRex10/GT). The expression of α(1,3)GT in transfected cells was detected by a lectin-binding assay using the lectin *Griffonia simplicifolia* 1 isolectin B4 (GS-1B4), which recognizes the same terminal structure Gal(α1–3)Gal as recognized by the XNAs (19). Analysis of one representative clone (CHO/GT) is shown in Fig. 1*A*. Although untransfected CHO cells do not bind the lectin GS-1B4, CHO/GT cells clearly bind the lectin and therefore produce the gal epitope.

To determine the extent to which α(1,2)FT would compete with α(1,3)GT for N-lac, the CHO/GT cells were transfected with pRex10/FT. A pool of transfected cells (CHO/GTFT) was selected and analyzed for α(1,3)GT and α(1,2)FT expression by lectin-binding assay. The expression of α(1,2)FT was determined based on the binding of a lectin *Ulex europaeus* I (UEA-I), which detects the H antigen (20). As shown in Fig. 1*A*, CHO/GTFT cells bind UEA-1, but not the parent CHO/GT (nor the untransfected CHOs). CHO/GTFT cells that express high levels of H antigen display up to a 70% reduction in GS-1B4 binding compared with CHO/GT. No difference in the level of α(1,3)GT mRNA or gene copy number was observed between CHO/GT and CHO/GTFT cell lines suggesting that decreased binding of GS-1B4 [and thus low α(1,3)GT activity] is due to competition at the enzyme level (data not shown).

We next examined by lectin blotting the glycosylation of membrane proteins that may be more important than glycolipids in xenotransplantation (21). Expression of porcine α(1,3)GT in CHO cells (CHO/GT) resulted in galactosylation

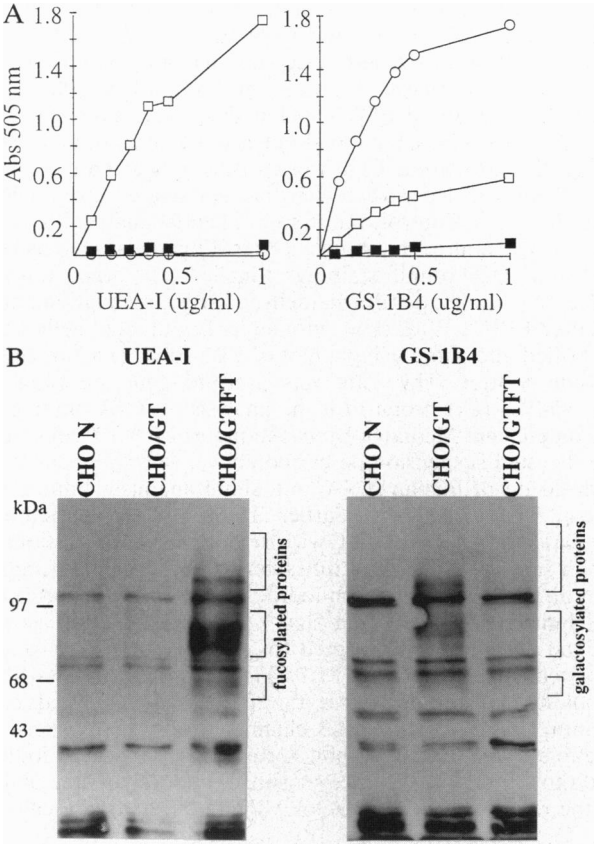

FIG. 1. Competition between porcine α(1,3)GT and human α(1,2)FT in CHO cells. (*A*) Binding of UEA-I or GS-1B4 lectin to untransfected CHO cells (■; CHO N), CHO cells transfected with GT (○; CHO/GT), and CHO/GT cells transfected with FT (□; CHO/GTFT) as measured by lectin binding assay. Amount of lectin used (μg/ml) is indicated. The absorbance was read at 505 nm. (*B*) UEA-I and GS-1B4 lectin binding to membrane glycoproteins of CHO-N, CHO/GT, and CHO/GTFT cells. The proteins were resolved by SDS/PAGE (8% gel) and transferred to membranes. Membranes were stained with UEA-1 or GS-1B4. Molecular weight markers are shown on the left. The galactosylated or fucosylated proteins are indicated.

of proteins mostly in the >60 kDa range (Fig. 1*B*, right panel). Expression of α(1,2)FT in CHO/GT cells (CHP/GTFT) abrogated GS-1B4 binding to these proteins resulting instead in the expression of the H antigen (fucosylated proteins).

These results clearly suggest that α(1,2)FT can compete with porcine α(1,3)GT and that it may be possible to use α(1,2)FT to reduce the gal epitope in transgenic animals. To test our idea we next generated transgenic animals that express α(1,2)FT.

Expression of α(1,2)FT in Transgenic Mice. We tested the concept of using α(1,2)FT to compete with α(1,3)GT in mice that normally express the gal epitope on endothelial cells and on secreted glycoproteins such as thyroglobulin, fibrinogen, etc. (22, 23). The chicken β-actin (AcFT, 876) and the murine H2k^b promoter (H2FT, 881) were used to drive the expression of the α(1,2)FT cDNA (Fig. 2) (24, 25). These promoters have been used in previous experiments to express human CD59 and DAF in transgenic mice and pigs resulting in widespread tissue expression including but not limited to endothelial cells (14).

By PCR and Southern blot analysis 3/29 and 7/30 mice had incorporated the construct 876 (AcFT) and 881 (H2FT), respectively, into their genome. Transgenic mice carrying construct 881 were analyzed for the expression of the α(1,2)FT gene. Northern blot analysis showed that α(1,2)FT mRNA was present in all the tissues examined, including heart and kidney (Fig. 3). No α(1,2)FT signal was detected in any of the tissues from normal mice. The RNA from 1–2 mice, which contained a truncated transgene, was also negative for α(1,2)FT message.

The expression of the H antigen in transgenic mice was analyzed by lectin staining of tissue sections and fluorescence microscopy. As shown in Fig. 4, the transgenic mouse (1–4) exhibited a high level of expression of H antigen as indicated by binding of UEA-I to myocytes and endothelial cells in heart (*C*), whereas no binding was observed in the tissues of normal mice (*A*). Similar results were obtained with kidney, lung, liver, and spleen from transgenic versus normal mice with expression being predominantly, though not exclusively, endothelial in nature (not shown).

To determine if the expression of α(1,2)FT in tissues from transgenic mice was associated with reduction of the gal epitope we stained tissue sections of α(1,2)FT transgenic and control mice with the lectin GS-1B4. As Fig. 4*C* indicates there was a dramatic reduction in the level of GS-1B4 binding in the heart of the α(1,2)FT transgenic mouse compared with the control mouse (Fig. 4*B*). This result suggests that expression of α(1,2)FT causes a dramatic decrease in the level of Gal(α1,3)Gal.

Binding of Xenoreactive Antibodies to Mouse Endothelial Cells. To determine whether expression of α(1,2)FT results in reduced binding of xenoreactive antibodies the endothelial cells from a normal and an FT transgenic mouse heart were isolated and incubated with XNAs and analyzed by flow cytometry as described in *Materials and Methods*. As shown in Fig. 5*A*, the endothelial cells (EC) from the transgenic mouse exhibit a significant reduction in the level of XNA (IgM) binding as compared with EC from normal mouse. Incubation of XNAs with a disaccharide galactobiose (Galα1,3Gal) completely abolished the binding to EC of normal as well as transgenic mice indicating the specificity of XNAs for the gal epitope (Fig. 5*A*).

Next we determined the effect of reduced XNA binding on complement mediated lysis of EC from transgenic mice. As shown in Fig. 5*B*, incubation of EC of control animals with XNAs followed by addition of rabbit serum (as a source of complement) resulted in lysis of 14% of the cells. In contrast, less than 5% lysis of the EC cells of FT transgenic mice was

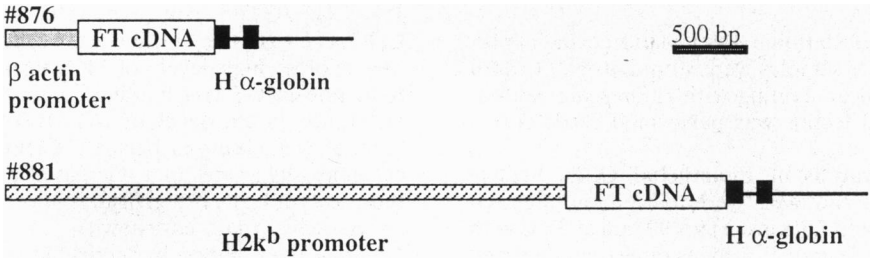

FIG. 2. Expression constructs for α(1,2)FT production in transgenic animals. A 1.1-kb fragment containing human α(1,2)FT cDNA was fused to either 0.5 kb of chicken β-actin promoter (construct 876) or to 4.3 kb of murine H2k^b promoter (construct 881). A 900-bp fragment of human α-globin gene comprising 2 exons and an intron with 400 bp of 3′ untranslated region was ligated at the 3′ end to provide splice and poly(A) signals.

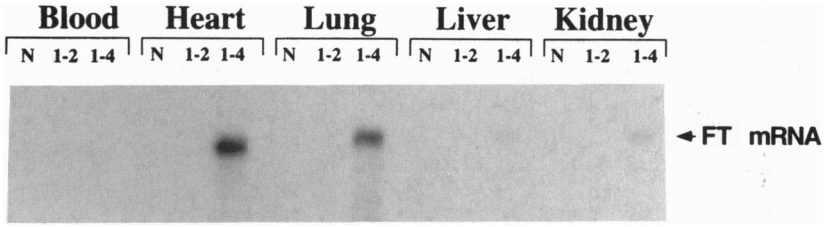

FIG. 3. Northern blot analysis of α(1,2)FT mRNA produced in transgenic mice. Transgenic mice (1–2 and 1–4), containing construct 881 (H2K^b-FT), were analyzed for α(1,2)FT mRNA expression. Total RNA was prepared from indicated tissues and subjected to Northern blot analysis using radiolabeled α(1,2)FT cDNA as a probe. The nontransgenic (N) mouse was used as a control. Mouse 1–2 had a truncated copy of the transgene. The position of α(1,2)FT mRNA (1.8 kb) is indicated.

observed, which is similar to the level of lysis of normal or transgenic EC incubated with Factor VIII antibody and rabbit serum without XNAs.

Generation of Transgenic Pigs. Based upon the results obtained with transgenic mice, transgenic pigs were generated by coinjecting the α(1,2)FT constructs 876 and 881 into the pronuclei of fertilized porcine eggs. A total of 656 injected eggs were transferred into pseudopregnant females and 58 (33 male and 25 female) pigs were born. Five transgenic pigs (3 male and 2 female) were identified by PCR and confirmed by Southern blot analysis of tail DNA. One pig contained construct 876 only (AcFT) and one pig contained 881 (H2FT); three pigs were positive for both.

Expression in Transgenic Pigs. As a preliminary analysis of the effectiveness of this approach to suppress Gal(α1,3)Gal synthesis in pigs we analyzed tissues obtained from transgenic pigs containing construct 876 (AcFT). The β-actin promoter has been shown to give high levels of widespread expression in endothelial cells and muscle (13). As in mice, high level expression of H antigen was detected by binding of the lectin UEA-I to tail sections of transgenic pigs compared with a nontransgenic littermate (Fig. 6 *C* versus *A*). At the same time expression of Gal(α1,3)Gal, as indicated by staining with GS-1B4, was markedly decreased (Fig. 6 *B* versus *D*). These data strongly suggest that the level of the xenogeneic gal epitope can be reduced by the expression of a competing glycosyltransferase.

DISCUSSION

The immediate barrier to the transplantation of vascularized pig organs to primates is hyperacute rejection, which is mediated by the binding of XNA and the activation of complement (1). In this report we have shown that the expression of the gal epitope, the predominant carbohydrate target of XNA, can be downregulated by α(1,2)FT expression in cells in culture, transgenic mice, and pigs (3, 26). This downregulation results in a reduction in XNA binding to endothelial cells and subsequent complement activation. Similar results in tissue culture cells were obtained in a recently published report (27). However, no data were presented that showed a reduction in antibody binding and complement activation on EC of transgenic mice.

The effect of the α(1,2)FT gene expression in downregulating the expression of the gal epitope in transgenic animals is particularly striking. The cell surface glycosylation pattern is related to the cell-type specific expression of glycosyltransferases. Pigs have a functional gene for α(1,2)FT and display ABH blood groups. However, these epitopes apparently are not expressed on vascular endothelium (19, 28). This is in agreement with the lack of endogenous expression of α(1,2)FT in the nontransgenic murine tissues examined at both the RNA and the protein level (Figs. 3 and 4). These data suggest that the high degree of competition observed between the endogenous α(1,3)GT and the transgenic α(1,2)FT in endothelial cells *in vivo* occurs because we are expressing a glycosyltransferase that is normally not expressed in endothelial cells. Using similar constructs expressing α(2,3)ST and α(2,6)ST, at least in cells in culture, we were unable to reduce the level of the gal epitope (data not shown) suggesting that the effect on the expression of the gal epitope may be specific to the α(1,2)FT gene. This effect could either be due to the location of the various enzymes in the Golgi such that α(1,2)FT is located before α(1,3)GT and the sialyltransferases are located after

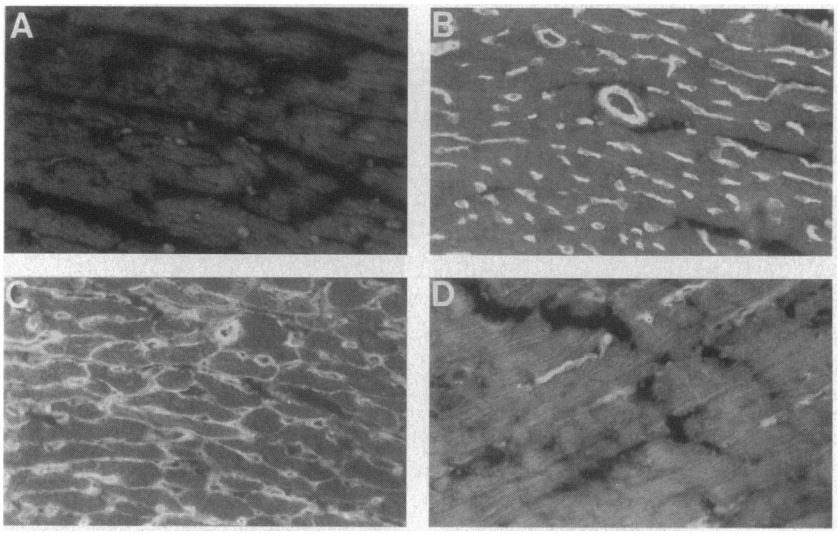

FIG. 4. Downregulation of the gal epitope by α(1,2)FT expression in transgenic mice containing construct 881 (H2FT). Fluorescence microscopic analysis of murine heart sections stained with FITC conjugated lectins. The transgenic mouse (1–4), expressing high levels of α(1,2)FT mRNA (Fig. 3), was used. Nontransgenic (*A*) and transgenic heart (*C*) stained with UEA-I. Nontransgenic (*B*) and transgenic heart (*D*) stained with GS-1B4.

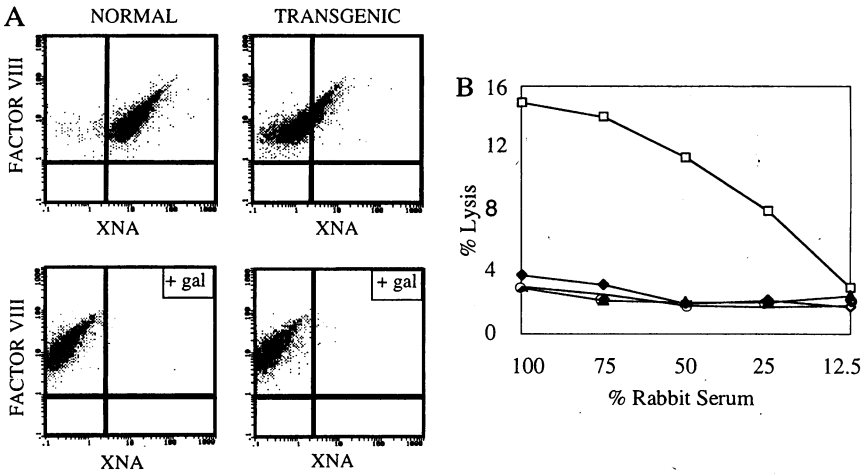

FIG. 5. (*A*) Analysis of XNA binding to heart endothelial cells of normal and FT transgenic mice by flow cytometry in the absence (upper panel) or presence (+ gal) of 5 mM galactobiose. (*B*) Complement mediated lysis of liver endothelial cells of normal mice incubated with (□) or without XNA (♦) and transgenic mice with (▲) or without XNA (○).

α(1,3)GT or that the enzymes have a different affinity for the glycosylation substrates.

In xenotransplantation a number of approaches have been used to abrogate the antibody–antigen interaction and prevent hyperacute rejection. These approaches include the bulk removal of total protein, including immunoglobulin, by plasmapheresis; specific immunoadsorption of total immunoglobulin using Sepharose-based columns containing sheep anti-human Ig; or the removal of XNA by perfusion through a porcine organ (29–31). All of these techniques, particularly the removal of total immunoglobulin, potentially put the patient at a greater risk of infection and may have to be performed multiple times after the transplant to maintain long-term graft survival. It is possible that the modification of the graft by a substantial reduction in the level of the gal epitope may prevent antibody binding to the graft and potentially prevent hyperacute rejection, or likely, more importantly be useful for long-term graft survival. Indeed, although ABO incompatible allografts in humans are subject to hyperacute rejection, ABO incompatible allografts in nonhuman primates, which express a lower level of A antigen, are not susceptible (32). Whether decreasing the level of Gal(α1,3)Gal in transgenic pig organs by expressing α(1,2)FT will itself prevent hyperacute rejection must await the generation of offspring from these founder animals.

Clearly, the use of the pig as a potential donor animal for transplantation makes it possible to focus immunosuppressive strategies not only on the recipient but also on the donor. This approach, if successful, may substantially reduce the immunosuppressive requirements imposed on the recipient. This has

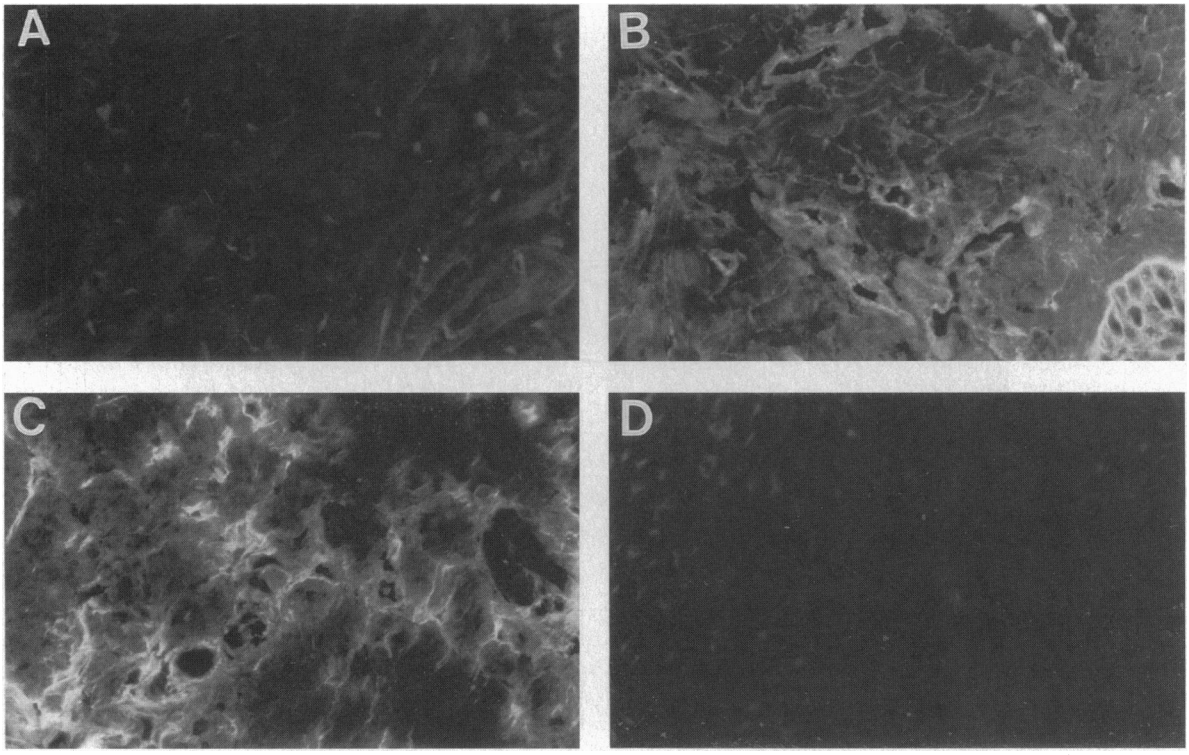

FIG. 6. Fluorescence microscopic analysis of a tail section of a transgenic pig containing construct 876 (AcFT) and a nontransgenic littermate. Nontransgenic (*A*) and transgenic tissue (*C*) stained with UEA-I. Nontransgenic (*B*) and transgenic tissue (*D*) stained with GS-1B4.

been accomplished for the complement system (14, 33, 34), and now we show that this may also be possible for the antibody–antigen reaction by the generation of transgenic pigs with reduced levels of xenoantigens.

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