Carbohydrates in transplantation

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Carbohydrate materials have become increasingly utilized in transplantation and cell/tissue engineering within the past year. This has been well documented in recent applications of immobilized or soluble α-galactosyl epitopes (i.e. oligosaccharides with a terminal Galα1–3Gal sequence) in preventing hyperacute rejection in pig-to-primate xenotransplantation. In addition, α-galactosyl polymers have been shown to exhibit much greater activity (up to 10^4 times) than α-galactosyl monomers in inhibiting the binding of anti-galactosyl antibodies to pig kidney epithelial cells and assisting in the prevention of cytotoxicity in human serum.

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Abbreviations

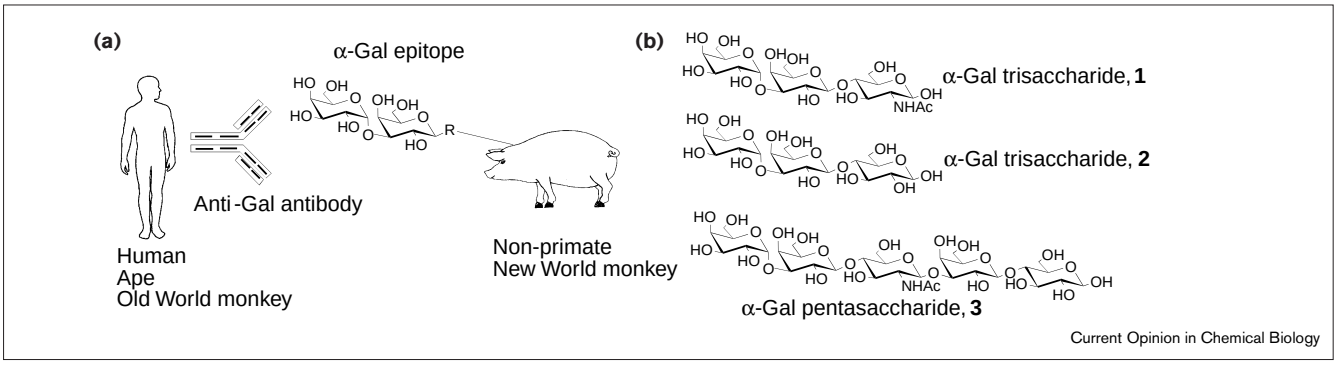
anti-Gal	anti-α-galatosyl antibodies
ELISA	enzyme-linked immunosorbent assay
Gal	galactose
Glc	glucose
HAR	hyperacute rejection
PEG	polyethylene glycol
PK15 cell	pig kidney epithelial cell
UDP	uridine diphosphate

Introduction

Tissue and organ transplantations have become routine surgery to save human lives from organ failure [1]. The majority of the clinically successful surgeries are of the allotransplantation type, using cadaveric human donor organs. The quantity of human donor organs is far from sufficient, however, and the number of patients on the waiting list for organs or tissues is increasing rapidly [2,3]. Because the supply of animal organs and tissues is unlimited, xenotransplantation (the transplantation of organs and tissues between animal species) is an alternative solution and has drawn considerable interest in clinical research. For ethical, viral (zoonoses are easily transmitted into closely related immunosuppressed recipients), breeding (few offspring, lengthy gestation time) and economic reasons, nonhuman primates are considered to be unsuitable for organ transplantation. The common pig is identified as the most suitable organ donor candidate for humans and has drawn significant attention because of its compatible organ size (especially miniature swine) and short breeding time. In addition, the risk of viral transmission can be controlled in the pig [2,4]. Pig endogenous retrovirus (PERV) is the most studied pig retrovirus and has been shown to be non-transmissible in a pig-to-baboon cell transplantation model as well as in infection experiments performed *in vitro* [5]. There was also no evidence of porcine cytomegalovirus (PCMV; a most common p-herpes virus) infection of the human cells in up to 15 passages [6]. However, the possibility of infection by other viruses, for which there does not exist any diagnostic test, cannot be ignored. Adequate precautions and safety monitoring need to be taken. It is necessary to include experts in the field of infectious diseases and microbiology on the xenotransplantation team.

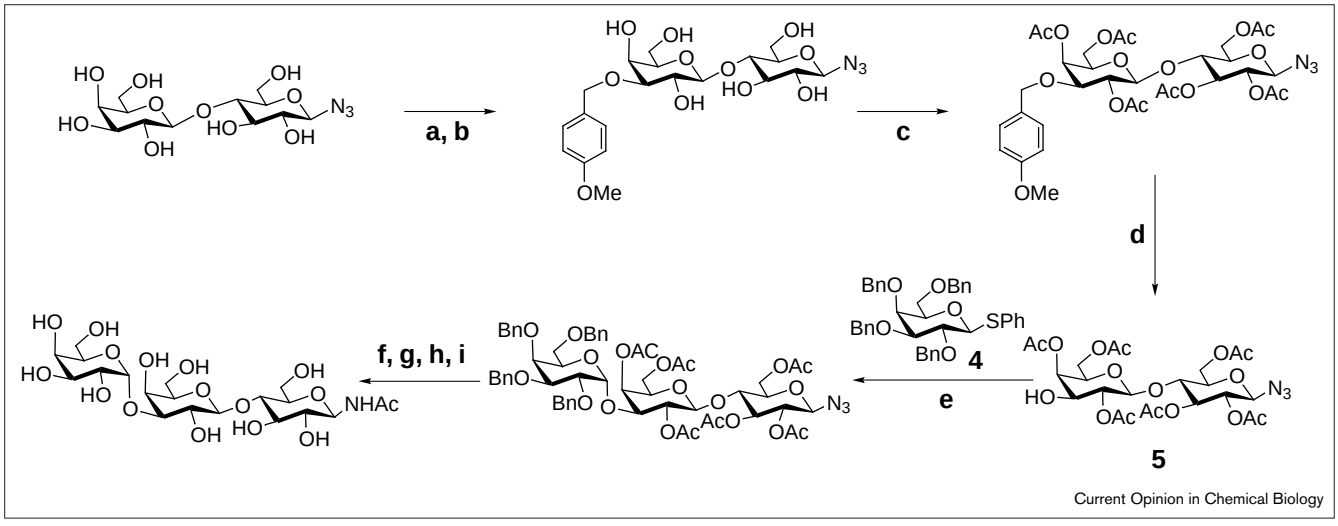
As in other discordant xenotransplantations (xenotransplantations performed between species with large differences), however, hyperacute rejection (HAR) destroys the pig organ in a timescale of minutes to hours in pig-to-human xenotransplantation [7]. It has been established that carbohydrate structures, namely α-galacosyl epitopes, are abundantly

Figure 1



The interplay of anti-Gal antibodies and α-Gal epitopes. **(a)** Large quantities of anti-Gal antibodies exist in the human body; they react with the α-Gal epitope, initiating the complement cascade and thus providing a barrier to xenotransplantation. **(b)** α-Gal structures **1**, **2** and **3** are abundantly expressed on the cell surfaces of mammals other than humans, apes and Old World monkeys.

Figure 2



Chemical synthesis of α-Gal trisaccharide. **(a)** Bu_2SnO, MeOH, reflux. **(b)** *p*-methoxybenzyl chloride (MPMCl), tetrabutylammonium iodide (TBAI), benzene. **(c)** Ac_2O, Py. **(d)** cerium (IV) ammonium nitrate (CAN), CH_3CN/H_2O. **(e)** *N*-iodosuccinimide/triflic acid (NIS/TfOH), CH_2Cl_2, 4 Å molecular sieve (MS). **(f)** PtO_2, H_2, EtOH. **(g)** Ac_2O, Et_3N, CH_2Cl_2. **(h)** Pd/C, H_2, AcOH, MeOH. **(i)** NaOMe, MeOH.

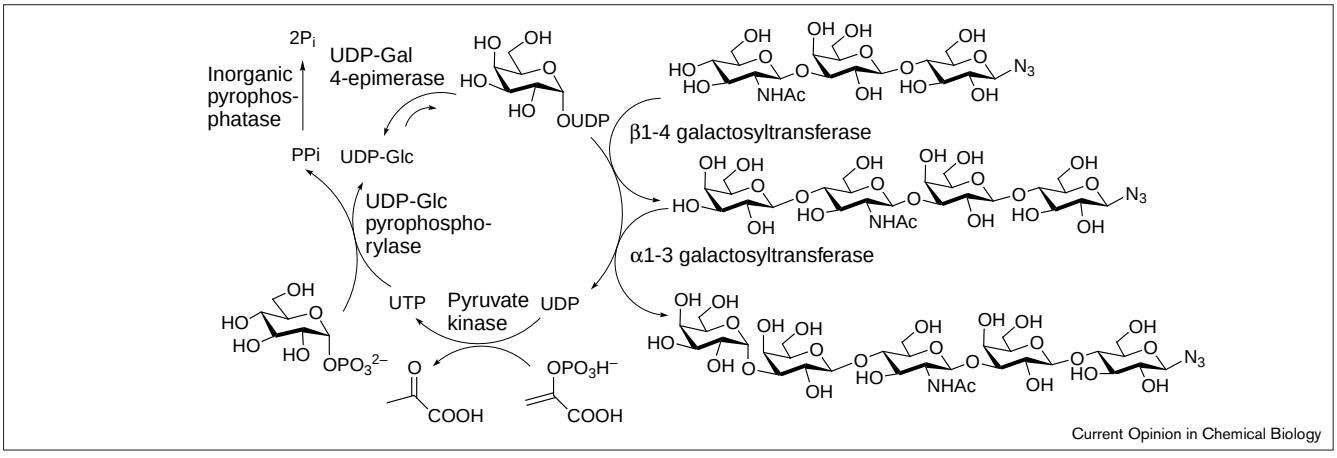
expressed on the surface of pig vascular endothelial cells (1×10^7 epitopes per cell) as well as other mammalian cells other than those of humans, apes and Old World monkeys [8–11]. These include disaccharides Galα1–3Gal–R (Gal, galactose), trisaccharides Galα1–3Galβ1–4GlcNAc–R (**1**, Figure 1; Glc, glucose), or Galα1–3Galβ1–4Glc–R (**2**) and pentasaccharides Galα1–3Galβ1–4GlcNAcβ1–3Galβ1–4Glc–R (**3**). Humans, however, naturally produce large quantities of anti-α-galactosyl antibodies (anti-Gal), which represent 1–3% of all circulating immunoglobulins and are produced by about 1% of all B cells ([12]; Figure 1). When pig organs or tissues are transplanted into the human body, the IgM isotype of anti-Gal bonds to α-Gal epitopes, which causes activation of the complement cascade, resulting in cell lysis [13]. This rapid activation of complement by anti-Gal IgM is an immunological barrier that poses the greatest risk of initiating HAR.

In order to overcome HAR in pig-to-human xenotransplantation, several approaches have been proposed including removal of anti-Gal antibodies through an α-Gal affinity column, inhibition of anti-Gal binding, depletion or inhibition of complement, genetic engineering of pigs with human complement regulatory proteins, generating α-Gal transferase in suppressed or knock out pigs and induction of T-cell and B-cell tolerance as well as elimination of anti-Gal producing B-cells through α-Gal-resin conjugates.

Chemical and enzymatic syntheses were developed to produce adequate amounts of α-Gal oligosaccharide and its derivatives for both experimental and pre-clinical studies. Chemical preparation of α-Gal trisaccharide has been accomplished by several research groups employing varying glycosylation methods [14,15]. A relatively larger scale (50 g) scheme has been developed in Wang's laboratory ([16]; Figure 2). This synthesis involved a stereoselective and high yielding glycosylation between a donor **4** and an acceptor **5**, which was prepared through an efficient dibutylin-oxide-mediated regioselective protection/deprotection strategy. Successful overexpression of α1–3 galactosyltransferase has made enzymatic synthesis of α-Gal epitopes a viable approach [17,18]. To overcome the high cost of uridine diphosphate (UDP)-Gal, researchers either made use of UDP-Glc in conjunction with UDP-Gal 4-epimerase [19] or a UDP-Gal regeneration cycle, which involved multiple enzymes in the UDP-Gal regeneration cycle. Figure 3 shows a double galactosylation pathway to the synthesis of α-Gal pentasaccharide **3** ([18]; Figure 1). This strategy employed pyruvate kinase, UDP-glucose pyrophosphorylase, UDP-Gal 4-epimerase and inorganic pyrophosphatase to regenerate UDP-Gal from glucose-1-phosphate and UDP. This scheme can provide the pentasaccharide on a multi-gram scale. It is expected that more economical synthesis of α-Gal oligosaccharides will be developed as the biomedical need for such materials increases [20,21].

Just as important as xenotransplantation is the development of bioartificial organs by cell engineering using immunoisolated living cells or tissues (microencapsulated cells or macroencapsulated tissues with a semipermeable membrane to protect cells or tissues from the immune system but still allow sufficient passage of nutrients, oxygen, and therapeutic products such as insulin). This method has also shown great potential in treating organ disability. *In vivo* studies have been applied to various organs including the artificial pancreas, liver and parathyroid. The immunoisolated cell transplantation does not require the immunosuppression of recipients and allows the selection

Figure 3



One-pot enzymatic synthesis of pentasaccharide Galα1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcβN$_3$ with *in situ* cofactor regeneration. β1-4 galactosyltransferase adds a Gal from UDP-Gal to its acceptor trisaccharide by β1,4-linkage to form a tetrasaccharide. Subsequently, α1-3galactosyltransferase (α1-3GalT) adds another Gal from UDP-Gal to the chain to form a pentasaccharide (α-Gal epitope pentasaccharide). The donor UDP-Gal for both galactosyltransferases is produced by the regeneration system consisting of pyruvate kinase (which transforms the product UDP of the galactosyltransferase-catalyzed reaction to UTP in the presence of phosphoenolpyruvate), UDP-Glc pyrophosphorylase (which transforms UTP to UDP-Glc in the presence of Glc-1-phosphate), UDP-galactose 4-epimerase (which catalyzes the interconversion of UDP-Gal and UDP-Glc) and inorganic pyrophosphatase (which converts pyrophosphate to phosphate, omitting any possible inhibition effect caused by the pyrophosphate).

of cells from nonhuman mammals. The bioartifical organ usually has a capsule membrane consisting of natural or modified polysaccharides as an ideal cage to protect both the organ cells and the host, and to allow the cells to have normal physiological functions [22].

This review focuses mainly on the recent applications of carbohydrate materials in xenotransplantation. In addition, novel applications of carbohydrate polymers in making artificial organs by cell/tissue engineering will be briefly discussed.

Carbohydrates in blood-group-incompatible allotransplantations

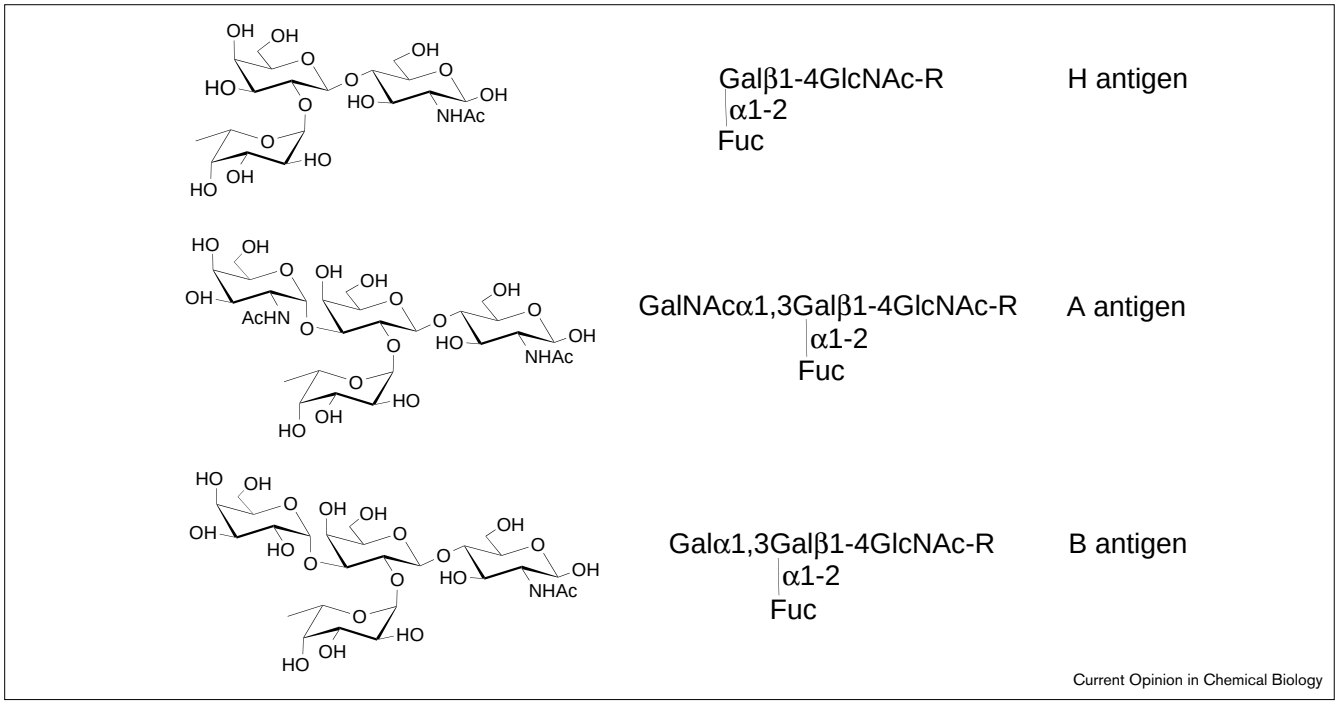
The very first example of carbohydrates in transplantation was performed in ABO blood-group-incompatible allotransplantation. A, B, AB and O are major human blood-group types and A, B and H antigens are structurally related oligosaccharides (Figure 4). People with type A blood have A antigens on the surface of their red blood cells and anti-B antibodies in serum. People with type B blood have B antigens and anti-A antibodies. People with type O blood have H antigens and anti-A as well as anti-B antibodies, while people with type AB blood have A and B antigens and neither anti-A nor anti-B antibodies. Thus, when type A or AB blood is transfused into a person with blood type B or O, the anti-A antibodies of the recipient bind to the transfused erythrocytes and trigger their destruction elicited by the immune system. Similarly, type B or AB blood cannot be transfused into patients with blood type A or O. HAR occurred in such transplantations and caused many inconveniences (such as having the patient wait for the suitable blood-group type of a donor organ or receive large quantities of immunosuppressive drugs) because the blood-group types of the human donor organs available were, in most cases, different from those of the recipients. Bannett *et al.* [23,24] demonstrated the possibility of clinically successful ABO-incompatible renal allotransplantation by depleting anti-A or anti-B antibodies from the recipient's blood using a column of synthetic A or B trisaccharide (Figure 4). The efficacy of this methodology was also confirmed in bone marrow transplantation. Cooper *et al.* [25] used synthetic A or B trisaccharides to inhibit HAR in baboons and prolonged the function of ABO-incompatible heart grafts. Using this 'specific intravenous carbohydrate therapy', onset of HAR was delayed from 19 min to a mean of > 28 days. 'Accommodation' [26] or 'self-tolerance' (under such conditions the grafted tissue would be treated in the same way as other self-components) was achieved in all of these cases. This state of specific tolerance is the ultimate goal of the transplant surgeon [1].

Solid α-Gal affinity column

Prevention of HAR is the first step for any successful xenotransplantation of porcine organs into humans. Various methods have been developed for this purpose, including genetic manipulation of the pig, inhibition of the interaction of α-Gal and anti-Gal, and induction of the tolerance of recipient by bone marrow transformation [27–29]. Among these approaches, the simplest and the most applicable one uses the same concepts as for ABO-incompatible allotransplantation; that is, to remove anti-Gal from the recipient's blood with an α-Gal immunoabsorption column.

Removal of anti-Gal using a melibiose Galα1–6Glc column combined with the intravenous infusion of

Figure 4



Structures of H, A and B antigens. A antigen differs from H antigen in having a GalNAcα1,3 group connected to the non-reducing end of Gal. B antigen differs from H antigen by having a Galα1,3 group connected to the non-reducing end of Gal. A, B and H antigens are expressed on the erythrocyte surface of blood types A, B and O, respectively. Fuc, fucosyl.

melibiose or arabinogalactan extended the survival of a pig heart graft to 12 hr in a baboon [30••,31]. A more applicable approach, however, was to use a column consisting of macroporous glass beads linked with synthetic α-Gal disaccharides by flexible hydrophilic polymer poly[*N*-2-hydroxyethylacrylamide] (PAA) ligands. In this case, the serum anti-Gal level and serum cytotoxicity were significantly reduced and resulted in the delay of HAR of a transplanted pig organ to about 3 weeks in an immunosuppressed splenectomized baboon [32].

Xu *et al.* [30••] demonstrated *in vitro* that an α-Gal trisaccharide (**2**; Figure 1) column was more effective than an α-Gal trisaccharide **1** column in removing anti-Gal. Compared to hemoperfusion through a pig liver, the α-Gal, trisaccharide **2** column caused less hypotension and reduction in platelets in an *in vivo* cynomolgus monkey (*Macaca fascicularis*, Old World monkey) plasma perfusion, although both were sufficient to deplete anti-Gal IgM and IgG and efficiently prevented the HAR of a transplanted pig kidney [30••].

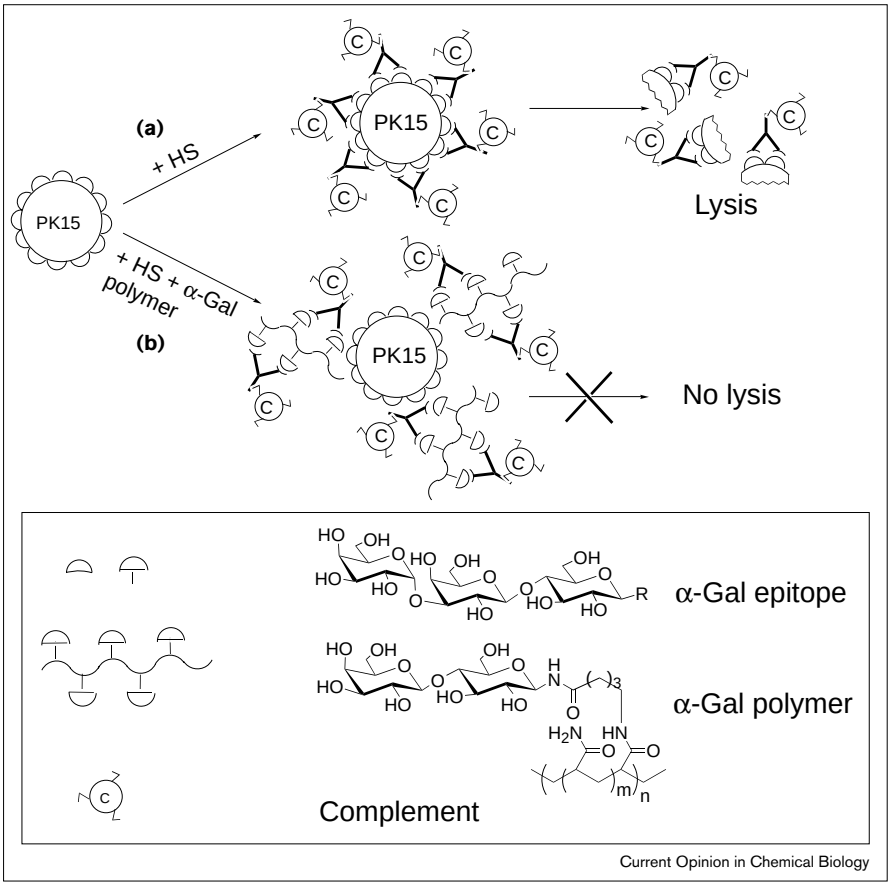
More detailed studies on α-Gal immunoadsorption columns were presented by Kozlowski *et al.* [33••,34] who investigated the antibody removal in baboons under immunologically naïve (no previous treatment), immunosuppressed (treated with conventional pharmacologic immunosuppressive therapy such as cyclosporine administration to reduce the pool of anti-Gal) or tolerance-induced (treated with a conditioning regimen such as whole body irradiation, porcine bone marrow transplantation, etc.) conditions. They demonstrated that removal of anti-Gal by α-Gal affinity columns had the same efficacy using whole blood or plasma (the anti-Gal antibody was decreased to 0–19% of pre-treatment levels for both IgG and IgM after a single immunoadsorption for either blood or plasma), whereas plasma perfusion was preferred if multiple adsorptions were required. The plasma perfusion method avoids potential activation of recipient leukocytes. The first three or four adsorptions provided additional reductions in the level of antibody. Splenectomy and/or immunosuppressive treatment delayed the return of anti-Gal.

In addition to traditional synthetic α-Gal oligosaccharides and common chromatographic beads, Klein, Euler and Vercellotti [35•] immobilized a hydrazide-modified microporous nylon membrane with Galα1–3Gal-bearing oligosaccharides derived from the degradation of λ-carrageenan, a natural polysaccharide known to possess alternately α1–3 and β1–4-linked D-galactopyranose sulfate residues. Complete inhibition of HAR was observed in these experiments.

Soluble α-Gal antagonists

Similar to the neutralization of anti-ABO antibodies for prolonging the survival of ABO-incompatible allografts, an alternative approach for the prevention of HAR in pig-to-primate xenotransplantation would be infusing synthetic or natural soluble α-Gal oligosaccharides continuously into the

Figure 5



Schematic illustrating the protection of pig cells from human serum cytotoxicity by α-Gal polymers. **(a)** In the absence of α-Gal polymers, the anti-Gal antibodies (Y-shaped structures) in human serum (HS) bind to PK15 cells, inducing complement fixation and cell lysis. **(b)** In the presence of α-Gal polymers, polyvalent α-Gal polymers bind to anti-Gal with high affinity, protecting PK15 cells from complement lysis. The structures of the α-Gal epitope and polymer are shown in the inset.

recipient's circulation. In this case, infused synthetic oligosaccharides are bound by anti-Gal antibodies, which are thus prohibited from binding to the identical structure (epitope) on the transplanted organ and preventing HAR. Progress has been made in both *in vitro* and *in vivo* studies, although the procedure was not as successful as the cases involving ABO-incompatible allotransplantation [27].

Disaccharide melibiose, polysaccharide arabinogalactan, and glycans derived form porcine gastric mucin, as well as glycomimetic peptides, have been used intravenously to inhibit anti-Gal in primates [30••]. More recent *in vivo* infusion experiments indicated that trisaccharide **1**, **2** or pentasaccharide **3** (Figure 1) were 2–4 times more efficient than melibiose or arabinogalactan [36••]. The most detailed *in vitro* demonstration of the efficacy of the α-Gal oligosaccharides in the protection of cultured pig cells from cytotoxicity of human or baboon sera was provided by Neethling *et al.* [37]. Among the 28 compounds tested, the α-Gal-based carbohydrates had various effects in protecting PK15 cells (pig kidney epithelial cells) which express large amounts of α-Gal epitopes on the cell surface [9]. The disaccharide Galα1–3Galβ–O$(CH_2)_2COOCH_2$ obtained from Chembiomed Inc. and the Alberta Research Council (Edmonton, Alberta, Canada) showed the highest efficacy. Non-α-Gal-based carbohydrates did not significantly protect PK15 cells.

Simon *et al.* [36••] demonstrated that intravenous infusion of α-Gal trisaccharide **2** or pentasaccharide **3** assisted in delaying, but did not block, HAR in an *in vivo* pig-to-baboon cardiac xenotransplantation. Because they are small water-soluble molecules, the oligosaccharides were rapidly cleared from the blood, having a serum half-life of 45–50 min and a clearance rate of 2–5 ml/min/kg when administered at 0.5 mmol/kg into baboons. In order to prolong the inhibition of anti-Gal, polyethylene glycol (PEG) conjugates of α-Gal disaccharides and trisaccharides were synthesized. The introduction of PEG increased the molecular weight, delaying the excretion of the α-Gal oligosaccharides. PEG also acted to precipitate complement components and reduce immunogenicity. Not only was the serum half-life prolonged upon the introduction of polyethyleneglycol, but also the inhibition effect against complement cytotoxicity was enhanced, although weaker binding affinity to IgM was noticed [38].

Unlike the infusion of soluble synthetic A or B blood group oligosaccharides in ABO-incompatible cardiac allografting in baboons [27], no accommodation was achieved in the

infusion of α-Gal oligosaccharides to prevent the HAR of a xenotransplanted pig organ. This may be for two reasons. One possibility is that there are many more anti-Gal antibodies in human serum than anti-A or anti-B antibodies. Anti-Gal is the most abundant natural polyclonal antibody in human sera (including IgG, IgM and IgA) [39]. Anti-Gal IgG comprises 1.0–2.4% of total serum IgG, whereas anti-Gal IgM comprises 3.9–8.0% of total serum IgM [40,41]. In contrast, anti-A or anti-B IgG comprises about 0.001% of total serum IgG, whereas anti-A or anti-B IgM comprises about 0.01% total serum IgM [42]. A second explanation is the presence of discordant complement in a pig-to-baboon xenotransplantation model as a transplanted baboon allograft can be protected from the recipient baboon complement by the presence of species-specific complement regulatory proteins on the surface of its cells. A transplanted pig organ has no such protection, however, and renders the vascular endothelium to complement-mediated injury. A transgenic pig with human complement regulatory protein may be helpful for graft accommodation [29].

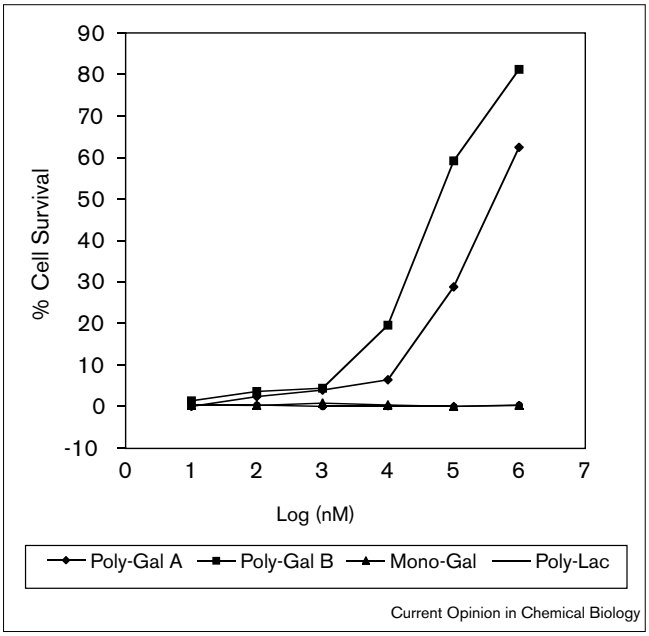
Multivalent α-Gal polymers

The synthetic monomeric α-Gal oligosaccharides used in the inhibition of the human serum cytotoxicity displayed a generally low binding affinity for anti-Gal, thus α-Gal monomers were always required in high dosages in order to be effective. In order to increase the efficacy of α-Gal antagonists, a series of multivalent α-Gal polymers were synthesized in our laboratory with a polyacrylamide backbone connected to varying densities of Galα1–3Galβ1–4Glcβ trisaccharide epitopes. Inhibition enzyme-linked immunosorbent assays (ELISAs) and flow cytometry assays demonstrated a dramatically enhanced inhibitory effect in α-Gal epitope and anti-Gal interaction by α-Gal polymers compared with the monomeric α-Gal system [43••]. For example, the IC_{50} (concentration of inhibitor at 50% inhibition of the binding between antibodies and antigens) of α-Gal 'polymer B' (contains 28% α-Gal epitopes) in the inhibition ELISA for IgM was 35 nM compared to 268 μM for the α-Gal monomer. In the flow cytometry assay, the IC_{50} was enhanced from >>1 mM for the monomer to 2.2 μM for polymer B.

Further *in vitro* cytotoxicity assays demonstrated the enhanced effect of α-Gal polymers in protecting PK15 pig kidney cells from human serum cytotoxicity (Figure 5). The trypan-blue dye exclusion assay for complement-mediated cytolysis showed that 1 mM of 'polymer A' (containing 36% α-Gal epitopes) prevented 62% PK15 cells from human serum cytotoxicity, and polymer B protected 81% cells at 1 mM concentration. Under the same conditions, no effect was obtained by α-Gal trisaccharide monomer or polylactose (Figure 6; PG Wang *et al.*, unpublished data).

It is worth mentioning that apart from α-Gal epitopes, several other carbohydrate antigens involved in transplantation have been identified, including Galα1–3Le^x, Hanganutziu–Deicher (gangliosides containing the sialic acid, *N*-glycolylneuraminic acid) [44], Tn (transcription factor precursor, GalNAcα–R), and Forssman (GalNAcα1–3GalNAcβ1–3Galα1–4Galβ1–4Glcβ1–R) antigens [27,28] (Figure 7). These carbohydrate derivatives may also be useful targets for human natural antibodies in transplantation although their roles and functions have not yet been clearly outlined.

Figure 6

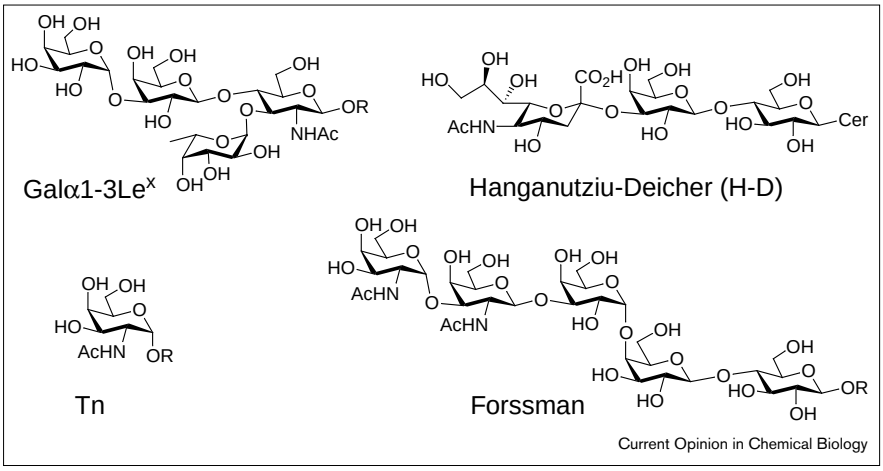


Protection of PK15 cells from the cytotoxicity of human serum at different concentrations of α-Gal polymer A and B (Poly-Gal A and Poly-Gal B) compared to that of α-Gal monomer (Mono-Gal) and polylactose (Poly-Lac). The percentage inhibition for each sample was calculated according to the following equation: % cell survival = $(C_I - C_{MIN})/(C_{MAX} - C_{MIN}) \times 100\%$ (where C_I = number of live cells in the mixture of inhibitor and human serum, C_{MAX} = number of live cells in the mixture of phosphate buffered saline (PBS) and heat-inactivated human serum, and C_{MIN} = number of live cells in the mixture of PBS and human serum).

Carbohydrate polymers in tissue/cell engineering

Because of their biocompatibility and other properties, carbohydrate polymers have great advantages in making supporting materials or capsule membranes in animal bodies. Since alginate was first introduced by Lim and Sun [45] in microencapsulation of isolated islets, many modifications and improvements have been made [46]. Alginates are a family of polysaccharides composed of homopolymeric regions of β-D-mannuronic (M) and α-L-guluronic (G) acid as well as mixed sequences (M–G blocks). The structure is compatible *in vivo* although it was noticed recently that mannuronic acid residues in alginate are able to induce cytokine release [47]. To overcome the heterogeneous distribution of the capsular membrane by an entrapment model, the capsule membrane of an artificial pancreas, made from sodium-alginate-based materials with a thicker capsule wall and bigger pore size, was able to allow the free exchange of nutrients and metabolic waste,

Figure 7



Structures of Galα1–3Le^x, Hanganutziu–Deicher, Tn (transcription factor precursor, GalNAcα–R) and Forssman antigens.

and was durable enough to survive handling, transplantation and the hostile environment inside the human body [22]. Alginate-based materials are also becoming increasingly popular in wound dressings to prepare a suitable surface for skin transplantation, especially in the treatment of chronic wounds producing large amounts of wound fluid. A fiber-free alginate wound dressing verified that improved absorption rates result in quicker haemostasis. This technology has also made dressing removal easier and better for wound re-epithelialisation [48].

Agarose is a carbohydrate polymer predominantly composed of repeating units of alternating β-D-galactopyranosyl and 3,6-anhydro-α-L-galactopyranosyl units. It is stable *in vivo* and able to form a firm gel at low concentration. An agarose-based microcapsule containing allo-islets (islets of individuals from the same species) was successfully applied as a bioartificial pancreas in a mouse [49], as well as in a dog model [46].

Glycosaminoglycan, a biodegradable and biocompatible carbohydrate polymer, plays a critical role in cell attachment, differentiation, and morphogenesis. As an alternate to an allodermis, a collagen–glycosaminoglycan matrix is an attractive synthetic skin substitute in cultured epithelial autografts (or cultured autologous keratinocytes) and occupies an important position in skin transplantation. It also has the advantages of facile manufacturing and storage. The vascularized collagen–glycosaminoglycan matrix was shown to provide an excellent wound bed for cultured epithelial cells in a pig model [50].

Chitosan — (1,4)-linked 2-amino-2-deoxy-β-D-glycopyranose — was evaluated as a scaffold biomaterial for anchorage-dependent hepatocyte support in a fetal-pig-hepatocyte-to-rat xenotransplantation model, as well as evaluation by *in vitro* experiments. Similar to glycosaminoglycans, chitosan-based biomaterial surfaces have good hepatocyte attachment properties and are feasible scaffolds for creating liver tissue organoids [51,52].

Conclusions and future perspectives

In summary, carbohydrate materials play important roles in transplantation and tissue engineering. Mimetics of graft carbohydrate antigens can be immobilized on columns or membranes to deplete the corresponding antibodies in the recipient, both in ABO-incompatible allotransplantation and pig-to-primate xenotransplantation. Monomeric, soluble antigen antagonists can be infused into the recipient's body and result in the delay of HAR. Future work in this area is expected to involve the development of highly efficient polyvalent α-Gal immunosorbent columns to remove specific isotypes of anti-Gal, development of rational design and/or combinatorial screening of α-Gal epitope mimetics for better affinity and bioavailability, and investigations utilizing α-Gal glycoconjugates to specifically destroy anti-Gal-producing B-cells to eliminate chronicle rejection in xenotransplantation. It should be emphasized that a successful xenotransplantation is a very complex process that will require several different approaches in unison. We believe that a combination of antibody removal by immunoadsorption column and intravenous infusion of soluble α-Gal oligosaccharides with other techniques, such as using powerful immunosuppressive reagents and inducing B-cell or T-cell tolerance, will lead to a practical solution to successful clinical xenotransplantations. Moreover, it can be expected that natural and synthetic carbohydrate polymers will find increasing applications in creating bioartificial organs, making wound dressings, and constructing the supports for cultured epithelial or tissue cells.

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