The reducing end of \( \alpha \text{Gal} \) oligosaccharides contributes to their efficiency in blocking natural antibodies of human and baboon sera

Abstract  Synthetic galactosyl oligosaccharides were tested for their ability to inhibit the cytotoxic reaction of human and baboon natural antibodies on PK15 cells in culture. Methyl-\( \alpha \)-Gal gave weak inhibition, Gal\( \alpha \)-3Gal substantially inhibited the reaction (400 \( \mu \)M), and Gal\( \alpha \)-3Gal/3-4GlcNAc was ten times more efficient (30 \( \mu \)M). The modification from \( \alpha \) to \( \beta \) anomeric configuration of the nonreducing end resulted in a complete loss of activity, while substitutions at the reducing end induced only a partial loss of activity. These observations suggest that natural anti-\( \alpha \)-Gal antibodies recognize the epitope from its nonreducing end, but that substitutions at the reducing terminus can modify the antibody-binding capacity. Modified tri- and tetrasaccharides are better inhibitors than the disaccharide but not as good as Gal\( \alpha \)-3Gal/3-4GlcNAc. The reducing terminus therefore contributes some energy to the reaction, indicating that certain oligosaccharides will be of more potential clinical use than others.

Keywords  Xenotransplantation, natural antibodies, oligosaccharides - Oligosaccharides, xenotransplantation - Natural antibodies, xenotransplantation - Baboon, xenotransplantation

Introduction  New World monkeys and lower mammals express the Gal\( \alpha \)-3Gal epitope on vascular endothelium [5, 14, 18]. Old World monkeys and humans have nonfunctional \( \alpha \),3-galactosyltransferase genes [7, 9, 12], do not express the Gal\( \alpha \)-3Gal epitope in tissues, and have developed natural antibodies that react with this antigen [6]. These antibodies can be responsible for the hyperacute rejection of pig vascularized organs transplanted into higher primates and for the cytotoxic reactions obtained on pig cells incubated in the presence of human or baboon normal serum and complement [1, 3, 4, 6, 11, 13, 15, 19, 20].

The main \( \alpha \)-Gal glycolipid extracted from pig vascular endothelium has been shown to be the neutral penta-glycosylceramide Gal\( \alpha \)-3Gal(3-4GlcNAc)3Gal(3-4Glc)1-Cer [17], and a similar oligosaccharide structure may be present on pig cell membrane glycoproteins. However, the components of this pentasaccharide that contribute most significantly to the binding of natural anti-\( \alpha \)-Gal antibodies to the tissue target epitopes have not yet been defined.

The recent chemical synthesis (ChembioMed, Dexstra Laboratories, and Syntosome) [10] and enzymatic synthesis [8] of some di-, tri-, and tetrasaccharides with terminal nonreducing \( \alpha \)-Gal structures have allowed us to investigate various \( \alpha \)-Gal oligosaccharides for their efficiency as blockers of the “in vitro” cytotoxic reaction of natural anti-\( \alpha \)-Gal antibodies on the pig kidney cell line (PK15) that expresses the \( \alpha \)-Gal epitope.
**Materials and methods**

**Live/dead cytotoxicity test**

PK15 cells were seeded in Terasaki microcytotoxicity plates at 750 cells per well and grown for 24 h at 37°C in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum, penicillin/streptomycin 10,000 U/ml, and glutamine 200 mM. After washing, 10 μl of human, baboon, or owl monkey serum in serial dilutions was added and the mixture was incubated for 60 min at 37°C, washed again, and incubated for 30 min at room temperature with a mixture of 10 μl of live/dead fluorescent reagents, calcein AM 1 μM, and ethidium homodimer 2 μM (Molecular Probes, Eugene Ore., USA). Live (green cytoplasm) and dead (red nuclei) cells were counted in an inverted fluorescence microscope as previously described [11, 13].

**Results**

**Cytotoxic reaction**

The PK15 cell line expressed large amounts of αGal epitopes, which were stained with the labeled lectin I-B4 of *Griffonia simplicifolia* (GSIB4, Vector Laboratories, Burlingame, Calif., USA) or with labeled human or baboon anti-αGal antibodies, affinity-purified on the Galα1-3Galβ1-4GlcNAc-Synsorb immunoabsorbent (Chembiomed, Alberta Research Council, Edmonton, Canada).

Incubation of PK15 cells in the presence of fresh human or baboon serum resulted in almost 100% lysis of cells (Fig.1). This reaction is complement-mediated since inactivation of complement by heating at 56°C for 30 min or by the addition of EDTA abolished the reaction. The full cytotoxic reaction was restored by the addition of fresh rabbit serum as a source of complement. Incubation of cultured PK15 cells under similar conditions with owl monkey serum (*Aotus trivirgatus*, a New World monkey) did not give a significant cytotoxic reaction (Fig.1).

Oligosaccharides

Monosaccharides were obtained from Sigma Chemicals (St. Louis, Mo., USA). Four synthetic oligosaccharides with αGal on the reducing end – G203 (Galα1-3Gal), G334 (Galα1-3Galβ1-4Gal), G443 (Galα1-3Galβ1-4Galα1-3Gal), and GN334 (Galα1-3Galβ1-4GlcNAc) – were obtained from Dextra Laboratories (Reading, UK). Other related oligosaccharides were obtained from Synsomes (Munich, Germany) and the solid immunoabsorbent Galα1-3Galβ1-4GlcNAcβ-Synsorb was obtained from Chembiomed (Alberta Research Council, Edmonton, Canada).

Incubation of PK15 cells in fresh serum (solid symbols) from human (▼), baboon (▲), and owl monkey (●) on PK15 pig cells in culture. The unshaded symbols indicate the cytotoxic reaction of human serum after one absorption (▼) and three absorptions (▲) on the Galα1-3Galβ1-4GlcNAcβ-Synsorb. Percent dead cells is represented on the ordinate and the reverse of the serum dilution on the abscissa.