importance in hyperacute rejections of ABO-incompatible grafts and accumulating evidence supports that anti-carbohydrate antibodies are of primary importance in the rejection of xenografts. We have analyzed serum samples from four patients transplanted with pig islet cells at Huddinge hospital, Stockholm. The binding of IgM and IgG antibodies to glycosphingolipid antigens prepared from pig tissues and separated on thin layer plates have been investigated in pre- and posttransplant serum samples.

Results: In the pretransplant serum samples IgM antibodies bound weakly to glycolipids in the five sugar region known to contain the linear B structure, Galα1-3Gal. In the post-transplant serum samples the binding appeared stronger but with the same specificity. IgG antibodies showed stronger binding in the pretransplant serum samples to several glycolipid fractions from the 3-5-sugar region but also to compounds with longer carbohydrate chains. In the posttransplant samples stronger binding was apparent but there were no convincing evidence of the recognition of new specificities. Adsorption to a Synsorb^R-column with Galα1-3Gal specificity did not grossly change the binding pattern of the IgG antibodies. However, the eluate from the column showed strong binding to the linear B compound but also to glycolipids with longer carbohydrate chains presumably with the same terminal epitope. Identification of these are in progress.

Conclusion: The human anti-pig antibodies detecting carbohydrate epitopes expressed on pig cells show a complex pattern. The linear B structure appears to be one major target. More work is needed to identify other targets for the xenoreactive antibodies.

S12.7

Carbohydrate Antigens as Targets for Xenoreactive Antibodies in a Mouse to Rat Transplantation Model

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There is a great clinical interest in the possibility of using animals as organ donors to humans. The greatest problem is that all donors, except anthropoid apes, give rise to a hyperacute rejection of transplanted organs (1).

Mouse to rat is a model where the graft isn't hyperacutely rejected. However, repeated transplantation of mouse organs to the same rat induces hyperacute rejection. In our model, mouse heart is heterotopically grafted to the neck vessels of the rat. The graft works for several days without rejection. Transplantation of a second mouse heart to the contralateral neck vessels gives rise to rejection of the graft within a minute, expressing all features of a hyperacute rejection (2).

Total acid and non-acid glycosphingolipids of nine different organs have been prepared from the donor, NMRI mouse, and recipient, Lewis rat, strains. Thin layer plates were stained with sera from ungrafted rats and rats grafted twice with mouse hearts. Sera from grafted rats show clear antibody binding to a neutral glycolipid migrating in the five-sugar region. The same structure is the dominating glycolipid in vascular tissue. The antibodies are mainly of IgG and IgM class, in a pattern similar to that after pig to human xenotransplantation. Sera from ungrafted rats only show a weak and non-specific IgM and IgA binding.

Work is currently in progress to determine the structure of the glycolipid antigens and investigating reactivity of the same rat serum to glycoproteins from the donor strain.

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S12.8

Pig to Human Xenotransplantation. Confirmation of the Major Target Epitope of Pre-Formed Human Natural IgG and IgM Anti-Pig-Terminal Galactoseα1,3 Galactose

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We have now accumulated evidence, both structural and serological, that endothelial glycoconjugates terminating in galactoseα 1,3 galactose (Galα1,3 Gal) form the dominant target antigens in the human natural IgG *and* IgM anti-pig repertoire.

1. ELISA demonstrates that humans have both IgG and IgM that bind to Galα 1,3 Gal. We have found that the titres of IgG and IgM anti-Galα 1,3 Gal in 10 normal human volunteers (of blood group AB) are both about 1 in 100. (A Galα 1,3 Gal-BSA conjugate [Biocarb] was used). This contradicts the existing literature which suggests variously, that human antibodies to oligosaccharides terminating in αgalactose are predominantly IgM[1], primarily IgG[2], or exclusively IgG[3].

2. Immunohistology demonstrates that a mouse monoclonal IgM against Galα 1,3 Galβ 1,4, GlcNAc (TH5 — the kind gift of Drs H. Clausen & S. Hakomori) binds to vascular endothelium in pig kidney and aorta.

3. Glycosphingolipids have been prepared from pig kidney and aorta. Human IgG and IgM, both from whole sera and in eluates from sera adsorbed on Galα 1,3 Gal-conjugated Synsorb, bind to fractions that have been shown by NMR and tandem mass spectrometry to contain glycosphingolipids that terminate in Galα 1,3 Gal.

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S12.9

Natural Anti-GalNAcα1-3Gal Specific Antibodies in the Serum of Blood Group A Individuals