MS data set on tissue-derived heparan sulfate oligosaccharides. The software, code and a test data set are publically archived under an open source license.

(288) Evolutionally Conserved Blood Group Glycolipids are Endogenous Antigens forInvariant Natural Killer T Cells

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CD1d-restricted invariant natural killer T cells (iNKT) express evolutionally conserved V 14 $^{+}$ (mouse) or Va24^{+} (human) T-cell receptors (TCR), which are defined by specific recognition of the marine sponge.derived glycosphingolipid a-galactosylceramide. During development of iNKT cells in the thymus, endogenous lipids are necessary for positive selection of randomly generated TCRs from mainstream T-cell precursors. The identities of the self-lipids that select iNKT cells *in vivo* remain elusive. Our glycosphingolipidomics analysis of thymuses of mouse, pig, and human identified blood group glycosphingolipids that are structurally but not genetically related to isoglobotriaosylceramide (iGb3), a previously identified ligand for iNKT cells. We found that some of these iGb3-related glycosphingolipids can activate iNKT cells and stimulate their production of cytokines. These findings indicate a surprising function of evolutionally conserved blood group glycolipids as agonist ligands for iNKT cells and reveal the presence of multiple ligands that might be related to the physiology of iNKT cells.

(289) Formation of an enzyme complex for efficent production of keratan sulfate glycosaminoglycan

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Keratan sulfate (KS) proteoglycan is one of the major components of the corneal extracellular matrix and suggested to have an important role for function of the cornea. Corneal KS glycosaminoglycan (GAG) is highly sulfated by two distinct carbohydrate sulfotransferases, corneal GlcNAc 6-*O* sulfotransferase (CGn6ST, also known as GlcNAc6ST-5/GST4) and KS Gal 6-*O* sulfotransferase (KSG6ST). We prepared a lentiviral vector that produces both sulfotransferases in the infected cells and infected the lentiviral vector to different cultured cell lines, HeLa cells and human corneal epithelial (hCE) cells. We confirmed the sulfotransferases are expressed in the infected cells and also detected production of highly sulfated KS GAG in hCE cells. However, production of highly sulfated KS GAG is minimal in HeLa cells, suggesting that HeLa cells lack required components for efficient production of the carbohydrate. We then analyzed gene expression of β1,3-GlcNAc transferases by RT-PCR and found that HeLa cells lack expression of *B3gnt7*, which encodes β1,3-GlcNAc transferase-7 (β3GnT7). To confirm the requirement of β3GnT7 for highly sulfated KS GAG production in HeLa cells, we overexpressed β3GnT7 in sulfotransferase-expressing HeLa cells and observed enhanced production of highly sulfated KS GAG in the cells. By immunoprecipitation analysis, we found that three tagged enzymes, β3GnT7-HA, CGn6ST-flag and KSG6ST-T7, were co-precipitated by anti-FLAG antibody-conjugated beads, indicating that the three enzymes form a complex to process efficient KS GAG production in the cells.

(290) Complex *N*-glycans are essential for spermatogenesis

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Complex *N*-glycans are generated in the Golgi following the action of MGAT1 (GlcNAcT-I), the enzyme that transfers GlcNAc to initiate hybrid and complex *N*-glycan synthesis. This class of glycans is essential for life. Mouse embryos that lack MGAT1 cannot survive beyond embryonic day 9.5 (E9.5). We have investigated the role of MGAT1 during spermatogenesis using conditional deletion in spermatogonia via a testis-specific *Cre* recombinase transgene driven by the Stra8 promoter (Stra8-*iCre*). The deletion efficiency of MGAT1 floxed alleles was 100%, as shown by the transmission of the deleted allele by heterozygous mice, as well as by lectin histochemistry of conditional mutants. Males, in which the synthesis of complex *N*-glycans was blocked by deletion of *Mgat1* in spermatogonia, did not produce sperm. Sertoli cells, spermatogonia and spermatocytes appeared normal on histological analyses, while most spermatids formed giant multinucleated cells (MNCs) or symplasts associated with increased apoptosis. The phenotype became visible during the first wave of spermatogenesis at 22–26 days post-partum (dpp), when spermatids begin to appear. Both primary and secondary spermatocyte numbers were unchanged at 28 dpp. The mutant mice failed to produce complex *N*-glycans as shown by lectin histochemistry of testis sections using L-PHA and GSA, while high-mannose structures are increased in testes of mutant mice, as revealed by Con A histochemistry. Western blot analyses using a monoclonal antibody against basigin, an *N*-glycoprotein highly expressed in elongated spermatids, combined with PNGase F or Endo H digestions, confirmed that complex *N*-glycans were not detectable in mutant mice and revealed that a small fraction of basigin in wild-type mice remained modified with high-mannose glycans. This high-mannose fraction is consistent with the presence of an inhibitor of MGAT1 that is expressed in spermatocytes. The inhibitor is a Golgi glycoprotein termed GlcNAcT-I inhibitory protein (GnT1IP). A membrane bound form (GnT1IP-L) is the active and specific inhibitor and it is expressed in spermatocytes but not spermatids of adult mice. We are investigating the hypothesis that down-regulation of complex *N*-glycans in spermatocytes is required for their interactions with Sertoli cells and the progression of spermatogenesis. Supported by grant RO1 30645 to P.S.