main cause of hyperacute rejection during xenotransplanation. The major xenoactive antigens have been identified as carbohydrate structures containing disaccharide sequence aGal1,3bGal (terminal structure of a-Gal epitopes). The unique enzyme responsible for the formation of this disaccharide on mammalian cell surface is UDP-Gal:Galb1(r)4GlcNAc a-1,3-galactosyltransferase (a-1,3GT) (EC 2.4.1.151), a protein which is absent in humans, apes and Old World monkeys due to mutational inactivation of the gene. Full-length a-1,3GT is a type II membrane protein containing a short N-terminal cytosolic domain, a membrane spanning region, a stem and a C-terminal catalytic region. The amount of a-1,3GT available from natural is very limited. It is necessary to get a large quantity of active a-1,3GT for both organic synthesis and glycobiological study. Thus, the gene of a truncated catalytic domain of bovine a-1,3GT (80-368) was inserted into expression vector pET15b that contained the coding sequence for 6-Histidine tag, an ampicillin resistant sequence and T7 promoter. The constructed plasmid was transformed subsequently into *E. coli* strain DH5a as a cloning host and then BL21(DE3) cell line as an expression host. In the cell lysate, the enzyme was expressed at a level of approximately 60 Unit/L. The purified enzyme has an expected MW 36,000 with a specific activity of 10.6 Unit/mg. Such a high level of expression of a soluble glycosyltransferases indicated that the cloning and expression system we have been using was very suitable for such galactosyltransferases. Using the same expression system we recently cloned and expressed both truncated (82-371) and full-length (1-371) porcine a-1,3GT. The acceptor specificity, enzyme specific activity and kinetic parameters will be compared with each other and those of the natural enzyme. The recombinant enzyme has been successfully used in enzymatic synthesis of a variety of a-Gal epitope derivatives for immunotherapy study.

Although some published data suggested that cell surface carbohydrates involved in the metastatic process, the directed experimental confirmations for the role of particular carbohydrates such as a-Gal epitopes in metastasis formation are actually missing. Gene transfection into the cells that fail to express the appropriate glycosyltransferase and synthesize cell membrane carbohydrate will be a more precise analysis of possible role of cell surface carbohydrates in the metastatic process. By our observation, human prostate cancer cell DU145 did not have a-Gal epitopes on cell surface. In order to investigate the relationship among a-1,3GT, a-Gal epitopes and metastasis development of DU145 cancer cells, full-length porcine a-1,3GT gene was transfected into DU145 using Tet-On gene expression system. The level of expression of a-Gal epitopes on the cell surface was controlled by exogenous tetracycline (Tc) or its derivative doxycycline (Dox). Such a-Gal epitopes coated human prostate cancer cell lines provided a valuable tool in investigating the biological functions of a-Gal epitopes in human cancer development.

(112) Engineering Cell Surface Glycoforms for Tumor Selective Immunotherapy.

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Tumor cells often present abberrant glycosylation patterns either in the form of unusual carbohydrate epitopes or increased expression of certain sugars. In particular, the overexpression of the terminal monosaccharide residue sialic acid is a marker for a variety of cancers including gastric, colonic, epithelial, liver and lung cancers. By combining the principles of chemoselective ligation and metabolic engineering, our laboratory has developed a technology for selectively targeting heavily sialylated cancer cells (Mahal, L. K.; Yarema K. J.; Bertozzi, C. R., *Science*, 1997, 276, 1125-1128.) We introduced a uniquely reactive functional group, the ketone, into cell surface-associated sialic acids, providing a chemical handle for the covalent attachment of chemotherapeutic agents under physiological conditions. In this presentation, we outline a strategy to target synthetic carbohydrate antigens to highly sialylated cells using our metabolically installed ketone handle. We demonstrate our strategy by targeting the galactose alpha (1-3) galactose epitope, a potent immunogen in humans, to tumor cells.

(113) Fuc-TVII dependent expression of E- and P-selectin ligands on Th1 and Tc1 cells.

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We have investigated the role of the a(1,3) fucosyltransferase, Fuc-TVII, in directing E- and P-selectin ligand expression by lymphocytes under cytokine conditions dominated by IL-12 and IFN-gamma (T helper and T cytotoxic Type I conditions), using mice deficient in Fuc-TVII (Fuc-TVII-/-). Less than 1% of $CD4^+$ or $CD8^+$ T-cells purified from mesenteric or peripheral nodes of wild type (WT) mice express ligands for E- and P-selectin. By contrast, these ligands are undetectable in Fuc-TVII-/- mice. After culture for 6 days in Type I culture conditions (IL-2, IL-12, and anti-IL4), 30-40% of $CD4^+$ (Th1) cells and 70-80% of $CD8^+$ (Tc1) cells express E- and P-selectin ligands, while those from Fuc-TVII(-/-) mice are completely deficient in such ligands. Similar percentages of Th1 and Tc1 cells from WT and Fuc-TVII-/- mice produce IFN-gamma, but not IL-4, showing that these cells were appropriately differentiated. The contact hypersensitivity (CHS) response is generally considered to be a Th1 response, dominated by $CD4^+$ cells and the cytokines IL-12 and IFN-gamma, although there is evidence that $CD8^+$ T-cells may also play a role (Tc1 response). We studied the in vivo up-regulation of E- and P-selectin ligands (E- and P-sel-lig) on T-cells using dinitrofluorobenzene (DNFB) as a model of CHS. Mouse ears were painted with either vehicle or DNFB on day -1 and 0, and draining lymph node cells were analyzed on day 4 using 3-color flow cytometry. Activated $CD4^+$ and $CD8^+$ cells, identified by bright CD44 staining, increased 4 fold in DNFB treated WT mice and 2 fold in Fuc-TVII(-/-) mice. The number of $CD4^+$ E- or P-sel-lig+ cells and $CD8^+$ E- or P-sel-lig^+ cells increased 5 and 11 fold, respectively, in DNFB treated WT mice. No cells with these phenotypes were detected in Fuc-TVII-/- mice. Intracellular cytokine production was also analyzed. The numbers of $CD4^+$ and $CD8^+$ cells producing IFN-gamma increased 8 fold in treated WT mice, and 2 fold in Fuc-TVII(-/-) mice. $CD4^+$ cells producing IL-4 increased from essentially zero in untreated mice to extremely low, but detectable numbers in WT mice, but remained undetectable in Fuc-TVII(-/-) mice. $CD4^+$ and $CD8^+$ IFN-$gamma^+$ cells from nodes were also analyzed for E-sel-lig expression. $CD4^+$ and $CD8^+$ IFN-$gamma^+$ E-sel-lig^+ cells were virtually undetectable in nodes of untreated animals. After DNFB treatment, an average of 1% of $CD4^+$ and 2.6% of $CD8^+$ cells were IFN-$gamma^+$ E-sel-lig^+ in WT mice. This population was not detectable in Fuc-TVII(-/-) mice. These data show that during a CHS response, both Th1 and Tc1 cells proliferate and up-regulate E- and P- selectin ligands, and that this expression is dependent on Fuc-TVII.

(114) New strategies for the modelling of antibody structure in dilute solution

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Antibodies provide the physical chemist with a very difficult problem in terms of unravelling the intricacies of conformation in solution. Largely arising from conformational flexibility about the hinge region x-ray crystallography is tantalisingly out of reach except for those antibodies with little or no hinge region (such as the hingeless mutants "Dob" or "Mcg"). Intact antibodies are too large for structural determination by high-resolution nmr. However, hydrodynamics can play a key role in establishing the overall solution conformation (average angles between domains) and, particularly interesting for engineered or chimaeric antibodies, changes in the oligomeric state. A problem with the hydrodynamic approach is dealing with molecular hydration which can provide serious and misleading ambiguities when interpreting a hydrodynamic parameter in terms of shape. Strategies which do not deal with this properly are not useful. The new *size-independent* bead-shell modelling algorithm SOLPRO appears however particularly useful, and we demonstrate its use by application to the Fab domain. The strategy for extending this to sorting out the conformation of intact immunologically active antibody molecules will be then be indicated. [1] SOLPRO: theory and computer program for the prediction of SOLution PROperties of rigid macromolecules and bioparticles. Garcia de la Torre, J, Carrasco, B, Harding SE (1997) Eur. Biophys. J. 25, 361-372