no Tn antigenicity[1]. These results are consistent with our previous study, in which GalNAc-Ser-(GalNAc)-Thr-(GalNAc)-Thr was essential for the Tn antigenicity of ovine submaxillary mucin[2]).

1) *Proc. Natl. Acad. Sci. USA*, H. Nakada *et al.* in press.
2) *J. Biol. Chem.*, **266**, 12402, 1991, H. Nakada *et al.*

S12.16

Carbohydrate Specificity of the Receptor Sites of Mistletoe Toxic Lectin-I*

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The carbohydrate specificity of mistletoe toxic lectin-I (ML-I) was studied by quantitative precipitin, precipitin-inhibition, and hemagglutination-inhibition assays. The results indicated that ML-I has a broad range of affinity for Gal α,β linked sequences. The galabiose (**E**, Galα1→4Gal) sequence, a receptor of the uropathogenic *E. coli* ligand, was one of the best disaccharide inhibitors tested. The lectin also exhibited affinity for **Lac**(Galβ1→4Glc), **T**(Galβ1→3GalNAc), **I/II**(Galβ1→3/4GlcNAc) and B(Galα1→3Gal) sequences. Galα1→4Gal and Galβ1→4Glc are frequently occurring sequences of many glycosphingolipids located at the mammalian cell membranes, such as intestinal and red blood cell surface membranes available for ligand binding and toxin attachment. This finding provides important information concerning the possible mechanism of intoxication of cells by the mistletoe preparation.

1. Wu, A. M., Chin, L-K, Franz, H., Pfüller, U. and Herp, A.: Carbohydrate Specificity of the Receptor Sites of Mistletoe Toxic lectin-I. *Biochim. Biophy. Acta.*, **1117** 232–234, 1992; 2. Wu, A. M. and Sugii, S.: Coding and classification of GalNAc and/or Gal specific lectins. *Carbohydr. Res.*, **213**: 127–143, 1991; 3. Karlsson, K.-A. (1989). Animal glycosphingolipids as Membrane Attachment Sites for Bacteria. *Annu. Rev. Biochem.*, **58**: 309–350.

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S12.17

Monoclonal Antibodies to Synthetic Carbohydrate Antigens

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Use of synthetic carbohydrate antigens for immunization gives the possibility of obtaining strictly directed specific response; screening with the help of synthetic antigens permits to select most specific and affinity Mabs and also antibodies having desired epitope specificity, e.g. recognizing a small common fragment of several antigens. Besides, a problem of antigen quantity and homogeneity can be solved.

Immunogens were constructed as oligosaccharides coupled with lipophilic (phosphatidylethanolamine-connected) polyacrylamide and adsorbed on *S. minnesota* before immunization. Mabs specificity was studied by direct binding and inhibition methods.

From seven hybridomas obtained by immunization with synthetic Le^y six were directed to tetrasaccharide Le^y. Three Mabs bound strongly to Le^y hapten and not cross-reacted with any of related structures tested, in particular Le^x, H (type 2), Le^a, Le^b, and disaccharide fragments. Three others showed preferential reactivity with Le^y, but slightly cross-reacted with H (type 2), Le^x or Le^a. K_a for Mabs Pl10 was 8×10^8 M^{-1}. Four hybridomas producing Mabs to trisaccharide H (type 1) were obtained. One anti-H Mab showed reactivity with synthetic H (type 1), H substance from saliva, but cross-reacted with A glycoproteins from RBC, another anti-H Mab cross-reacted with synthetic $Le^{x,y}$. Two Mabs, obtained by immunization with synthetic $SiaLe^a$ bound with high affinity to $SiaLe^a$ and weakly to Le^a and H (type 1) but not bound to $Le^{x,y}$.

S12.18

A Monoclonal Antibody Reacting Specifically for Ganglioside *O*-Acetylated GD_2 in Neuroectodermal Tumors

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Murine monoclonal antibodies (Mab) were generated against various epitopes of the disialoganglioside GD_2, considered yet as poorly immunogenic. Three reactivity patterns were assessed by several approaches: binding to tumor cell lines, ganglioside immunoassays, high performance thin layer chromatography (HPTLC) immunostaining. The Mab 8B6, representative of one of three groups was shown to be reactive to an alkali labile epitope of GD_2 purified from human neuroectodermic tumors. (i) Alkali sensitivity was demonstrated by two dimensional HPTLC and immunooverlay to separate alkali labile gangliosides from alkali stable gangliosides. (ii) After chromatography of gangliosides from melanoma or neuroblastoma, the disialylation of the alkali labile ganglioside antigen by *Vibrio cholerae* neuraminidase was monitored by immunostaining with a Mab anti-asialo-GM2. Similarly this latter reacted with the derivative of GD_2 and GM_2. (iii) By chemical acetylation of purified GD_2, the new compound had a migration on HPTLC in the appropriate position and became reactive specifically with Mab 8B6. Conversely its alkaline treatment resulted in the complete loss of its reactivity but the modified structure was recognized by Mab 10B8 specific to GD_2. Both data suggest that *O*-acetylation of disialoganglioside can create a unique epitope without cross reactivity with neuroectodermic tumors and according to Varki (1) can be considered as an onco-fetal tumor marker. From a clinical standpoint, the tissue restriction of this novel *O*-acetylated ganglioside defined by the Mab 8B6 may be of practical relevance for diagnostic assays as well as for immunotherapy or radio-immuno-