

peptidorhamnomannan to precipitate with this antiserum was observed by double diffusion agarose gel and quantitative precipitin test. By use the precipitin inhibition reaction with these O-linked oligosaccharides it was shown that the tetra and pentasaccharide were the best inhibitors in the range of 0.1 to 1.0  $\mu\text{M}$ . The relative percentual of inhibition obtained with 1.0  $\mu\text{M}$  hapten was 63% and 82% respectively; while the trisaccharide was a poor inhibitor. These results show that the major rhamnose-containing oligosaccharides are indeed the main antigenic determinants of *S. schenckii* peptidorhamnomannan.

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#### S12.26

### Interaction of Carboxymethylated $\beta$ 1,3-Glucan with Scavenger Receptors of Murine Peritoneal Macrophages

M. I. Dushkin<sup>1</sup>, A. F. Safina, T. A. Korolenko and G. V. Pravotorov

*Dept. Cell Biochem., Institute of Physiology and Laboratory of Experimental Models of Atherosclerosis, Institute of Therapy<sup>1</sup>, Siberian Branch of RAMS, Novosibirsk 630117, Russia.*

Effect of chemically modified polysaccharide  $\beta$ 1,3-carboxymethylglucan (CMG, production of Chemical Institute, Slovak Academy of Sciences, Bratislava, Slovakia — Dr. J. Sandula) was compared with action of nonmodified glucan, zymosan, LPS, mannan Rhodexman (production of St. Petersburg Chem.-Pharm. Inst., St. Petersburg, Russia) on metabolism of acetylated low density lipoproteins (acLDL), known as a ligand of scavenger receptors in culture of murine peritoneal macrophages was studied. It was found that CMG at a low concentrations (5  $\mu\text{g}/\text{ml}$ ) decreased in 20 fold the rate of incorporation of <sup>14</sup>C-oleate into cellular cholesterol esters in presence of 50  $\mu\text{g}/\text{ml}$  of acLDL. Preincubation of macrophages during 24 h with CMG was followed by decrease of this effect. On the contrary, zymosan and LPS had slight effect on acLDL metabolism; there was no action in case of preincubation with mannan Rhodexman and nonmodified glucan. At concentrations between  $25 \cdot 10^{-3}$  –  $50 \cdot 10^{-3}$   $\mu\text{M}$  CMG (like dextran sulfate, known ligand of scavenger receptors) decreased degradation of acLDL in lysosomes; the results were confirmed also by electron microscopic study with using of acLDL labelled by colloid aurum. It was concluded that CMG possessed high affinity to macrophage scavenger receptors and its immunomodulating effect was mediated through the interaction with this receptor.

#### S12.27

### Generation of Murine Monoclonal Antibodies to Ganglio-, Globo-, and Gala-Series Glycosphingolipids

T. Tai, I. Kawashima, H. Ozawa, M. Kotani and K. Ogura  
*Department of Tumor Immunology, Tokyo Metropolitan Institute of Medical Science, Honkomagome, Bunkyo-ku, Tokyo 113, Japan.*

Glycosphingolipids are classified into several groups such as

ganglio-, lacto-, neolacto-, globo-, and gala-series by biosynthetic pathway. Monoclonal antibodies (MAbs) to these glycosphingolipids have been shown to be powerful reagents not only in analyzing biological functions of glycosphingolipids on cell surface membranes, but also in the diagnosis and treatment of cancer patients. In the past decade, these MAbs have generally been established by immunizing BALB/c or C57BL/6 mice with tumor cells. It would be preferable to establish hybridomas by immunizing mice directly with purified glycosphingolipids. It is, however, still difficult to routinely generate murine MAbs to them except lacto- and neolacto-series glycosphingolipids.

The present study describes an improved method for generating murine MAbs by immunizing C3H/HeN mice with purified glycosphingolipids (1). Using the procedure, we have been successful in generating a number of sets of MAbs to glycosphingolipids as follows: (i) *ganglio-series* asialo-pathway (GlcCer, LacCer, Gg3Cer, and Gg4Cer), a-pathway (GM3, GM2, GM1, GD1a, and GT1a), and b-pathway (GD3, GD2, GD1b, GT1b, and GQ1b); (ii) *globo-series* (Gb3Cer, Gb4Cer, and Gb5Cer); (iii) *gala-series* (GalCer, SM4s, and GM4); (iv) *NeuGc-containing ganglioside* (GM3, GM2, and GD3) (2, 3, 4). These MAbs have been proved to be useful for studying the different distribution of gangliosides in rat central nervous system as shown in the other presentation from our laboratory.

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#### S12.28

### Trypanosoma cruzi Glycoconjugates of 74 kDa and 120 – 200 kDa Carry $\alpha$ -Galactosyl Epitopes Recognized by Lytic Antibodies from Patients with American Trypanosomiasis (Chagas Disease)

I. C. Almeida and L. R. Travassos

*Escola Paulista de Medicina, São Paulo, SP 04023-062, Brasil.*

Antibodies lytic to *Trypanosoma cruzi* trypomastigotes are markers of active Chagas (Ch) disease, being protective by preventing host cells reinfection and reducing the number of viable infective parasites. We have shown previously (*J. Immunol.*, **146**: 2394, 1991) that lytic antibodies from chronic patients recognize  $\alpha$ -Gal<sub>P</sub> epitopes expressed at the surface of trypomastigotes, and have a much greater affinity for the parasite antigens than the natural antiGal IgG. Both antibodies, however, bind equally well to mouse laminin or to the Gal $\alpha$ (1 $\rightarrow$ 3)Gal $\beta$ (1 $\rightarrow$ 4)GlcNAc ligand. Different binding affinities could reflect idio-type variations and/or specific  $\alpha$ -Gal<sub>P</sub>-containing structures in the parasite. By using the same extraction procedure as for *Leishmania* GIPLs (*J. Biol. Chem.*, **266**: 15170, 1991), GPI-anchored glycoconjugates (F2 and F3) were isolated from cell cultured trypomastigotes, which reacted with Ch antiGal and very little with natural antiGal. Both F2 (74 and 95.6 kDa) and F3 (120 – 200 kDa) absorbed almost 80% of the lytic activity of patients' sera. The reactivity of F3 with purified Ch antiGal was abolished both by  $\alpha$ - (but not  $\beta$ -) galactosidase and neuraminidase suggesting a complex epitope. Treatment with  $\alpha$ -2,3-trans-