3959

INHIBITION OF LYMPHOCYTE ACTIVATION BY GANGLIOSIDES AND GLYCOPHORIN. F.J. Sharom and T.E. Ross. University of Guelph, Guelph, Ontario, Canada N1G 2W1.

Exogenously added gangliosides are known to inhibit lymphocyte activation and increased ganglioside levels in serum may be linked to the immunosuppression observed in cancer. Gangliosides are potent inhibitors of ^{3}H-thymidine incorporation in con A and periodate-stimulated mouse splenocytes in the order $GT_{1b} > G_{M1} = GD_{1a}$ > mixed (bovine brain), with total inhibition at 5–10 μg per 2.5 x 10^5 cells. Other glycolipids were much less effective, with asialoGM1 > globoside > cerebrosides. Addition of human erythrocyte glycophorin totally suppressed activation at 50 μg per 2.5 x 10^5 cells. Glycophorin is not known to insert into cell membranes, suggesting that incorporation of these molecules into the lipid bilayer may not be a requirement for immune suppression. Asialoglycophorin was ~ 5x more potent as an inhibitor, implying that sialic acid residues are not necessary for inhibition of activation. Gangliosides and glycophorin do not interfere with lectin binding, and are not toxic to lymphocytes. Inhibition is reversible by washing the cells 2h after addition of gangliosides/glycophorin. Time course studies showed that both molecules must be present for 18-24h following con A addition to cause immune suppression. Inhibition could not be prevented by 5 x 10^{-8} M phorbol myristate acetate. Further studies are aimed at determining the mechanism by which gangliosides and glycophorin inhibit activation and the point in the sequence at which they act. (Supported by NCI and NSERC of Canada)

3960

A NOVEL (PHOSPHORYLATED) GANGLIOSIDE MEDIATES SPECIFIC ADHESION IN CHICKEN HEPATOCYTES. T. Ghosh, C. Srnka, M. Kuhlenschmidt, and S. Roseman. Johns Hopkins Univ., Baltimore, Md. 21218.

We have previously reported that chicken liver hepatocytes and their plasma membranes show marked adhesive specificity in vitro; an adhesive specific factor (CF) was partially purified from both the membranes and liver. Crude CF migrated as a highly polar ganglioside with 0.5 the R_f of GT1 on TLC. CF has now been purified to apparent homogeneity by two different methods; one involves repeated silica gel chromatography (Iatrobeads), with or without a DEAE-agarose step. The second method, apparently new for ganglioside purification, is adsorption to a lectin (wheat germ) column and elution with N-acetylglucosamine. These methods separate CF from a tightly bound substance(s), possibly polypeptide, which substantially reduces both the biological activity and mobility of CF on TLC. Homogeneous CF migrates with GM3 in 16 solvent systems, although CF is far more complex than GM3 (see below), and GM3 is biologically inactive.

A variety of chemical and enzymatic methods show that CF contains approximately 1 mol each of the following components (in addition to fatty acids): sphingosine, D-glucose, D-Galactose, L-fucose, sialic acid, hexosamine (glucosamine?), and organic P linked to the hexosamine. CF also appears to contain as many as 3 O-acetyl groups. The presence of phosphate makes this a unique glycosphingolipid, and suggest new possibilities for the functions of these glycolipids via a cycle of phosphorylation and dephosphorylation (e.g., in the synapse)

3961

CEREBROSIDE SULFATASE ACTIVATOR: FIBROBLAST UPTAKE IS NOT MEDIATED BY THE PHOSPHOMANNOSE RECEPTOR. Fluharty, A. L., Tsay, K. K., and Kihara, H. UCLA/MRRC Group, Lanterman State Hospital, Pomona CA 91768.

Cerebroside sulfatase activator (CS-Act) is a protein required for cerebroside sulfate (CS) hydrolysis by arylsulfatase A (ASA). CS-Act is deficient in an ASA$^+$ form of the CS storage disease, metachromatic leukodystrophy (Pediatr. Res. 13:1179, 1979). Fibroblasts from patients with CS-Act deficiency show attenuated CS hydrolysis and this defect can be corrected by exogenous CS-Act, implying uptake into lysosomes (Am. J. Hum. Genet. 33:900, 1981). CS-Act from human urine corrected more effectively than material from human liver or pig kidney, suggesting a "high uptake" form analogous to those of lysosomal enzymes. Phosphomannose substituents on the latter facilitate fibroblast uptake by a specific receptor system. CS-Act was not retained by Con-A Sepharose or Glycogel B. If the activator is a glycoprotein, it must have a different pattern of glycosylation than the lysosomal enzymes. Moreover, metabolic correction was not abolished by treatment of CS-Act with alkaline phosphatase or by mannose 6-phosphate. Thus, correction by urinary CS-Act appears to occur by facilitated uptake but it is not by the phosphomannose system. (Supported by NIH grants NS-11665 and HD-4612).

3962

PARTIAL CHARACTERIZATION OF U937 GLYCOLIPID WHICH ENHANCES HUMAN MONOCYTE RESPONSE TO MIF. C.G. Wilde, D.Y. Liu. Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115

Previous work from this laboratory has implicated gangliosides in the structure of the guinea pig receptor for macrophage migration inhibitory factor (MIF) (Liu et al. JBC 257: 159, 1982). We have extended this finding in the human system in studies of the effect of glycolipids from the histiocytic lymphoma U937 on the response of blood monocytes to MIF. Glycolipids from U937 cells, when incubated with monocytes, enhance their response to MIF. This activity resides in an acidic ganglioside fraction after silicic acid chromatography. When further fractionated by thin layer chromatography, active glycolipid is found exclusively in the region characteristic of disialogangliosides and long chain monosialogangliosides. The enhancement of MIF response by active glycolipid is not due to preferential uptake by responding cells. Additionally, the effect is apparently not due to nonspecific effects on migrating cells. Thus, some myeloid cell lines (HL60) become MIF responsive upon treatment with active glycolipid while other cells do not (KG-1, K562, lymphocytes) though glycolipid is taken up equally well by all. Metabolic labelling of U937 cells with [^{3}H]-fucose shows that the major U937 fucoganglioside migrates in the region containing the enhancing activity and suggests that it may be the species involved in the MIF receptor. (Supported by NIH Grants AI-07685, AM-07031, BRSG RR05669 and NSF Grant PCM-8208262)

3963

CHARACTERIZATION OF Fucα1-2NEOLACTOTETRAOSYLCERAMIDE USING PURIFIED FucT-2 FROM BOVINE SPLEEN. T. De, M. Basu, S.Basu and T.Brown.Dept of Chem.;Univ.of Notre Dame,Notre Dame,IN 46556.

Fucose (α1-2)- and (α1-3)-linked blood group-related GSLs containing a common core structure of nLcOse$_4$Cer(Galβ1-4GlcNAcβ1-3Galβ1-4Glc-Cer) are almost ubiquitous on animal cell surfaces. Biosynthesis in vitro of H-active fucose-containing GSL (Fucα-nLcOse$_4$Cer) has been reported using a membrane-bound Golgi-rich fraction from bovine spleen (BSGR)(Basu,S., Basu,M. and Chien,J.L.(1975) J.Biol.Chem.250,2956-2962). Recently characterization of FucT-2(GDP-Fuc:nLcOse$_4$Cer(α1-2)fucosyltransferase)&FucT-3(GDP-Fuc:LcOse$_3$Cer(α1-3)fucosyltransferase) present in the cationic detergent solubilized supernatant from BSGR and their separation by column chromatography have been achieved. The FucT-2 activity (free of FucT-3 activity) catalyzed the transfer of fucose from GDP-fucose to the ^{3}H-labeled terminal galactose residue of nLcOse$_4$Cer. The ^{3}H-labeled enzymatic product was isolated, purified and then subjected to exhaustive methylation. After acetolysis the partially methylated sugars were separated by two dimensional TLC. ^{3}H-labelings were detected only in two areas, 3,4,6-tri-O-Me-Gal(54%) and 2,3,4,6-tetra-O-Me-Gal(28%).In a separate experiment 85% ^{14}C-Fuc was released when ^{14}C-labeled enzymatic product was treated with clam α-fucosidase.These data unambiguously prove the formation of the Fucα1-2Gal-linkage in the terminal region of H-active GSL during the FucT-2(Bov.Spleen) catalyzed reaction. The FucT-2 also transfers Fuc to lactosamine-containing glycoproteins.(Supp:NS-18005;CA-14764 to S.B. and CA-18357 to M.B.)

3964

IDENTIFICATION OF GalNAcβ1-3Gal-LINKAGE IN GLOBOSIDE SYNTHESIZED IN VITRO BY GalNAcT-2 FROM GUINEA PIG 104Cl CELLS. K. Das, M. Basu, R.J. Schaeper and S. Basu. Dept of Chemistry; University of Notre Dame, Notre Dame, IN 46556.

The stepwise biosynthesis in vitro of globoside and Forssman glycosphingolipids (GSLs) was achieved in guinea pig 104Cl cells(Basu,S.,Basu,M.,Higashi,H. and Evans,C.H.(1982) in New Vistas in Glycolipid Research, Plenum Press,pp.131-137) and in guinea pig kidney by others. However, the exact chemical structures of biosynthesized products, globoside and Forssman GSLs,are not yet known.Based on pH optima and kinetic parameters the following three GSL:GalNAc transferases (GalNAcTs) were distinguished:

1. UDP-GalNAc:GM3 GalNAcT(GalNAcT-1; pH-8.1 in HEPES buffer)
2. UDP-GalNAc:Gb3 GalNAcT(GalNAcT-2; pH-7.0 in HEPES buffer)
3. UDP-GalNAc:Gb4 GalNAcT(GalNAcT-3; PH-7.0 in Cac-HCl buffer)

In 104Cl cells the GalNAcT-2 activity (free of GalNAcT-1 activity) catalyzed the transfer of GalNAc from UDP-GalNAc to the ^{3}H-labeled terminal galactose residue of Gb3[(6-^{3}H)Galβ1-4-Galβ1-4Glc-Cer)].The ^{3}H-labeled globoside was purified and then subjected to exhaustive methylation.After acetolysis the partially methylated sugars were separated by two-dimensional TLC.The ^{3}H-labelings were detected in two major areas, 2,4,6-tri-O-Me-Gal(40%) and 2,3,4,6-tetra-O-Me-Gal(46%).In a separate experiment 80% GalNAc was released when labeled Gb4 was treated with clam β-hexosaminidase.The present results establish the formation of GalNAcβ1-3-linkage in the terminal region of biosynthesized Globoside.(Supp:CA-14764 and CA-18357)

3965

FLUORESCENT DERIVATIVES OF GANGLIOSIDE GM1 ARE FUNCTIONALLY EQUIVALENT TO GM1 AS RECEPTORS FOR CHOLERA TOXIN. Sarah Spiegel and Peter H. Fishman. NINCDS, NIH, Bethesda, MD 20205

Fluorescent derivatives of GM1 containing rhodamine or Lucifer yellow CH attached to the sialic acid residue were synthesized and purified. The biological activity of the fluorescent derivatives were compared with that of native GM1 using GM1-deficient rat glioma C6 cells. Exposure of the cells to either native or fluorescent GM1 resulted in a similar, marked increase in binding of ^{125}I-cholera toxin. The affinity of the toxin for cells treated with either native or fluorescent GM1 also was similar (Kd = 0.65 and 1.1 nM, respectively). More importantly, the fluorescent derivatives were as effective as native GM1 in enhancing the responsiveness of the cells to cholera toxin. Cells were incubated with gangliosides, washed, incubated with toxin and assayed for cyclic AMP accumulation. Cyclic AMP levels in cells treated with fluorescent or native GM1 increased 7- to 9- fold more than levels in cells not exposed to GM1. Cells treated with Lucifer yellow CH-labeled GM3 did not exhibit a similar increase in cholera toxin binding or responsiveness. Thus, the introduction of a fluorophore into the oligosaccharide moiety of GM1 does not abrogate the function of the native ganglioside as the receptor for cholera toxin. The present demonstration that such modified gangliosides retain their biological activity should promote their use as probes for investigating the distribution, dynamics and function of these membrane components.

3966

ISOLATION AND CHARACTERIZATION OF GLYCOLIPIDS OF PENICILLIUM CHARLESII. C.A. Abbas* and J.E. Gander. Univ. of Florida, Dept. Microbiol. & Cell Sci., Gainesville, Fl. 32611

Phytosphingolipids are common in higher plants and fungi. The occurrence of membrane-bound galactofuranosyl residues in P. charlesii membranes suggests that either a galactofuranosyl-containing glycolipid or glycoprotein occurs. This investigation was directed toward determining whether galactofuranosyl-containing glycolipids occurred in this fungus.

The organic phase from a $CHCl_3$:methanol:0.1 N KCl(2:1:0.1, v/v/v) extract of 2-3 day shake cultures of P. charlesii contains a number of glycolipids. They were separated from other lipids by silicic acid chromatography and analyzed by one and two dimensional thin-layer chromatography.

Glucose and a substance which migrated similar to erythritol were detected by paper chromatography following treatment of glycolipid preparations to deacylate O-acyl residues.

The alkali resistant glycolipids were fractionated by one dimensional TLC into primarily three fractions. Aliquots from these fractions were subjected to analysis by gas chromatography-mass spectrometry, gas, paper, column, and thin-layer chromatography. The results of these analyses indicated that these glycolipids were simple glycosphingolipids with dehydrophytospingosine as the primary nitrogenous base and fatty acyl residues of C12 to C26. The data suggest that the major glycolipid is a glucosyl ceramide. The other glycolipids are being analyzed to determine their carbohydrate constituents.

This research was supported by NIH research grant GM-34068.

3967

N-ACETYL- AND N-GLYCOLYLNEURAMINOSYL LACTO-N-NOROCTAOSYL-CERAMIDES FROM BOVINE ERYTHROCYTES. S. Dasgupta, J-L. Chien and E.L. Hogan. Neurology, Med. Univ. S.C., Charleston, S.C. 29425.

Four glucosamine containing gangliosides have been found in bovine erythrocytes (Chien et al. J. Biol. Chem. 253:4031, 1978). They were N-acetyl- and N-glycolylneuraminosyl lacto-N-tetraosylceramides (NeuAc- and NeuGc-nLcOse cer), and NeuAc- and NeuGc-nLcOse cer. Here, we purified two additional monosialoganglioside with longer oligosaccharide chain. The purified compounds migrate as single bands in three solvent systems. Ganglioside A, with higher Rf contains NeuAc while ganglioside B, with lower Rf contains NeuGc. In the absence of detergent both were hydrolyzed by the neuraminidase from Cl. perfringens to a neutral glycolipid with Rf slightly less than that of the heptaglycosylceramide isolated from bovine erythrocytes (Chien, et al., J. Lipid Res. 29:669, 1979). The chromatographic behavior of A is identical to that of NeuAc-lacto-N-norooctaosylceramide from rabbit skeletal muscles (Iwamori and Nagai, 1981). Ganglioside B was analyzed by GC to contain NeuAc, GlcNAc, Gal, Glc, (molar ratio 1:3:4:1). Sphingosine is the predominant long chain base detected while the major fatty acid were found to be C 24:0. These results along with sequential Glycosidase and methylation study established the structure of ganglioside B to be NeuGcα2→3Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glc-cer (NeuGcnLcOse cer). (Supported in part by NIH Grant NS-16120)

3968

GLYCOLIPIDS OF BOVINE PINEAL. M.M. Whalen, G.C. Wild and W.O. Spall (SPON: L.F. Smith). Univ. New Mexico Med. Sch., Albuquerque, N.M. 87131 and Los Alamos National Labs, Los Alamos, NM 87544.

Some glycolipids, especially gangliosides, are enriched in synaptic membranes. Excised pineal bodies contain a large proportion of synaptic endings, which may be all noradrenergic. These endings contain serotonin as well, which is possibly taken up by the synaptic terminals, after being made in the pinealocytes. Pineal acetone powder was extracted with chloroform-methanol (C:M) mixtures for gangliosides. The extract was applied to silicic acid columns and eluted batchwise with C:acetone and C:M mixtures. Column fractions were purified by either TLC or an HPLC procedure which we have developed. Final preparations were methanolyzed and analyzed by gas chromatography. GD_{3} is the predominant ganglioside, with less GM_3. GD1a and GM_1 are present in smaller amounts. Also present is a novel trisialoganglioside which has a glucose:galactose:GalNAc ratio of 2:1:1. On HPLC, it elutes between disialo and trisialo ganglioside standards. Neutral glycolipids, in addition to cerebroside, include an acetone-extractable polar form of cholesterol, previously undescribed. The cholesterol is linked to sugar, primarily galactose. These results may indicate a relationship between gangliosides GD_3 and GM_3 and noradrenergic synapses.

3969

CHEMICAL ANALYSIS OF HUMAN PLATELET GLYCOLIPIDS. H.M. Weinfeld and T.A.W. Koerner. Dept. of Pathology, Tulane University School of Medicine, New Orleans LA 70112

Glycolipids (GL) are complex conjugates of hydrophilic oligosaccharide and hydrophobic ceramide moieties, found in the membranes of mammalian cells in cell-specific distributions. It is possible that GL function as platelet receptors for antibodies, viruses, biogenic amines, cations and other ligands. Basic to such functional studies is knowledge of platelet GL composition, data which we now report. More that 670 grams wet wt. of platelets were purified from platelet concentrates (1010 units) by differential centrifugation. RBC and WBC contamination was <0.02%. GL were obtained from purified platelet pellets by extraction ($CHCl_3$:CH_3OH, 1:1) and column chromatography (DEAE-Sephadex and Iatrobead), then were fractionated and quantitated by high performance TLC and densitometry. Structures were established by proton NMR at 500 MHz and mass spectroscopy. In order of mobility, five neutral GL were present with percentages as follows: 1, 19, 14, 63, and 3%. The latter four components correspond to globoside, trihexosyl-, lactosyl-, and glucosyl-ceramide. The first component is a novel GL with 6-9 sugar residues, the structure of which is still under study. Similarly, six gangliosides are present with percentages as follows: 0.2, 0.7, 0.3, 0.2, 2.3, and 96.3%; the two major components being GM3 (96%) and LM1 (2.3%).

3970

ACUTE ALCOHOL REDUCES BRAIN GANGLIOSIDES. W. R. Klemm and D. M. Foster. Depts. Vet. Anat. and Lab. Svc. OETVAMC (Temple), Texas A&M University, College Station, TX 77843.

Previously, Klemm and Engen reported that a single intoxicating dose of alcohol could deplete the steady-state levels of brain sialic acid (J. Neurosci. Res. 3:341-351; 4:371-382).

We now show that this effect is due at least partly to specific decrements in a variety of species of gangliosides. Rats were given a single IP dose of alcohol (3g/kg) and sacrificed at 1, 4, 8, 16, or 24 hours later; controls received a comparable volume of physiological saline and were sacrificed at similar times.

A clear diminution of all classes of gangliosides occurred at both 4 and 8 hours, and triganglioside levels were still decreased even at 16 hours. We thus propose that gangliosides in the neuronal membrane are secondarily affected by the well-known fluidizing effect of alcohol. Because gangliosides provide nerve cell membranes with many of their distinctive properties, we propose that at least some of alcohol's effects on brain, behavior, and even addiction may be attributable to the "secondary" effects on such membrane components as gangliosides.