(LCB) species, C18 and C20, present in the brain gangliosides of different animals, the C20 absent, or present in trace amount, in prenatal life appears postnatally and slowly but progressively increases during development and aging. These differences and behavior may derive from the two following possibilities. (a) Some glucosyltransferases or activator proteins with a different specificity or affinity for the different ceramide species are expressed differently during brain development. (b) The specific enzymes or some cofactors for the condensation of palmitoyl-CoA or stearoyl-CoA to L-serine (producing the C18 and C20 LCB, respectively), are modulated by specific inhibitors(activators).

We are studying the above problem by addition to cerebellar granule cells during differentiation and aging in culture, of radioactive fatty acids, used as precursors for the synthesis of LCBs and sphingolipids. After fatty acid incorporation, cells are submitted to lipid extraction and gangliosides, glycosphingolipids, sphingomyelin and the free ceramides and LCBs separated and analyzed for radioactive LCB content and structure.

S.12 CARBOHYDRATES IN IMMUNOLOGY

S12.1
Structural Features of Anti-Carbohydrate Antibodies

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Many important antigenic determinants are carbohydrate in nature, and it is of great interest to determine the molecular basis of carbohydrate recognition by antibodies. This presentation will review two approaches to analysis of the structure of antibody-oligosaccharide complexes, x-ray crystallography and NMR spectroscopy. Dr. D. Bundle and collaborators have analyzed crystal structures of three Fab Ab fragments complexed with oligosaccharide haptens of bacterial *O*-antigens. The binding site of an anti-*Salmonella* Ab is a pocket 8A long and 7A wide that is filled by a trisaccharide, primarily by an abequose residue. The binding site is lined by aromatic residues and contains a structured water molecule. A cluster of hydroxyl groups on one aspect of the oligosaccharide form crucial hydrogen bonds with the Ab. Crystal structures of antibodies complexed with larger oligosaccharides derived from Shigella *flexneri* also reveal that no more than three hexose residues could be "seen" in the complex. Weak interactions between the Fab fragments and sugars outside the binding site could be detected by calorimetry. Our laboratory is studying panels of murine mAb against two carbohydrate determinants, 3-fucosyllactosamine (3-FL, Le^x) and GalGb4. We are using a combination of site-directed mutagenesis, computer modeling and NMR techniques in our studies. Both sets of mAb exhibit highly restricted use of immunoglobulin genes. The VH segment of both antibodies are encoded by the VH441 gene. Anti-3-FL L chains are encoded by Vk24b, and anti-GalGb4 L chains by a VKOx-1-like gene. A preliminary model of the anti-GalGb4 Ab 3A9 indicates that it presents a pocket on the surface created by side chains of Arg 93 in L3 and Tyr 103 in H3. Replacement of Tyr by Ala led to total loss of binding activity. Replacement of the VH segment of 3A9, which contains several somatic mutations, by genes encoding the germline sequence of VH441 or VHX24 resulted in no major change in binding activity, which indicates that the somatic mutations were not required for effective binding of antigen. A model of the anti-3-FL Ab PM81 indicates that it has a large cleft in the putative binding surface of the Fv fragment. The base of the cleft is formed by L3 and H3, and the sides are formed primarily by L1 and H2. Data on the role of H3 in Ab affinity will be presented.

S12.2
Role of Sugar Chains in Immune Response

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The mechanism, by which maternal body accepts allogenetic fetus within uterus, has not been well elucidated. Muchmore and Decker (1) isolated an immuno-suppressive material from the urine of pregnant women and named it uromodulin. Uromodulin is a glycoprotein containing sugars up to 30% of its molecular weight. Pennica *et al.* (2) reported that uromodulin is identical to Tamm-Horsfall glycoprotein (THGP). Uromodulin suppresses the antigen specific *T*-cell growth and the activity is assumed to reside in its sugar moiety based on the result that it was destroyed by periodate oxidation (3). We confirmed that the oligosaccharide fraction obtained from THGP by hydrazinolysis inhibits the growth of mononuclear cells induced by tetanus toxoid. The inhibitory activity was distributed in the sialidase resistant acidic oligosaccharide fraction (AR). Since the activity of fraction AR was lost by mild methanolysis, sulfated oligosaccharides are considered to express the inhibitory activity. Since the inhibition was not observed when the fraction AR was added 24 hrs after mixing human mononuclear cells and tetanus toxoid, the fraction should work at the early stage of immune response. Measurement of cytokines in the medium revealed that the amount of IL-1β increases and that of IL-2 decreases by the addition of fraction AR. These results indicated that fraction AR inhibits the binding of IL-1β to IL-1 receptor of mononuclear cells. Actually, IL-1β specifically binds to fraction AR immobilized on an NH_2-HPTLC plate. Since IL-1β did not bind the oligosaccharide fraction released from porcine thyroglobulin, it should recognize a particular sulfated *N*-linked sugar chain. Search for the oligosaccharide reacting with IL-1β revealed that it is a sulfated complex type sugar chain with the GalNAcβ1→Galβ1→4GlcNAc group in its outer chain moiety.

(1) Muchmore, A.V. and Decker, J. M. (1985) *Science,* **229**, 479.
(2) Pennica, D. *et al.* (1987) *Science,* **236**, 83.
(3) Muchmore, A.V. *et al.* (1987) *J. Immunol.,* **138**, 2547.