results obtained showed that the nonapeptide has activity similar to the PRP and that NP could replace PRP in studies on the mechanism of action of PRP.

S13.10

Generation of Normal and Glycosylphosphatidyl Inositol (GPI)-Deficient T-Lymphocyte Clones from a Patient with Paroxysmal Nocturnal Hemoglobinuria

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Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired condition of the hematopoietic tissue in which early progenitor(s) give rise to defective subpopulations in the various cell lineages in which assembly of the glycan moiety of the glycosylphosphatidyl inositol (GPI) anchor is disturbed. As a consequence, plasma membranes of affected PNH cells lack GPI-anchored proteins including the leukocyte antigen CD48. To investigate the underlying biochemical lesion, we established, in analogy to investigations carried out in the Tn-syndrome[1], T-lymphocyte clones harboring the PNH defect from the peripheral blood from an affected individual. Using a monoclonal antibody directed to CD48 and FACS, GPI-deficient T-cells were enriched and cloned by limiting dilution. Normal CD48-expressing T-cell clones were also established from the same patient. Several features of PNH resemble the Tn-syndrome, such as an acquired (as opposed to inherited) "mosaic" status, the involvement of all hemopoietic lineages and the presence of idiopathic as well as symptomatic forms. These T-cell clones should be useful to i) biochemically characterize the PNH defect, ii) identify the DNA element that encodes the responsible enzyme(s), and iii) study the role of CD48.

[1]Thurnher, M., Clausen, H., Fierz, W., Lanzavecchia, A., Berger, E. G.: T cell clones with normal or defective *O*-galactosylation from a patient with Permanent Mixed-Field Polyagglutinability. *Eur. J. Immunol.* 1992, **22**, 1835-42.

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S13.11

Involvement of Carbohydrate Blood Group Antigens in the Phenomenon of Cellular Heat Resistance

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Recent data indicate that cells may resist heat shock via more than one route: hsp synthesis and other still ill-defined mechanisms. We investigated this phenomenon using four types of cells derived from a single rat colon carcinoma: clones REGb and PROb; PRO A+, a glycosylation variant of PROb selected for its high expression of blood group A antigen; and Ph8, a thermoresistant variant of PROb selected by repeated sublethal heat-treatments. Basal heat resistance was not correlated with 73 kD hsc and 70 kD hsp synthesis, but was clearly associated with the level of cell surface expression of blood group H and A antigens. Synthesis of these two carbohydrate structures requires two glycosyltransferases, H and A enzymes, which activities, also correlated with basal heat resistance. After heat shock, increased levels of 70 kD hsp synthesis were associated with development of thermotolerance. However, an increase of A and H cell membrane antigens was also observed six hours following heat treatment. Furthermore transient increase of T and H glycosyltransferase activities occurred one hour after heat shock, strongly suggesting a participation of carbohydrate structures in acquisition of thermotolerance. Increase of H enzyme, after heat shock, was unaffected by inhibitors of transcription and translation. The major part of the blood group antigens were *O*-linked and to a lesser extent *N*-linked to the glycoproteins. Only trace amounts of glycolipid based blood group antigens were found. Thus, carbohydrate blood group antigens could be major cellular heat protectors and would participate in the development of thermotolerance by a mechanism of induction independent of protein synthesis.

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S13.12

Comparison of the Carbohydrate Chains of Cell Surface Glycoproteins in PC12 Pheochromocytoma with Those in its Variant, PC12D

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PC12D cells, a new subline of PC12 pheochromocytoma cells, extend neurites faster than PC12 cells responding to nerve growth factor (NGF). PC12D cells differ also morphologically from PC12 cells, being flatter in shape and have extended short processes without any stimulation (1, 2). In view of the potential importance of oligosaccharide chains of cell surface glycoproteins, we have carried out comparative studies to characterize these oligosaccharide chains. Cells were incubated in media containing ^{3}H-glucosamine to label carbohydrate chains or with ^{3}H-threonine to label peptide chains, and then cell surface glycoproteins were separated from other cellular proteins by the Triton X114 phase separation method. The glycoproteins were digested with pronase after delipidation, and then the digests were fractionated on Sephadex G-50. The amount of ^{3}H-glucosamine as well as that of ^{3}H-threonine incorporated into the void volume fraction (peak 1) which is regarded as derived from cell surface glycoproteins was significantly lower in PC12D than in PC12 cells. The decrease was accounted for by a lower content of poly (*N*-acetyllactosamine) containing carbohydrate chains, as revealed by endo-β-galactosidase digestion. Further, the NGF stimulation caused the decrease of the peak 1 material from PC12 cells while PC12D cells were insensitive in this regard. Thus, it appears that the content of poly (*N*-acetyllactosamine) may be correlated with the changes in cell morphology.

(1) R. Katoh-Semba *et al.* (1987) *J. Neurosci. Res.*, **17**, 36–44.
(2) M. Sano and S. Kitajima (1992) *J. Neurochemistry*, **58**, 837–844.