The carbohydrate-deficient glycoprotein syndrome: an experiment of nature in glycosylation

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The study of diseases in which the glycosylation of glycoproteins is altered, experiments of nature in glycosylation, can provide information about the factors regulating glycosylation and the functional consequences of changes in glycosylation for specific cells and molecules as well as for the whole organism. Genetic defects in glycosylation include: (i) mutations in the N-glycosylation sites of specific proteins; (ii) decrease in the synthesis of a precursor e.g. UDP-galactose in galactosaemia or GDP-fucose in leucocyte adhesion deficiency type I; (iii) a defect in enzyme-modifying glycosylation of a class of glycoproteins, e.g. lack of phosphorylation of lysosomal proteins due to a deficiency of UDP-GlcNAc:phospho-N-acetylglucosaminyltransferase; and (iv) a defect in general glycoprotein processing, which may be partial [e.g. deficiency of N-acetyl-glucosaminyltransferase II or Golgi α-mannosidase II in HEMPAS (hereditary erythrocytic multiseriality with positive acidified serum test)], tissue/cell-specific (e.g. in cancer cells) or secondary (e.g. cystic fibrosis and chronic alcoholism).

The carbohydrate-deficient glycoprotein syndromes (CDGS) are a group of genetic multisystemic diseases characterized by the altered glycosylation of a wide range of serum and probably membrane-associated glycoproteins [1,2]. The change in electrophoretic mobility or pl of serum transferrin due to decreased sialylation is a convenient and reliable biochemical diagnosis of CDGS. The predominant transferrin isoform in normal serum is tetrasialotransferrin, which has two N-linked, biantennary, disialylated glycans. In contrast disialotransferrin in serum from CDGS patients.

The universal nature of the altered glycosylation and the diverse functions of the affected glycoproteins probably explain the wide involvement of different organs and tissues. There is severe involvement of the nervous system in all patients, such as psychomotor retardation, axial hypotonia, ataxia, hyporeflexia, squint and, in older patients,
abnormal eye pigmentation and stroke-like episodes leading to epilepsy in many cases. Visible symptoms, which are generally recognizable soon after birth and which aid in clinical diagnosis, include an unusual distribution of body fat, long fingers and toes, prominent occiput, restricted movements of hips and knees and peau d’orange skin. In addition, there is generally hepatomegaly, proteinuria, cardiac myopathy and effusions and hypogonadism, especially in females. Post-mortem examination has revealed olivopontocerebellar atrophy, mild neuronal loss and gliosis in the cerebral cortex, renal cysts, fibrosis of testes, lymph node abnormalities and lysosomal inclusions in hepatocytes. The severity of symptoms varies and about 20% of patients die in infancy or early childhood through liver or heart failure or infections [3–5]. Whether this severe infantile form of CDGS is a genetic variant or results from environmental or other genetic factors is not known. Recently, two sub-types of CDGS, types II and III have been described, which differ from the major form of CDGS, type I, in their combination of characteristic clinical symptoms and their pattern of transferrin isoforms [6,7]. A marked deficiency of N-acetylgalcosaminyltransferase II has been demonstrated in fibroblasts of the two patients with type-II CDGS [8]. The two N-linked glycans of serum transferrin from these patients are truncated on the α1-6 mannose arm and consequently only contain one sialic acid residue each. The underlying defect in the two patients with type-III CDGS is unknown.

To investigate the altered glycosylation in type-I CDGS, transferrin was purified from the serum of patients and controls and the N-linked glycans were analysed. Treatment of rivanol-purified serum transferrin with α-neuraminidase converted the disialo- and tetrasialo-transferrin from patients into asialotransferrin, confirming that the different pattern of transferrin isoforms in the patients was due to undersialylation. The N-linked glycans were released from trypsin-digested transferrin by

**Figure I**

F.a.b.m.s. of N-linked glycans released by N-Glycanase from serum transferrin of patients with CDGS type I

![Graph showing the distribution of N-linked glycans](attachment:image.png)
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N-Glycanase, separated from the peptides and analysed by fast-atom-bombardment m.s. (f.a.b.m.s.). The peptides were analysed by h.p.l.c. The predominant molecular ion observed by f.a.b.m.s. from both the normal and CDGS transferrin had an m/z of 2771, corresponding to a biantennary and disialylated N-linked glycan (Figure 1). Fragment ions corresponding to Nana.Hex.HexNAc, but not Hex.HexNAc were also observed. It was concluded that the structures of the N-glycans were unaltered in CDGS type-I and that the predominant disialo-transferrin arises from the non-occupancy of one of the two N-glycosylation sites. Furthermore, analysis of the tryptic peptides from deglycosylated and glycosylated transferrin suggested that the non-occupancy of the two sites was random. These conclusions were confirmed by other groups using electrospray ionization m.s. [9-11]. The random under-occupancy of N-glycosylation sites in CDGS type-I could be due to a defect in oligosaccharyl-transferase, an inadequate supply of dolichylpyrophosphate-oligosaccharide or defective organization of the components of the glycosylation reaction.

The enzyme N-acetyl-β-D-hexosaminidase is a glycoprotein, which is located primarily in lysosomes but which is also found in extracellular fluids such as serum and the culture medium in which fibroblasts have been grown. The dual localization of this glycoprotein means that it is ideal for comparing the N-glycosylation of intracellular and secreted glycoproteins in CDGS. The N-acetyl-β-D-hexosaminidase isoenzymes in serum and fibroblasts from a CDGS type-I patient and a control were separated by ion-exchange chromatography and isoelectric focusing. A higher proportion of more positively charged forms was found in CDGS type-I serum but not in fibroblasts, suggesting that the underglycosylation and hence lower sialic acid content was restricted to the secreted form of the enzyme. The levels of several lysosomal enzymes were normal in the CDGS fibroblasts. However, some preliminary experiments using lectin-affinity chromatography indicate that the glycosylation of intracellular lysosomal enzymes in CDGS type-I fibroblasts may be altered.

CDGS type I appears to be an autosomal recessive disorder because it affects both sexes and the parents of affected children do not generally show symptoms, although partial biochemical features have been observed in some parents, particularly fathers [12]. An attempt at prenatal diagnosis for CDGS type I was carried out by analysing the glycoproteins transferrin, α1-antitrypsin and N-acetyl-β-D-hexosaminidase in fetal blood obtained at 19 weeks gestation [13]. All showed a normal electrophoretic or chromatographic pattern, suggesting the fetus was unaffected. However, 2 weeks after a premature delivery at 35 weeks, all three glycoproteins showed the pattern characteristic of CDGS type I, which then persisted. The female infant also had clinical symptoms confirming diagnosis of the disorder. These results show that the abnormal glycosylation of secreted glycoproteins cannot be detected in the blood of an affected fetus. Possible explanations are (i) the failure in CDGS type I to switch from a normal fetal glycosylation pathway to a normal post-natal pathway, (ii) maternal glycoproteins in the fetal blood or another form of maternal protection, or (iii) the abnormal glycosylation is secondary to some other primary defect.

The biochemical diagnostic phenotype of CDGS type I, an increase in serum disialotransferrin, is also seen in chronic alcoholism [14] and galactosaemia, which results from a deficiency of galactose-1-phosphate uridylyltransferase and is also a multisystemic disorder (Figure 2) [15]. Many of the problems in galactosaemia are prevented by the removal of galactose from the diet but long-term

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**Figure 2**

Electrophoresis in agarose followed by immunodetection on nitrocellulose of transferrin

Lane 1, normal serum; lane 2, normal cerebral spinal fluid; lane 3, CDGS type-I serum; lane 4, serum from untreated galactosaemic patient; lane 5, serum from same patient 3 weeks after commencement of treatment.
problems such as developmental delay, ovarian dysfunction and speech abnormalities persist. This suggests that the alternative pathway for the formation of UDP-galactose by UDP-glucose-4-epimerase for galactosylation is inadequate in certain cells or in the fetus. The structure of the serum disialo-transferrin and other glycoproteins in untreated and treated galactosaemia patients is under investigation.

The CDGS are a group of primary and secondary genetic and acquired disorders resulting from defects in events in the endoplasmic reticulum or Golgi apparatus. Elucidation of the mechanisms of altered glycosylation in CDGS will provide information on the regulation of glycosylation and will lead to methods for heterozygote detection, prenatal diagnosis and possibly treatment of this major new group of inborn errors of metabolism.

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**Mutational analysis of the epitopes recognized by anti-(rat CD2) and anti-(rat CD48) monoclonal antibodies**

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**Introduction**

CD2 was discovered with monoclonal antibodies (mAbs) which precipitate a 50 kDa molecule from T cells and block the formation of rosettes between these cells and sheep red blood cells (reviewed by [1]). CD2 is now one of the best characterized T-cell cell-surface molecules. On the basis of early sequence analyses it was predicted that CD2 consists of two immunoglobulin superfamily (IgSF) domains [2] and this was subsequently confirmed in structural studies of the molecule [3,4]. The rosetting phenomenon implied that CD2 functions as a recognition molecule and it is now clear that in humans and rodents CD2 interacts with CD58 [5,6] and CD48 [7,8] respectively.

The work of Meuer et al. [9] provided the first insights into the cellular events initiated by CD2 recognition. It was shown that a subset of anti-human CD2 antibodies, now known to bind to domain 1 and referred to as T11-type antibodies, induced the appearance of a 'neo-epitope' on