



The Role of Glycosylation in Disease

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Carbohydrates are known to play a part in several pathological processes, some of which are indicated in this article. A few topics of interest have therefore been covered; for a recent review on the role of carbohydrates in medicine see 1 and references therein.

CARBOHYDRATES AND ONCOLOGY.

A wide variety of carbohydrate markers have been found to be present on cancer cells. These markers have been correlated with the tumor grade, metastatic potential and prognosis. A good review of the large number of markers detected so far has recently been published⁽²⁾. These markers are largely O-linked oligosaccharides and tend to be more strongly expressed in tumor cells with either a poor prognosis or greater metastatic potential than in the corresponding normal cells. In contrast, cells that normally express carbohydrate structures, such as the ABH antigens, tend to lose this expression when they turn malignant.

The use of carbohydrate markers for clinically important prognostic purposes is well developed, with monoclonal antibodies to sialosyl-Tn markers being used in colonic carcinoma⁽³⁾, and the agglutinins from *Helix pomatia*⁽⁴⁾ and

Datura stramonium⁽⁵⁾ being used in breast cancer. Other carbohydrate markers for other forms of cancer are also known. Undoubtedly, the use of such markers for prognostic and chemotherapeutic planning purposes will increase.

CARBOHYDRATES AND INFLAMMATION.

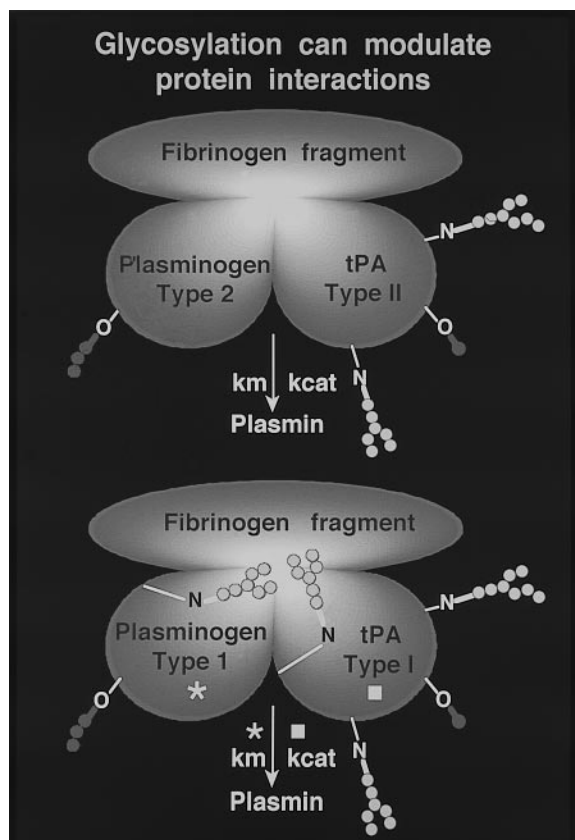
There has been a huge increase in the number of papers published on this topic since 1989, largely as a result of the identification of the molecules responsible for the initial processes involved in the adhesion of leukocytes to vascular endothelium. These molecules, the selectins, can recognize the oligosaccharides sialyl-Lewis^x and sialyl-Lewis^a. Many recent reviews of these processes have been published (see e.g. 6, 7). Modification of these natural ligands has been described^(8,9) with a view to synthesizing a small molecule antagonist for anti-inflammatory therapeutic purposes. In addition to the obvious anti-inflammatory properties of such a drug, it might also be used for oncological purposes, as E-selectin plays a role in capillary morphogenesis⁽¹⁰⁾. A further level of complexity has recently been added to the understanding of lymphoid-endothelial cell interactions by the finding of raised levels of soluble isoforms of adhesion molecules during disease processes⁽¹¹⁾ and in particular in sepsis⁽¹²⁾.

A congenital defect in the adhesion molecules LFA-1 and Mac-1 has been reported to result in a profound leukocyte adhesion deficiency⁽¹³⁾. Such patients have recurrent severe infections due to the neutrophils having severe adhesion and motility defects. Since that time, a further leukocyte adhesion deficiency has been reported⁽¹⁴⁾ in which sialyl-Lewis^x is absent. Again, the clinical symptoms are of recurrent severe infections, but in addition, the Bombay phenotype (hh) was present in the blood, together with mental retardation, short stature and a distinctive facial appearance.

Rheumatoid arthritis (RA), tuberculosis (TB) and Crohn's disease, together with erythema nodosum leprosum (ENL) are known to be associated with a deficiency of terminal galactose on the oligosaccharides present on the Fc part of the serum IgG molecule⁽¹⁵⁻¹⁸⁾. It appears that the reduction in the amount of terminal galactose may be due to the reduced galactosyltransferase activity toward asialo-agalacto-IgG in the B cells from RA patients which in turn can be ascribed to the lowered affinity for UDP-Gal. The measurement of serum levels of asialo-agalactosyl-IgG may have clinical utility in the differential diagnosis of early synovitis when combined with measurement of rheumatoid factor⁽¹⁹⁾.

CARBOHYDRATES AND INFECTION.

Some bacteria are known to bind to surface epithelial cells by means of carbohydrate-lectin interactions. A recent example of this is the preferential binding of *Helicobacter*



The activation of type 1 and type 2 plasminogen by type I and type II tPA. (Courtesy of The Glycobiology Institute, Oxford, UK.)

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pylori to the surface of gastric epithelial cells in the stomach using the Lewis^b antigens^(20,21). These are part of the blood group antigens that determine blood group O. Another binding site has also been found⁽²²⁾ as *H. pylori* codes for a protein that binds specifically to sialic acid which is also found on glycoproteins on the gastric epithelial cells.

Cholera toxin is a classic A-B toxin produced by *Vibrio cholerae* which produces severe fluid loss and dehydration in the human. The B-subunit binds the glycosphingolipid GM₁⁽²³⁾, enabling the A-subunit to enter the cell and activate cAMP production. Similar activity is also found for the pertussis toxin produced by *Bordetella pertussis*, the agent responsible for whooping cough.

Influenza virus recognizes and binds to glycoconjugates on cell surfaces through one of its surface proteins, haemagglutinin. It requires a second surface enzyme, sialidase (neuraminidase) for effective infection and replication. The viral sialidase shows a characteristic substrate specificity^(24,25), allowing the release of newly synthesized virions from infected cells⁽²⁶⁾ and facilitates the movement of the virus to the mucosal cells by attacking the protective mucus layer at the mucosal surface^(25,27). Rational drug design has now allowed the synthesis of new, more potent inhibitors of the influenza sialidase⁽²⁸⁾.

The role of carbohydrates in human immunodeficiency virus (HIV) infection has recently been well reviewed. Briefly, the glycoprotein gp120 from HIV is heavily glycosylated with 24 relatively conserved N-linked glycosylation sites, all of which are probably used⁽²⁹⁾ and a few O-linked sites. Glycosylation appears to be necessary for gp160/gp120 from human immunodeficiency virus (HIV) to be functional as defined by CD4 binding competence⁽³⁰⁾ but other workers have found conflicting results⁽³¹⁾. It does appear however, that the glycosylation is essential for generation of the proper conformation of gp120 to provide a CD4-binding site.

CARBOHYDRATES AND CONGENITAL DISORDERS.

There are three congenital disorders so far that are known to involve the biosynthetic mechanism for glycoproteins possessing N-linked oligosaccharides. The very rare congenital dyserythropoietic anaemia type II (HEMPAS) is caused by a deficiency of the Man α :N-acetylglucosaminyltransferase II enzyme^(32,33), while I-cell disease is caused by a deficiency of the enzyme phospho-N-acetylglucosaminyltransferase⁽³⁴⁾. Recently, a deficiency in the carbohydrate moiety of secretory glycoproteins, lysosomal enzymes and membranous glycoproteins has been found to lead to a set of multisystemic diseases with major nervous system involvement. These syndromes are known as the

carbohydrate-deficient glycoprotein syndromes and appear to be due to a deficiency of asparagine-N-linked oligosaccharide transfer in type I disease and a deficiency of N-acetylglucosaminyltransferase II in type II disease⁽³⁵⁾. A recent discovery of a third variant of the disease (type III)⁽³⁶⁾ awaits the discovery of the basic biochemical defect.

SUMMARY

It is clear that carbohydrates contribute significantly to the structure and function of glycoproteins that are involved in physiological and pathological processes. Careful structural and functional analyses of these carbohydrates will enable their role within the medical field to be better defined.

REFERENCES

1. Dabelsteen, E. and Clausen, H., eds. (1992) Carbohydrate Pathology, APMIS supplement no. 27, vol. 100, Munksgaard Publishers, Copenhagen, Denmark, ISBN 87-16-14947-5
2. Muramatsu, T. (1993) Carbohydrate signals in metastasis and prognosis of human carcinomas, *Glycobiology* **3**, 294-6
3. Itzkowitz, S.H., Bloom, E.J., Kokal, W.A., Modin, G., Hakomori, S. and Kim, Y.S. (1990) Sialosyl-Tn. A novel mucin antigen associated with prognosis in colorectal cancer patients, *Cancer* **66**, 1960-6
4. Leatham, A.J. and Brooks, S.A. (1987) Predictive value of lectin binding on breast-cancer recurrence and survival, *Lancet* **i**, 1054-6
5. Hiraizumi, S., Takasaki, S., Ohuchi, N., Harada, Y., Nose, M., Mori, S. and Kobata, A. (1992) Altered glycosylation of membrane glycoproteins associated with human mammary carcinoma, *Jpn. J. Cancer Res.* **83**, 1063-72
6. Lasky, L.A. (1992) Selectins: interpreters of cell-specific carbohydrate information during inflammation, *Science* **258**, 964-9
7. Bevilacqua, M.P. (1993) Endothelial-leukocyte adhesion molecules, *Annu. Rev. Immunol.* **11**, 767-804
8. Brandley, B.K., Kiso, M., Abbas, S., Nikrad, P., Srivastava, O., Foxall, C., Oda, Y. and Hasegawa, A. (1993) Structure-function studies on selectin carbohydrate ligands. Modifications to fucose, sialic acid and sulphate as a sialic acid replacement, *Glycobiology* **3**, 633-9
9. Nelson, R.M., Dolich, S., Aruffo, A., Cecconi, O. and Bevilacqua, M.P. (1993) Higher-affinity oligosaccharide ligands for E-selectin, *J. Clin. Invest.* **91**, 1157-1166
10. Nguyen, M., Folkman, J. and Bischoff, J. (1993) A role for sialyl Lewis-X/A glycoconjugates in capillary morphogenesis, *Nature*, **365**, 267-9
11. Gearing, A.J.H. and Newman, W. (1993) Circulating adhesion molecules in disease, *Immunol. Today* **14**, 506-12
12. Newman, W., Beall, L.D., Carson, C.W., Hunder, G.G., Graben, N., Randhawa, Z.I., Gopal, T.V., Wiener-Kronish, J. and Matthey, M.A. (1993) Soluble E-selectin is found in supernatants of activated endothelial cells and is elevated in the serum of patients with septic shock, *J. Immunol.* **150**, 644-54
13. Springer, T.A., Thompson, W.S., Miller, L.J., Schmalstieg, F.C. and Anderson, D.C. (1984) Inherited deficiency of the Mac-1, LFA-1, p150,95 glycoprotein family and its molecular basis, *J. Exp. Med.* **160**, 1901-18
14. Etzioni, A., Frydman, M., Pollack, S., Avidor, I., Phillips, M.L., Paulson, J.C. and Gershoni-Baruch, R. (1992) Brief report: recurrent severe

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- infections caused by a novel leukocyte adhesion deficiency, *New Engl. J. Med.* **327**, 1789-92
15. Furukawa, K., and Kobata, A. (1991) IgG galactosylation - its biological significance and pathology, *Mol. Immunol.* **28**, 1333-40
 16. Rook, G.A., Attiyah, R.A. and Foley, N. (1989) The role of cytokines in the immunopathology of tuberculosis, and the regulation of agalactosyl IgG, *Lymphokine Res.* **8**, 323-8
 17. Filley, E., Andreoli, A., Steele, J., Waters, M., Wagner, D., Nelson, D., Tung, K., Rademacher, T., Dwek, R. and Rook, G.A. (1989) A transient rise in agalactosyl IgG correlating with free interleukin 2 receptors, during episodes of erythema nodosum leprosum, *Clin. Exp. Immunol.* **76**, 343-7
 18. Furukawa, K., Matsuta, K., Takeuchi, F., Kosuge, E., Miyamoto, T and Kobata, A. (1990) Kinetic study of a galactosyltransferase in the B cells of patients with rheumatoid arthritis, *Int. Immunol.* **2**, 105-12
 19. Young, A., Sumar, N., Bodman, K., Goyal, S., Sinclair, H., Roitt, I. and Isenberg, D. (1991) Agalactosyl IgG: an aid to differential diagnosis in early synovitis, *Arthritis Rheum.* **34**, 1425-9
 20. Boren, T., Falk, P., Roth, K.A., Larson, G. and Normark, S. (1993) Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens, *Science* **262**, 1892-5
 21. Saitoh, T., Natomi, H., Zhao, W.L., Okuzumi, K., Sugano, K., Iwamori, M. and Nagai, Y. (1991) Identification of glycolipid receptors for *Helicobacter pylori* by TLC-immunostaining, *FEBS Lett.* **282**, 385-7
 22. Evans, D.G., Karjalainen, T.K., Evans, D.J. Jr., Graham, D.Y. and Lee, C.H. (1993) Cloning, nucleotide sequence, and expression of a gene encoding an adhesin subunit protein of *Helicobacter pylori*, *J. Bacteriol.* **175**, 674-83
 23. Masco, D., van de Walle, M. and Spiegel, S. (1991) Interaction of ganglioside GM1 with the B subunit of cholera toxin modulates growth and differentiation of neuroblastoma N18 cells, *J. Neurosci.* **11**, 2443-52
 24. Corfield, A.P., Michalski, J.-C. and Schauer, R. (1981) The substrate specificity of sialidases from microorganisms and mammals, In: Tettamanti, G., Durand, P. and Di Donato, S. (eds), *Sialidases and sialidases* **4**, 3-70, Edi Ermes, Milan
 25. Corfield, A.P. (1992) Bacterial sialidases - roles in pathogenicity and nutrition, *Glycobiology* **2**, 509-21
 26. Palese, P., Jobita, K., Ueda, M. and Compans, R.W. (1974) Inhibition of influenza and parainfluenza virus replication in tissue culture by 2-deoxy-2,3-dehydro-N-trifluoroacetylneuraminic acid (FANA), *Virology* **61**, 397-410
 27. Corfield, A.P., Lambre, C.R., Michalski, J.-C., and Schauer, R. (1992) Role of sialic acid and sialidases in molecular recognition processes, Institut National de la Sante et de la Recherche Medicale, Paris, 113-75
 28. von Itzstein, M., Jin, B., Wu, W.-Y., Kok, G.B., Pegg, M.S., Dyason, J.C., Jin, B., Phan, T.V., Smythe, M.L., White, H.F., Oliver, S.W., Colman, P.M., Varghese, J.N., Ryan, D.M., Woods, J.M., Bethell, R.C., Hotham, V.J., Cameron, J.M. and Penn, C.R. (1993) Rational design of potent sialidase-based inhibitors of influenza virus replication, *Nature* **363**, 418-23
 29. Leonard, C.K., Spellman, M.W., Riddle, L., Harris, R.J., Thomas, J.N. and Gregory, T.J. (1990) Assignment of intrachain disulfide bonds and characterization of potential glycosylation sites of the type 1 recombinant human immunodeficiency virus envelope glycoprotein (gp120) expressed in Chinese hamster ovary cells, *J. Biol. Chem.* **265**, 10373-82
 30. Morikawa, Y., Moore, J.P., Wilkinson, A.J. and Jones, I.M. (1991) Reduction in CD4 binding affinity associated with removal of a single glycosylation site in the external glycoprotein of HIV-2, *Virology* **180**, 853-6
 31. Li, Y., Luo, L., Rasool, N. and Kang, C.Y. (1993) Glycosylation is necessary for the correct folding of human immunodeficiency virus gp120 in CD4 binding, *J. Virol.* **67**, 584-8
 32. Fukuda, M.N., Dell, A. and Scartezzii, P. (1987) Primary defect of congenital dyserythropoietic anaemia type II. Failure in glycosylation of erythrocyte lactosaminoglycan proteins caused by lowered N-acetylglucosaminyltransferase II, *J. Biol. Chem.* **262**, 7195-206
 33. Fukuda, M.N. (1990) HEMPAS disease: genetic defect of glycosylation, *Glycobiology* **1**, 9-15
 34. Ben-Yoseph, Y., Potier, M., Mitchell, D.A., Pack, B.A., Melancon, S.B. and Nadler, H.L. (1987) Altered molecular size of N-acetylglucosamine 1-phosphotransferase in I-cell disease and pseudo-Hurler polydystrophy, *Biochem. J.* **248**, 697-701
 35. Jaeken, J., Carchon, H. and Stibler, H. (1993) The carbohydrate-deficient glycoprotein syndromes: pre-Golgi and Golgi disorders? *Glycobiology* **3**, 423-8
 36. Ramaekers, V.T., Stibler, H., Kint, J. and Jaeken, J. (1991) A new variant of the carbohydrate deficient glycoproteins syndrome, *J. Inher. Metab. Dis.* **14**, 385-8.



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