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Book Review

GlycoImmunology 2, Advances in Experimental Medicine and Biology, Vol. 435 (1998) Axford, John S. (ed), New York: Plenum Press.

This book is a collection of papers that were presented at the Fourth Jenner International GlycoImmunology Meeting in Loutraki, Greece, in November 1996, which was the latest in a series of multidisciplinary meetings providing the possibility for discussion and exchange of ideas between everyone from research scientists to clinicians. This was encouraged by the arrangement of the program and is reflected in this book which gives a well-rounded view of glycoimmunology, rather than specializing in any particular aspect.

The first section has the heading of "Glycogiology: The Basics." Rather than reiterating material found in standard textbooks or reviews, it concentrates on a few areas of interesting research. The first chapter by Dirk Van den Eijnden discusses how studies of novel pathways, which were first discovered in invertebrate systems, may also apply to mammals. In invertebrates, rather than finding galactose in the *N*-acetyl lactosamine unit, it is common to find *N*-acetyl galactosamine, the so-called LacdiNAc structure. In other studies, an enzyme, β1-4 GlcNAcT, was found that transfers some GlcNAc residues to form chitobiose structures. It seems possible, therefore, that there are other glycosyltransferase gene families to be discovered and that some members of these could be found in mammals. In the next chapter, Harry Schachter presents a concise but comprehensive review of some diseases with defects in the systems for *N*-glycan biosynthesis. In a review of carbohydrate deficiency syndromes (CDGS types I and II) and HEMPAS (hereditary erythroblastic multinuclearity with a positive acidified serum lysis test), he shows how it can be difficult to localize the exact site of the defect, due to the complex interactions between the products of the transferase genes and the presence of alternative pathways. Another important aspect of studies of such syndromes are the clues they provide to understanding the functional role of glycans. In the final chapter in this section, Steve Homans gives a clear account of how NMR may be used to look at the structure of a carbohydrate protein complexes in solution. The systems chosen were those formed between a cytotoxin enzymatic subunit and a trisaccharide from globotriaosylceramide, and by a steriod glucuronide with an antibody. These serve to illustrate how multidimensional multinuclear NMR techniques can be used to gain considerable conformational data on binding in solution. This is very important in considering how glycans are interacting with proteins and which part of the glycan is important in such interactions.

The next section continues the theme on oligosaccharides and protein recognition. The chapter by Brian Sutton considers detailed studies of the role of oligosaccharides of IgG Fc fragment in its associations with the rheumatoid factor. The crystal-structure studies indicate that there are few antibody contact residues, suggesting that the binding of this autoantibody to IgG may represent cross-reactivity Although this antibody does not recognize the carbohydrate, accessibility to some of the peptide epitopes is resticted by the glycan, and this may be altered with the agalactosylated structure found in rheumatoid arthritis patients. In the next chapter, Ten Feizi briefly describes the application of the neoglycolipid technology in the determination of glycans involved in lectin and collectin binding. Next, Jean-Philippe Girand describes interesting studies on the way in which sulphated carbohydrate ligans are biosynthesized in high endothelial venules. So far this group have cloned the enzyme responsible for the synthesis of activated sulphate, PAPS (3′ phosphoadenosine 5′ phosphosulphate) synthetase. This enzyme seems to play an important role in controlling oligosaccharide sulphation. It will be of considerable interest to see studies of related enzymes and the sulphotransferases themselves. Studies on the Sialyl Lewsi X (SLe^x) epitope are reported in the chapter by Risto Renkonen, in which a series of sLex glycans were synthesized on a polylactosamine backbone, some of which have multimeric presentation of the epitope. Complex syntheses can be performed in sufficient yields to give sufficiently well-characterized material for binding studies. It was possible to determine the relative affects of multivalency and chain length in these studies. These suggested that parts of the molecule other than the SLe^x epitope could be important in the binding process. In the final chapter in this section, Claudine Keida describes interesting functional assays, using fluorescent beads coated with neoglycoproteins having different glycans attached. This type of system can be used to monitor the binding of different cell types and their specificities for different oligosaccharides both in vitro and in vivo, thus monitoring activity under physiologic conditions.

Further aspects of biological function are considered in the next section, starting with an update of research into the role of O-GlcNAc by Bradley Hayes and Gerry Hart.

A considerable number of proteins have now have been found to carry this modification, and they perform a number of different roles. There is an increasing amount of evidence to suggest that OGlcNAc performs a regulatory role, and there seems to be a close relationship to protein phosphorylation in a number of cases. Various effects on protein folding, conformation, subunit association, and enzyme activity have been described. The enzymes involved in the addition and removal of this residue in a dynamic process are also described. Farooq et al. describe a study of IgG glycosylation in a longitudinal study of an IgG3 paraprotein from a multiple myeloma patient. This showed that there were changes in glycosylation over a period of time, which appeared to correlate with disease activity. The glycosylation profile, therefore, may be a useful indicator of the disease. On a more basic level, the role of calnexin on sorting of misfolded protein in the endoplasmic reticulum is described by John Bergeron. The calnexin recognizes a monoglucosylated intermediate in the pathway and acts as a lectin. On correct folding, the nascent glycoprotein will dissociate from the calnexin, and the subsequent action of a glucosidase releases the glucose from the folded protein. This may serve as a means of "quality control," as the authors term it, to prevent production of incorrectly folded proteins.

The next section deals with inflammation, and the first chapter by Eric Berger is a most useful overview of the immunodetection of glycosyltransferases. The availability of specific antibodies to glycosyltransferases means that they can be used as invaluable tools for monitoring the distribution and expression of glycosyltransferases in a tissue and even on a cell-specific basis. As protein glycoforms may be regulated by the transferase expression, these can give valuable insights into the control of glycosylation. At the end of this chapter is a comprehensive list of currently available antibodies and their specificities. Some very clean exmples of how glycosylation can serve as a regulation mechanism are presented by Phillipp Van den Steen. Control of cytokine and tissue protease activities are vital in regulation of inflammation, and many key components are glycosylated in a controlled manner. The effects of changes in N and O glycosylation on the pharmokinetics and in the activity of extracellular matrix proteases; these provide some of the best examples of functional role of glycans. Staying with the inflammation theme, Willem Van Dijk reports studies on the expression of SLe^x on acute phase proteins, in particular α1-acid glycoprotein, which show changes in the relative amounts of different glycoforms correlated with the inflammatory process. Of particular interest are changes in fucosylation and increased SLe^x expression, which occur in the late phase of inflammation and which can be readily detected by the specific lectin Aleuria aurantia lectin. This increased SLe^x expression may have some effect on the selectin interactions with leukocytes.

The next section is entitled "Glycosylation and Disease"; it gives a number of examples of disease-related glycosylation in different areas. Pauline Rudd reports on a detailed study of the glycosylation of CD59, a regulator of complement-mediated lysis in human erythrocytes. This important molecule is not only N and O glycosylated, but also carries a glycosylphosphatidly inositol anchor. The glycosylation is very complex, and until recently, the complete analysis of such glycosylation would have taken a large amount of material and a considerable period of time. The developments in analytical technology with fluorescent labeling, predictive high perfo liqui chromatograph (HPLC) chromatography, and enzymatic sequencing of the complete glycan pool have enabled complete characterization of the CD59 glycosylation in a relatively short period and with the small amounts of the material that are available. It was even possible to study the differences in the platelet and red cells forms of the protein. Such techniques will undoubtedly make analysis of many more glycoproteins of biological interest a realistic possibility. Another well-known example of disease-related glycosylation changes occurs in IgG in rheumatoid arthritis. John Axford reported on the progress of his group in studying the glycosylation pattern of IgG in a number of rheumatic diseases by HPLC. Intriguingly, there seem to be specific patterns associated with each disease, although this will require confirmation by studies currently underway with a larger number of patients. Other studies reported by Alice Allen suggest that changes in O glycosylation in the hinge region may correlate with IgA nephropathy, and this could be significant in the pathology of the disease, although once more further data will be required to substantiate this. Graham Turner reports a new technique, which would allow rapid oligosaccharide profiling, and demonstrated it with studies on acute phase proteins. Optimized and sensitive release procedures are combined with HPAEC-PAD analysis to give detailed and very reproducible glycan profiles of acute phase proteins in various disease states; this could be used to provide more sensitive and specific disease markers on a routine basis. In the next two chapters, the role of viral glycosylation is examined. Anand Mehta describes the role of *N*-linked glycosylation in secretion of Hepatitis B virus. Experiments with various inhibitors of *N*-linked glycosylation emphasize the importance of correct glycosylation for viral secretion. Site-specific effects were also demonstrated by studies on three different hepatitis B envelope glycoproteins, and mechanisms involving protein misfolding are discussed. These studies have obvious relevance to the therapeutic possibilities of preventing virus secretion by specific inhibition of their glycosylation. In a related chapter, Tim Block examines the role of glycan processing in protein trafficking and shows how studies on the hepatitis B envelope protein are valuable in this respect. Detailed studies of subcellular localization in the presence of glucosidase inhibitors show that the proteins with unprocessed

glycans are not secreted and are in fact recycled through the microsomes although precise mechanisms still need to be determined.

The final section in the book is devoted to "Glycotherapeutics" in which some of the approaches being taken in this expanding field are presented. Zhi-Guang Wang and Ole Hindsgaul describe some very elegant methods for the synthesis of oligosaccharide libraries, using combinatorial chemistry. These approaches promise the production of large arrays of related compounds, which can then be screened for activity and hopefully give lead compounds for therapeutics. Details of several techniques are given, their synthetic pathways covered with some illustrative examples of the types of compound obtained, and the relative merits of the different approaches. Synthetic schemes for producing libraries of glycomimetic compounds, which probably offer the greatest therapeutic potential, are also described. These will undoubtedly be taken up commercially, and it will be of interest to see results of biological studies with such compounds. In the next chapter, Richard Moxon describes an area of glycoimmunology with immediate application, namely, that of vaccines. Bacterial polysaccharides are highly specific to certain strains and can affect virulence. Using models of *Neisseria meningitidis* and *Haemophilus influenzae* as examples, it is shown how knowledge of the detailed structures present on the lipo polysaccharides (LPS), along with generation of mutant strains with altered glycosylation, can lead to the elucidation of a core structure that can be a good potential vaccine candidate. Dariusz Izycki considers a different aspect of therapy in the use of gene therapy with glycosyltransferase genes, which could be used to correct abnormal glycosylation. Construction of retroviral vectors allows a highly efficient transfer of glycosyltransferase genes into metastic cells in murine melanoma, which result in alteration of their surface glycosylation. The double copy dicistronic retroviral system has looks very promising for gene therapy delivery. Liz Hounsell uses a number of examples of glycosaminoglycans and proteins with O-linked glycans to demonstrate the importance of multivalent epitope presentation and local confirmation to give high-affinity interactions. The effects of oligosaccharide epitope diversity in these examples, where a number of different carbohydrates are present in close proximity, are also discussed. It is concluded that in complex interactions involving glycoproteins the quantitative aspect of expression is most important. In the final chapter, Robert Feldmann shows how Group B streptococcal capsular carbohydrate (GBS) can be used to probe the mechanism of the antibody response to GBS infection and to elucidate the role of regulatory T-cells in B-cell activation *in vitro*. This may help to understand the reason for poor immune response and lead to improved vaccine development.

In summary, this book is a comprehensive account of current progress in glycoimmunology. It provides detailed accounts of presentations from the Jenner Conference, and the diverse range of subjects should be of interest to both the scientific and medical communities. This is an area of active research, and the next meeting in this series is eagerly awaited.

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