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Review

The role of glycosylation in protein antigenic properties

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Abstract. Glycosylation of proteins is a common event and contributes to protein antigenic properties. Most data have been obtained from model studies on glycoprotens with well-defined structure or synthetic glycopeptides and their respective monoclonal antibodies. Antibodies raised against glycoprotein antigens may be specific for their carbohydrate units which are recognized irrespective of the protein carrier (carbohydrate epitopes), or in the context of the adjacent amino acid residues (glycopeptidic epitopes). Conformation or proper exposure of peptidic epitopes of glycoproteins is also frequently modulated by glycosylation due to intramolecular carbohydrate-protein interactions. The effects of glycosylation are broad: glycosylation may 'inactivate' the peptidic epitope or may be required for its reactivity with the antibody, depending on the structure of the antigenic site and antibody fine specificity. Evidence is increasing that similar effects of glycosylation pertain to T cell-dependent cellular immune responses. Glycosylated peptides can be bound and presented by MHC class I or II molecules and elicit glycopeptide-specific T cell clones.

Key words. Antibody; epitope; glycoprotein; glycosylation; MHC complex; N-glycan; O-glycan; Pepscan analysis; peptide; T cell response.

Introduction

Glycosylation is the most common posttranslational modification of proteins [1]. Nevertheless, its role was obscure for a long time. That it may protect proteins against proteolytic degradation or denaturation was known, but its contribution to the biological activity of proteins was not immediately recognized. The questions remained as to why nature 'decorates' proteins with a variety of complex oligosaccharide structures, why some inborn errors in biosynthesis or metabolism of glycoconjugates lead to severe diseases, and concerning the significance of alterations of glycosylation in several other diseases, including cancer [2–5]. The last two decades have seen an enormous development in glycobiology which has provided at least a partial understanding of the role of glycosylation. In the glycoprotein biosynthetic pathway, N-linked glycans, which are recognized by lectin-like chaperonins (calnexin, calreticulin), play the role of 'tags' in protein folding and 'quality control' processes [6–8]. The glycans are known to play a role in oligomerization, targeting, clearance and biological functions of proteins [9, 10]. Most biological processes are initiated by specific interaction between molecules, that are cell membrane linked and/or secreted to body fluids. Oligosaccharide chains directly participate in specific interactions with lectins and lectin-like proteins, and a variety of human and animal lectins have been detected and their importance in biological processes has been documented [11]. In addition, protein-linked oligosaccharides, which do not participate directly in the reaction, can modulate interactions between polypeptide chains, by 'masking' or 'proper exposure' of the peptidic fragment involved in the reaction.

Most molecules (e.g. cellular receptors, cytokines, antibodies) involved in the immune system are glycosylated

and the role of glycosylation in their assembly, stability, cell surface exposure or secretion, and recognition has been the subject of several recent reviews [12–16]. The most important immunological process is the cellular and humoral response against foreign antigens. This review focuses on the contribution of oligosaccharide chains to recognition of glycosylated protein antigens by antibodies and T cell receptors.

Heterogeneity of protein glycosylation

There are two major types of oligosaccharide moieties linked to proteins in eukariotic cells, O-glycosidic chains linked *via* GalNAc to Ser/Thr residues, and N-glycosidic chains, whose innermost GlcNAc residue is attached to the amide group of Asn. Each of these types presents a variety of structures (fig. 1). N-glycosylation of Asn residues requires the sequence Asn-X-Ser/Thr and N-glycosylated proteins vary from those containing only one N-glycan to ones carrying over 20 N-linked oligosaccharide chains. The multiple O-glycans are present in mucin-type glycoproteins (mucins, glycophorins, leukosialins), where they frequently form clusters of chains linked to adjacent Ser/Thr residues. There are also glycoproteins with mixed-type glycosylation, i.e. a single or few O-glycans are present in N-glycosylated proteins or, vice versa, some richly O-glycosylated proteins may also carry one or more N-glycans. There are no distinct rules for the structural requirements of O-glycosylation sites, but certain amino acid sequences proximal to Ser/Thr residues are known to favour or inhibit their O-glycosylation [9]. There are also other types of glycosylation. Collagen carries Galβ or Glcβ1-2Galβ units linked to hydroxylysine residues. Recently discovered types of glycosylation of mammalian proteins include O-fucosylation, O-glucosylation and O-mannosylation of Ser/Thr residues, or C-mannosylation of tryptophan residues [17–19]. A unique type of glycosylation is attachment of a single GlcNAcβ to Ser/Thr residues of nuclear and cytoplasmic proteins [20, 21]. An exact consensus motif for O-GlcNAc has not been identified. However, many attachment sites are identical to those used by Ser/Thr kinases, and several lines of evidence have been obtained for a reciprocal relationship between O-GlcNAc and O-phosphorylation. Although the biological role of this highly dynamic protein modification remains to be elucidated, the exisitng data imply that O-GlcNAc is a regulatory modification involved in cellular signalling.

The structure of protein-linked oligosaccharide chains depends on several factors involved in the biosynthetic machinery of the cell, such as expression of glycosyltransferases (and other enzymes), the availability of nucleotide-sugar substrates and the correct location of the

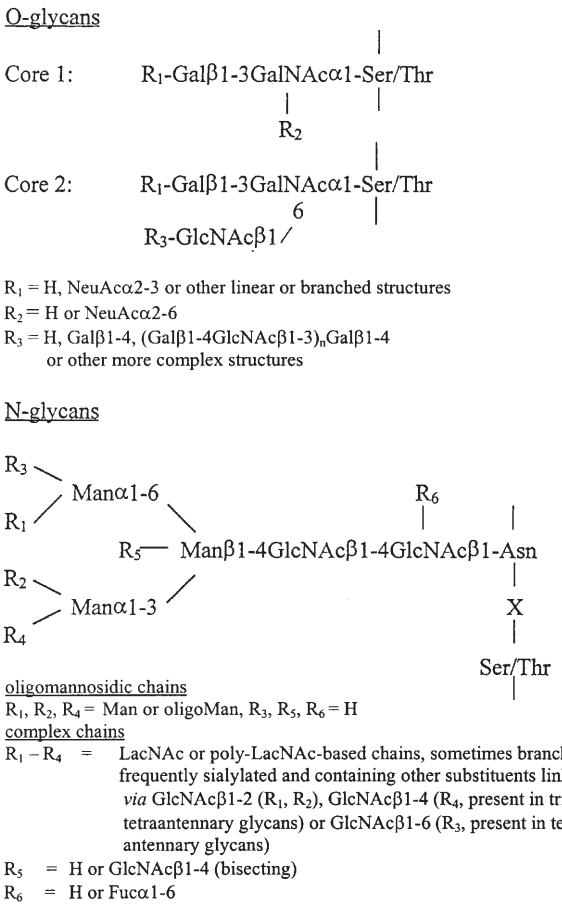

Figure 1. Structures of most common O- and N-glycans of human and animal glycoproteins.

protein or glycoprotein acceptor in the trafficking route of biosynthesized glycoproteins [22]. Glycosylation of individual proteins can be modulated by their own structure that involves not only the occupancy of potential glycosylation sites, but also the structure of the outer parts of glycans. For example, N-glycans carrying terminal GalNAc residues are typical for glycoprotein hormones [23], and mannosyl residues are selectively phosphorylated in N-glycans of lysosomal enzymes [24]. Such dependence on the amino acid sequence of the glycosylated protein may also apply to O-glycans. In glycophorin A (GPA), the efficiency of galactosylation of GalNAc residues linked to Ser/Thr residues 2–4 depends on adjacent amino acid residue(s) (fig. 2) and is lower in GPA of blood group N (Leu_1, Glu_5) than in GPA-M (Ser_1, Gly_5) [25]. The character of the cellular glycosylation system and modulation of this process by the structure of the protein recipient result in enormous heterogeneity in protein-linked oligosaccharide chains. Their structure depends on the cell type and its developmental state. In one cell, various glycoproteins are differently glycosylated and, moreover, each individual glycoprotein shows intermolecular and intramolecular microhetero-

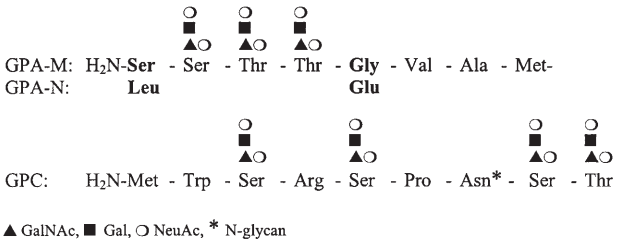

Figure 2. Structure of N-terminal fragments of GPA (M and N) and GPC.

geneity of the carbohydrate moiety, resulting from different degrees of occupancy of individual glycosylation sites and variations in the oligosaccharide structure.

Antigenic epitopes of glycoproteins

Glycosylated proteins carry different types of antigenic epitopes. Some oligosaccharide units can be antigenic themselves and elicit various anti-carbohydrate antibodies. Another type are glycopeptidic epitopes defined by antibodies which recognize specific oligosaccharide structures and adjacent amino acid residue(s). In contrast to anti-carbohydrate antibodies, antibodies directed against glycopeptidic epitopes do not react with free oligosaccharides. Finally, there are peptidic epitopes which represent either relatively short sequences of the polypeptide chain (linear epitopes), or include amino acid residues less or more distant in the polypeptide chain and brought into proximity due to the secondary structure of the protein (conformational epitopes). Many peptidic epitopes are independent of protein glycosylation and are recognized in fully glycosylated glycoprotein and in partially or totally deglycosylated antigen as well. However, not rarely, and especially in richly glycosylated proteins, peptidic epitopes are either apparently masked by glycans, or glycosylation of one or more flanking amino acid residues enhances the reaction or is even required for antibody binding. In the latter case, distinguishing between whether the oligosaccharide chain directly participates in the Ag-Ab reaction (glycopeptidic epitope) or is only required for the proper exposure or steric arrangement of the peptidic epitope may be difficult. However, one can tentatively assume that those antibodies which require a strictly defined carbohydrate unit(s) in a glycoprotein antigen, and do not bind to free or otherwise linked saccharide(s), deglycosylated antigen or to corresponding peptides are likely to react directly with both sugar and amino acid residues, and are anti-glycopeptidic antibodies. On the other hand, those antibodies which react with non-glycosylated peptides, or require glycosylation which is not limited to the defined carbohydrate structure, are considered to have anti-peptidic specificity, even if glycosylation modulates their interaction with the antigen.

Glycosidic and glycopeptidic epitopes of glycoproteins

Structures of many oligosaccharide chains linked to proteins are identical or closely similar among species and are not or are only weakly immunogenic. Despite difficulties in generating antibodies against such structures, there are reports on obtaining murine monoclonal antibodies against common oligomannosidic and complex-type N-glycosidic chains [26–28]. However, certain carbohydrate structures are expressed only in some individuals (e.g. ABH/Le, P1, or Sd^a blood group antigens [29]) or occur rarely (e.g. polyagglutination antigens). Such carbohydrate units are strongly immunogenic in individuals or animals missing these structures, and in most cases, 'natural' alloantibodies exist in human sera (table 1). The O-GlcNAc present in intracellular proteins is also immunogenic. An O-GlcNAc-specific antibody binds various O-GlcNAc-containing proteins which can be eluted from the antibody with free GlcNAc [21]. An interesting monoclonal antibody was obtained which recognized a unique α-linked GlcNAc in O-glycans (structure in table 1) of mucin from mammalian gastric gland mucous cells [30]. A species-specific structure will also generate carbohydrate antigenicity. The xenoantigenic non-reducing terminal unit Galα1-3Gal is widely present in glycoproteins and glycolipids in mammals, but not in humans, and natural antibodies against this structure, present in all human sera, are a major reason for acute rejection of xenotransplants [31]. In an attempt to use this epitope as a target for gene therapy of cancer, transfection of functional α1,3-Gal transferase into human cancer cells made them susceptible to lysis by natural human antibodies [32]. The specific features of plant and insect N-glycans are the β1,2-xylose and core α1,3-fucose (table 1) which cause glycoproteins from plants and some invertebrates to elicit strong IgG and IgE (allergic) responses in humans and animals [33–35]. The problem is particularly important in view of the potential immunogenicity of pharmaceutical recombinant glycoproteins produced in transgenic plants [36].

Many monoclonal anti-carbohydrate antibodies have been obtained, and most of which are directed against oligosaccharide structures typical for glycosphingolipids [37]. Several 'glycotopes' are typical for glycoproteins and some epitopes are present in N- and O-glycans as well, and also in glycosphingolipids. Therefore, anti-carbohydrate antibodies are not specific for a defined glycoprotein, because they usually recognize their target epitopes in different antigens, irrespective of the glycan core structure and its carrier. However, anti-carbohydrate antibodies specific for the same structure may differ in fine specificity, since they recognize different fragments or sides of the target structure [37].

Table 1. Examples of immunogenic carbohydrate antigens.

Antigen	Structure	Comments
H	Fucα1-2Galβ1-3/4GlcNAc-R	blood group ABH/Lewis antigens
A	GalNAcα1-3[Fucα1-2]Galβ1-3/4GlcNAc-R	
B	Galα1-3[Fucα1-2]Galβ1-3/4GlcNAc-R	
Le^a	Gal β1-3[Fucα1-4]GlcNAc-R	
Le^b	Fucα1-2Galβ1-3[Fucα1-4]GlcNAc-R	
Le^x	Galβ1-4[Fucα1-3]GlcNAc-R	
Le^y	Fucα1-2Galβ1-4[Fucα1-3]GlcNAc-R	
P1	Galα1-4Galβ1-4GlcNAc-R	P blood group system antigen, alloantibodies in P1-negative individuals
Sialyl-Le^a	NeuAcα2-3Galβ1-3[Fucα1-4]GlcNAc-R	sialo-Lewis antigens, ligands for selectins
Sialyl-Le^x	NeuAcα2-3Galβ1-4[Fucα1-3]GlcNAc-R (and related structures)	
Cad	NeuAcα2-3[GalNAcβ1-4]Galβ1-3[NeuAcα2-6]-GalNAc-Ser/Thr	rarely occurring on polyagglutinable red blood cells
Sd^a	NeuAcα2-3[GalNAcβ1-4]Galβ1-4GlcNAc-R	antigen frequent in humans, antibodies in Sd(a–) individuals
TF	Galβ1-3GalNAcα1-O-Ser/Thr	Thomsen-Friedenreich and Tn antigens cryptic in normal tissues, antibodies in all human sera
Tn	GalNAcα1-Ser/Thr	
Sialyl-Tn	NeuAcα2-6GalNAcα1-Ser/Thr	
	GlcNAcβ-O-Ser/Thr	epitope of nuclear and cytoplasmic proteins
	GlcNAcα1-4 Galβ1-4GlcNAcβ1-6 GalNAc **GlcNAcα1-4**Galβ1-3	GlcNAcα-related epitope of gastric mucin
	Galα1-3Galβ1-3GlcNAc-R	antigen common in nonprimates and absent in humans, antibodies in all human sera
	Manα1-6 Manβ1-4GlcNAcβ1-4GlcNAcβ-Asn Manα1-3 ↑ ↑ **Xylβ1,2** **Fucα1,3**	epitopes present in plant and some invertebrate glycoproteins eliciting humoral responses and allergic reactions in humans

Another type of carbohydrate epitopes are Thomsen-Friedenreich (TF) and Tn antigens (table 1) which represent core structures of O-glycans present in erythrocyte glycophorins and other mucin-type glycoproteins. Glycophorins are densely O-glycosylated type 1 membrane sialoglycoproteins, and a major structure of their O-glycans is the tetrasaccharide NeuAcα2-3Galβ1-3-[NeuAcα2-6]GalNAc [38]. The TF and Tn antigens were first identified in rare polyagglutinable erythrocytes, either transiently carrying asialoglycophorins (TF), or present in individuals with acquired Tn syndrome, carrying glycophorins with non-galactosylated O-glycans (Tn or sialyl-Tn). These normally cryptic structures are exposed in many cancer tissues and are used as targets for immunotherapy [39–41]. Anti-TF, anti-Tn and anti-sialyl-Tn antibodies recognize Ser/Thr-linked Gal-GalNAc, GalNAc or NeuAc-GalNAc units, respectively, and frequently require clusters of such units present on mucin-type glycoproteins [42, 43]. Some anti-Tn antibodies react similarly with Tn and sialyl-Tn units, i.e. they recognize GalNAc residues irrespective of their substitution at C6 with sialic acid. Moreover, immunization of mice with asialo-glycophorin A (asialoGPA, GPA-TF) or asialo-agalactoGPA (GPA-Tn) showed that two types of anti-TF or anti-Tn antibodies are induced, with anti-carbohydrate or anti-glycopeptidic specificity [44 and our unpublished results]. Antibodies with anti-carbohydrate specificity react with various glycoproteins carrying TF or Tn determinants and are convenient reagents for identifying of these sugar units in biological material, including cancer cells. There are also antibodies which react only with the antigen used for immunization, i.e. recognize the respective carbohydrate structures in the context of a specific sequence in the polypeptide chain. Such antibodies recognize glycopeptidic epitopes and can serve for detection of the defined glycoform (TF or Tn) of a specific glycoprotein.

Strongly immunogenic N-terminal fragments of GPA and glycophorin C (GPC) from human erythrocytes (fig. 2) are also examples of glycopeptidic epitopes. GPA exists in two forms differing in amino acids 1 and 5 (blood group M and N antigens), and there are many antibodies which are specific or react preferentially with one of these forms (M or N specificity). Anti-M and anti-N monoclonal antibodies represent a wide spectrum of fine specificities [38]. Most recognize a difference in the first amino acid residue, although some anti-M are specific for epitopes dependent on Gly_5. Moreover, the blood group M- or N-related epitopes differ in their requirement for the terminal α-amino group and most are sensitive to desialylation. Interestingly, examination of modified GPAs carrying exclusively monosialylated linear (NeuAc-Gal-GalNAc) or branched (Gal-[NeuAc]GalNAc) O-glycans showed that some antibodies, reactive with epitopes sensitive to total desialylation, require the presence in the epitope of Gal-linked sialic acid only, while for other antibodies, GalNAc-linked sialic acid residues are essential [45]. GPC has an entirely different amino acid sequence compared to GPA, but antibodies directed against its N-terminal fragment (fig. 2) also recognize epitopes dependent on the first amino acid residue (Met) and usually dependent on sialylation [38, 46]. The presence of only one (Ser_1 or Leu_1 in GPA) or two (Met_1-Trp_2 in GPC) terminal non-glycosylated amino acid residues in the epitopes and lack of reactivity of desialylated and/or de-O-glycosylated antigens suggest that N-terminal fragments of GPA and GPC represent glycopeptidic epitopes in which both amino acid residues and carbohydrate components are directly involved in the antigen-antibody reaction. However, this does not apply to the Gly_5-dependent blood group M-related epitopes (see below).

Effects of glycosylation on peptidic epitopes of glycoproteins

To understand these effects it is important to realize that intramolecular interactions between glycans and the peptide backbone occur in glycosylated proteins and affect the conformation and flexibility of both glycan and peptide. Most physiological interactions occur in a water environment. Recent studies on glycopeptide and glycoprotein conformation in solution [reviewed in refs 47–50] using circular dichroism and advanced nuclear magnetic resonance spectroscopy techniques combined with computational molecular dynamics simulation have allowed the following general conclusions to be drawn: glycosylation usually increases the local structural order of the polypeptide chain (e.g. induces turn-like structures in glycopeptides), and the spatial arrangement of glycans is affected by their interaction with the peptide; the intramolecular carbohydrate-protein interactions and their effects depend on the glycan structure and its microenvironment and may be different in individual glycosylation sites. These data are in full agreement with observations that the reactivity of many antibodies directed against peptidic epitopes is modulated in different ways by antigen glycosylation. Several examples will serve to illustrate this problem.

The enhancement of antibody binding to partially or totally deglycosylated antigen suggested recognition of a peptidic epitope which in the native glycoprotein is more or less shielded by glycans, especially the large ones. However, it is not always a simple steric hindrance effect, as shown by the example of influenza virus haemagglutinins [51]. The haemagglutinins of pathogenic avian influenza viruses of Dutch and Rostock strains differ in that only the haemagglutinin of the Rostock strain carries a complex-type N-glycan at Asn_{149}. Rabbit antibodies against peptide comprising amino acid residues 143–162 of haemagglutinin reacted only with the non-glycosylated epitope of the Dutch strain. An apparent 'masking' effect of N-glycan was independent of its size. The Asn-linked GlcNAc residue was sufficient to prevent recognition of the epitope and its removal was required for restoration of the antigenic activity. The opposite effect, i.e. creation of a neoepitope by a single monosaccharide, was shown in the example of an interesting monoclonal antibody (FDC-6) which reacted with oncofetal fibronectin but did not react with normal fibronectin [52]. The minimal structure required for the reaction with FDC-6 was found to be the hexapeptide VTHPGY with an O-glycosylated threonine residue. The same sequence is present in normal fibronectin, but is not glycosylated. mAb FDC-6 reacted only with the glycosylated epitope, irrespective of O-glycan size, which can be NeuAc-Gal-GalNAc (present in native antigen), Gal-GalNAc, or only a GalNAc residue. These results indicate that in both cases, a single monosaccharide residue directly linked to the peptide alters peptide conformation or flexibility to either inactivate the antigenic structure [51] or create a new epitope [52].

The effect of antigen glycosylation was extensively studied with anti-MUC1 monoclonal antibodies, using synthetic peptides and glycopeptides comprising the immunodominant amino acid sequences of MUC1 mucin. Identification of epitopes by means of peptides synthesized in immobilized form on multiple plastic pins (Pepscan analysis) showed that most anti-MUC1 antibodies are specific for various overlapping epitopes within the sequence APDTRPAP of the tandem repeat unit of MUC1 [53]. Two anti-MUC1 monoclonal antibodies were tested with the synthetic MUC1 fragment, TAP-PAHGVT_9S**APDTRPAP**G$S_{20}T_{21}$APPA, in a non-glycosylated form or carrying one, two or three GalNAc residues on amino acids 9, 9/21 or 9/20/21, respectively [54]. The antibodies recognized overlapping epitopes

PDTR (mAb HMFG1) and RPAP (mAb C595). All glycosylated peptides showed distinctly decreased reactivity with mAb HMFG1, while monoglycosylation showed no effect and di- or triglycosylation enhanced the reactions with mAb C595 compared to the non-glycosylated peptide. Circular dichroism studies revealed that a left-handed polyproline II helix adopted by this peptide in solution is further stabilized by addition of the GalNAc residues [55].

Another set of peptides and glycopeptides was used to study a large number of anti-MUC1 monoclonal antibodies recognizing peptidic epitopes in the same immunodominant MUC1 region [56, 57]. Substitution of peptides with carbohydrate residues gave variable results, depending on the antibody and glycopeptide used, but in many cases, glycosylation altered the reaction with antibody. Surprisingly, for some antibodies, even extensive glycosylation of the peptide may be without effect. For example, two mAbs, SM3 and HMPV, reacted equally well with the MUC1 peptide and its counterpart carrying five GalNAc residues [58]. Together, these results revealed that glycosylation of MUC1 may modulate the reactivity of peptidic epitopes, from decreasing, through no effect, to enhancing their reactivity, in a manner that depends on the site of glycosylation and fine specificity of the antibody. Similar effects of glycosylation were found for human anti-MUC1 antibodies from breast cancer patients which were examined with various MUC1 peptides, either non-glycosylated or containing GalNAc residues [59]. The MUC1 mucin, whose glycosylation is altered in cancer cells, is one of the targets for cancer immunotherapy. The data on the antigenic properties of MUC1 and a role for its glycosylation are important for choosing the correct MUC1 peptides or glycopeptides as the most effective anti-cancer vaccines.

In our laboratory, peptidic epitopes for anti-GPA and anti-GPC monoclonal antibodies were identified by Pepscan analysis [46, 60–64]. Many anti-peptidic GPA antibodies recognize various overlapping epitopes within two segments of the extracellular domain of the GPA polypeptide chain (highlighted below), where several adjacent Ser and Thr residues are glycosylated (asterisks):

^{35}AAT***PRAHEV**S*EIS*V**RT*VYPPEEE**TGE^{60}

Interestingly, reactions of these antibodies with erythrocytes or GPA are either unaffected or strongly enhanced by GPA desialylation, even if antibodies recognize identical or closely similar epitopes on synthetic peptides. For example, one of two antibodies (OSK4-1) recognizing the RAHEV sequence reacted comparably with untreated and desialylated GPA, while the second (GPA33) reacted weakly with the untreated antigen and showed strong enhancement of reactivity (up to 100-fold) after its desialylation or de-O-glycosylation [60, 65]. To explain this difference, the epitope was examined using 95 replacement analogues of the RAHEV peptide in which each amino acid residue was replaced by one of 19 other amino acids. The mAbs OSK4-1 and GPA33 showed entirely different binding patterns and recognized a Glu or His residue, respectively, as an immunodominant amino acid [60]. Most likely, these antibodies react with different 'sides' of the same sequence, and only that with an exposed His residue is apparently masked by sialic acid present in O-glycans carried by flanking amino acid residues.

Most anti-peptidic antibodies directed against GPC recognize epitopes related to the sequence EPDPGM which is repeated twice in the GPC polypeptide chain:

^{14}LS*L**EPDPGM**AS*AS*T*T*MHT*T*T*
IA**EPDPGM**SGVP^{45}

Pepscan analysis showed that some antibodies require the presence of Leu_{16} in the epitope, while other antibodies should potentially react with both sites. However, unpredictable reactions of these antibodies with rare deletion variants of GPC (Gerbich and Yus, each containing one LEPDPGM sequence) and differentiated dependence of epitopes on antigen glycosylation indicate that in the glycosylated antigen there are additional requirements for the proper exposure of the peptidic epitope [38, 46, 61]. An interesting example is anti-GPC mAb NaM26-4C6 which cross-reacted with the band 3 protein [64]. This antibody recognized the epitope LEPDPGM of GPC and EDPDIP of the cytoplasmic (non-glycosylated) tail of band 3 protein. However, desialylation or de-O-glycosylation of GPC abolished its reactivity. This suggests that the LEPDPGM epitope, reactive with mAb NaM26-4C6 in synthetic peptides, requires full glycosylation in the GPC molecule for proper exposure.

There are also antibodies which react with peptidic epitopes inactivated by desialylation of the glycoprotein antigen and 'reactivated' after complete de-O-glycosylation. For example, anti-leukosialin (CD43) antibodies have been obtained which react with native and recombinant non-glycosylated (expressed in *Escherichia coli*) leukosialin, but do not react with desialylated leukosialin [66]. This is not a unique phenomenon, because similar properties are shown when anti-M antibodies cross-react with the rare variant M^g antigen. The M^g antigen is carried by GPA-N in which Thr_4 is replaced by Asn, and Ser_2/Thr_3 residues are not glycosylated (the M^g N-terminal sequence is: LSTNEVAMH-). Some of the antibodies which recognize a blood group M-related epitope, dependent on Gly_5 and sialylation (see fig. 2), surprisingly react with the non-glycosylated M^g antigen. Pepscan analysis of the epitopes for anti-M/M^g antibodies revealed that they react similarly with the peptides representing N-terminal sequences of GPA-M, GPA-N or GPA-M^g and

the immunodominant amino acids in the epitope are Val_6 and Met_8 [67]. Immunoblotting experiments showed that such antibodies bind specifically to untreated GPA-M, but after de-O-glycosylation they bind to GPA-M and GPA-N as well [65]. In conclusion, the Val_6/Met_8-related epitope is recognized in sialylated GPA, provided that a Gly (and not Glu) residue is present at position 5, is not or is only weakly reactive in asialoGPA, and is strongly reactive in non-glycosylated GPAs, regardless of the amino acid sequence at positions 1–5.

The role of glycosylation in T cell-dependent cellular immune responses

There are two subtypes of the major histocompatibility complex (MHC)-restricted T cell-dependent response. Antigens of endogenous origin (including antigens of replicating viruses) are proteolytically degraded in the cytoplasmic compartment of the target cell, and the peptides are transported into the endoplasmic reticulum. Here, the peptides 'meet' the nascent MHC class I proteins which upon binding the peptide can progress along the secretory pathway to the cell surface and present the peptide to cytotoxic T lymphocytes (CTLs). Exogenous antigens from outside the cell, or cell surface antigens, are internalized, degraded in the endosomal compartment, bound to MHC class II proteins and presented to helper (Th) cells. The Th cells and CTLs recognize presented peptides *via* T cell receptors (TCRs) which show clonal specificity for foreign antigens and presenting MHC molecules. The clones of primed T cells expand and either directly kill the virus-infected cells (CTLs), or activate the immune function of other cells (Th cells). The peptides bound to the groove of MHC class I molecules usually contain eight to ten amino acids; MHC class II can bind longer peptides.

There was an established view that only peptides are recognized by effector T cells in the context of MHC molecules and no evidence has been obtained so far that oligosaccharides can be bound and presented in this way. However, studies performed over the past decade have supplied increasing evidence that glycosylated peptides can also participate in the cellular immune response and that the contribution of the carbohydrate to T cell recognition is greatly differentiated. This problem was reviewed in 1997 [68] and will be briefly discussed here, with a stress on new findings.

A frequent approach to study this phenomenon has been to use synthetic peptides as T cell epitopes which are known to bind in the restricted way to the respective MHC class I or class II molecules [refs. in 68]. The peptides are chemically linked with monosaccharides or short oligosaccharides and compared with non-glycosylated peptides for their binding to MHC molecules and elicitation of the T cell immune response. Glycosylation of peptides had variable effects on the binding to MHC proteins: it abrogated or had no effect on binding, or in a few cases, increased the binding. The effect was greatly dependent on the carbohydrate location. In peptidic T cell epitopes, certain amino acids are anchor residues directly involved in MHC binding, and glycosylation of these residues reduced or abolished the binding. Carbohydrate linked to other residues, located within or outside the MHC groove, was tolerated. In most cases, immunization of mice with glycosylated peptides known to bind to MHC elicited the T cell clones which required both amino acid residues and sugars in the recognized glycopeptidic epitopes. To elucidate the role of glycosylation in TCR recognition of effector T cells, the response of T cell hybridomas to the same peptides carrying various carbohydrate residues was compared (table 2). The hen egg lysozyme (HEL) peptide with Galα1-4Galβ (galabiose) linked to the terminal amino group elicited T cell clones which recognized this glycopeptide and its analogues substituted with other carbohydrates, but not the naked peptide [69]. These results suggested that T cells recognize a specific conformation of the peptide conferred by glycosylation and that this recognition does not involve specific interaction between the carbohydrate moiety and the TCR. However, when the carbohydrate moiety was linked at the central position of the same HEL peptide, or the self-peptide of mouse haemoglobin, the T cell hybridomas recognized specifically the carbohydrate structure present on the peptide used for immunization [70, 71]. Similarly, immunization of mice with rat type II collagen elicited T cell clones recognizing specifically the peptide (collagen epitope) carrying either Gal or Glc-Gal residues naturally present in collagen [72]. The requirement for a specific carbohydrate structure and peptide sequence suggests that both properly exposed carbohydrate residues and amino acid side chains are directly recognized by T cells.

Direct recognition of carbohydrates by the TCR has been recently supported by determination of the crystal structure of MHC class I/glycopeptide complexes. Two analogues of the peptide FAPGNYPAL (immunodominant epitope of the Sendai virus nucleoprotein), carrying GlcNAcβ1-O-Ser in place of the fourth or fifth amino acid (both known to elicit the H-$2D^b$-restricted CTL response [73]) were studied as complexes with the MHC H-$2D^b$ molecule [74]. The glycan was found to be solvent exposed and available for direct recognition by the TCR. Modelling the complex between MHC/glycopeptide complexes and their respective TCRs showed that a monosaccharide residue can be accommodated in the standard TCR-MHC geometry. Another crystallographic study was performed on the glycopeptide analog of RGYVYQGL (epitope of the nucleoprotein of vesicular stomatitis virus) where Gln was replaced by homo-

Table 2. Specificity of carbohydrate recognition in glycosylated peptides by glycopeptide-specific T cell hybridomas.

Origin of peptide	MHC restriction (number of hybridomas)	Glycopeptide[a]	**R** in active peptides	**R** in inactive peptides	Reference
Hen egg lysozyme	class II I-A^k (2)	**R**→52DYGILQINSR^{61}	**Galα1-4Galβ** Galβ1-4Glcβ Glcβ1-4Glcβ Galβ		69
Hen egg lysozyme	class II I-A^k (2)	**R** ↓ ^{52}DYGI(L→S)QINSR^{61}	**Galα1-4Galβ** Galα1-4Gal$_{red}$ β[b]	Gal$_{red}\alpha$1-4Galβ[b] Glcβ1-4Glcβ Galβ	70
Mouse haemoglobin	class II I-E^k (19)[c]	**R** ↓ ^{67}VITAF(N→T)EGLK^{76} **R** ↓ ^{67}VITAF(N→S)EGLK^{76}	**GalNAcα-Thr** and/or GalNAcα-Ser	Manα1-Thr Glcβ-Thr GlcNAcα-Thr Glcβ1-3GalNAcα-Ser Glcβ1-3(GlcNAcβ1-6)GalNAcα-Ser	71
Rat type II collagen	class II (2)	**R** ↓ ^{256}GEPGIAGF(K→hnV)G EQGPK^{261}	**type II collagen** clone 1: Glcα1-2Galβ clone 2: Galβ	Galβ Galα1-4Galβ Glcα1-2Galβ Galα1-4Galβ	72

The hybridomas tested did not react with non-glycosylated counterparts of glycopeptides.

[a] Carbohydrate was bound either to the terminal amino group [69], or to Ser, Thr [70, 71] and hydroxynorvaline [72] which replaced natural amino acid residues in the peptides. The peptides with carbohydrate residues shown in bold (or type II collagen) were used for immunizing of mice.

[b] Gal$_{red}$ is Gal reduced at C6.

[c] There were a few exceptions in the fine specificity of the hybridomas tested; for simplicity, the generalized results are given.

cysteine carrying galabiose [75, 76]. Immunization with this glycopeptide generated MHC-restricted CTLs specific for the glycopeptide and unrestricted carbohydrate-specific CTLs which also recognized the free or glycolipid-bound disaccharide. The crystal structure of the H-2K^b/glycopeptide complex showed that carbohydrate dominates the central region of the putative TCR-binding site that allows its direct interaction with the TCR [76].

Of interest is how the results of studies on model glycopeptides apply to naturally glycosylated protein antigens. Most MHC class II-restricted protein antigens are glycosylated [1], and nuclear and cytosolic class I-restricted proteins undergo the dynamic O-glycosylation with GlcNAc [20, 21]. It is also important that glycoproteins can be partially or totally deglycosylated or/and reglycosylated during processing in the antigen-presenting cell. There are several reports on the carbohydrate-dependent T cell response to natural antigens. As mentioned above, immunization of mice with type II collagen induced T cell clones specific for glycopeptide carrying natural collagen-type carbohydrate units, Gal or Glc-Gal [72, 77]. The peptide carrying galactose was found to be immunodominant in collagen-induced arthritis in mice [77]. HLA class II-restricted T cell clones specific for the glycosylated epitope of the bee venom allergen phospholipase A2 (PLA) were isolated from allergic patients [78]. PLA contains one N-glycan, Manα1-6(±Manα1-3)Man β1-4GlcNAcβ1-4(±Fucα1-3/6)GlcNAcβ, and its α-mannose residues were necessary for stimulation of the respective T cell clones. A possibility that glycopeptides with a relatively large glycan can accommodate within the binding groove of MHC molecules was also indicated in other studies. The N-glycosylated Man-6-P containing glycopeptides from lysosomal enzyme binding to MHC class II [79] and N-glycosylated tyrosinase glycopeptide associated with MHC class I molecules [80] have been identified. Although the data are still scarce, they indicate that proteins glycosylation plays a role in the cellular immune response. The possible effects of glycosylation are similar to those found with epitopes recognized by antibodies. Carbohydrate linked to MHC-presented peptide may have no effect, may alter conformation and recognition of the peptidic epitope, or form a glycopeptidic neoepitope recognized by specific MHC-restricted T cell receptors. Moreover, glycopeptides and other glycoconjugates may elicit an MHC-unrestricted anti-carbohydrate cellular response and this approach is used in trials of cancer immunotherapy with carbohydrate vaccines [81].

Conclusions

The examples of antigens and antibodies were selected to illustrate diverse effects of protein glycosylation on antigenic epitopes. Glycoproteins may elicit antibodies against defined carbohydrate stuctures, which are recognized irrespective of their carrier protein or in the context of their location in the antigen polypeptide chain. Moreover, N- and O-glycans frequently modulate the activity of adjacent peptidic epitopes due to intramolecular carbohydrate-protein interactions and these effects are greatly differentiated. The presence of glycan(s) may not only enhance or decrease the reactivity of the peptidic epitope (quantitative change), but may also form a neoepitope or abolish the reaction (qualitative effect). There are no distinct rules as to which monosaccharide components of N- or O-glycans are most important for these effects. The influence of glycosylation on peptidic epitopes may pertain to the innermost GlcNAc or GalNAc residue directly linked to the polypeptide chain, to terminal sialic acid residues, or may be more complex, e.g. various components or fragments of the oligosaccharide chain may exert different (sometimes opposite) effects. Moreover, reactions of antibodies specific for the same amino acid sequence may be differentially affected by antigen glycosylation, because they recognize different immunodominant amino acid residues within the sequence. Glycosylation effects, presented in the examples of antigen-antibody reactions, may also be pertinent to T cell-dependent cellular immune responses and may apply to other recognition processes, like the formation of biologically active intermolecular complexes and the reactions of various ligands with their receptors. Generally, glycosylation only not supplies oligosaccharide structures participating directly in some reactions, but can also modulate protein-protein interactions. This is particularly important in view of the fact that glycosylation of proteins depends on the cell type and its developmental state, and is profoundly altered in cancer and many other pathological conditions. Full understanding of the role of glycosylation and the biological significance of glycosylation alterations, despite the progress already made, awaits further studies.

1 Apweiler R., Hermjakob H. and Sharon N. (1999) On the frequency of protein glycosylation, as deduced from analysis of the SWISS-PROT database. Biochim. Biophys. Acta **1473:** 4–8

2 Arrol H. and Jefferis R. (1997) Antibody and protein glycosylation in health and disease. In: Immunoregulation in Health and Disease, pp 115–138, Lukic M. L., Colic M., Mostarica-Stojkovic M. and Cuperlovic K. (eds) Academic Press, San Diego

3 Dennis J. W., Granovsky M. and Warren C. E. (1999) Protein glycosylation in health and disease. BioEssays **21:** 412–421

4 Dennis J. W., Granovsky M. and Warren C. E. (1999) Glycoprotein glycosylation and cancer progression. Biochim. Biophys. Acta **1473:** 21–34.

5 Hakomori S. (2001) Tumor-associated carbohydrate antigens defining tumor malignancy: basis for development of anti-cancer vaccines. Adv. Exp. Med. Biol. **491:** 369–402

6 Parodi A. J. (2000) Role of N-oligosaccharide endoplasmic reticulum processing reactions in glycoprotein folding and degradation. Biochem. J. **348:** 1–13

7 Benham A. M. and Braakman I. (2000) Glycoprotein folding in the endoplasmic reticulum. Crit. Rev. Biochem. Mol. Biol. **35:** 433–473

8 Helenius A. and Aebi M. (2001) Intracellular functions of N-linked glycans. Science **291:** 2364–2369

9 Van den Steen P., Rudd P. M., Dwek, R. A. and Opdenakker G. (1998) Concepts and principles of protein O-glycosylation. Crit. Rev. Biochem. Mol. Biol. **33:** 151–208

10 Reuter G. and Gabius H. J. (1999) Eukariotic glycosylation: whim of nature or multipurpose tool? Cell. Mol. Life Sci. **55:** 368–422

11 Lis H. and Sharon N. (1998) Lectins: carbohydrate-specific proteins that mediate cellular recognition. Chem. Rev. **98:** 637–674

12 Feizi T. (2000) Carbohydrate-mediated recognition systems in innate immunity. Immunol. Rev. **173:** 79–88

13 Muramatsu T. (2000) Essential roles of carbohydrate signals in development, immune response and tissue functions, as revealed by gene targeting J. Biochem. **127:** 171–176

14 Tsuboi S. and Fukuda M. (2001) Roles of O-linked oligosaccharides in immune responses. BioEssays **23:** 46–53

15 Lowe J. B. (2001) Glycosylation, immunty, and autoimmunity. Cell **104:** 809–812

16 Rudd P. M., Elliott T., Cresswell P., Wilson I. A. and Dwek R. A. (2001) Glycosylation and the immune system. Science **291:** 2370–237

17 Vliegenthart J. F. G. and Casset F. (1998) Novel forms of protein glycosylation. Curr. Opin. Struct. Biol. **8:** 565–571

18 Endo. T. (1999) O-mannosyl glycans in mammals. Biochim. Biophys. Acta **1473:** 237–246

19 Furmanek A. and Hofsteenge J. (2000) Protein C-mannosylation: facts and questions. Acta Biochim. Polon. **47:** 781–789

20 Comer F. I. and Hart G. W. (1999) O-GlcNAc and the control of gene expression. Biochim. Biophys. Acta **1473:** 161–171

21 Wells L., Vosseller K. and Hart G. W. (2001) Glycosylation of nucleocytoplasmic proteins: signal transduction and O-GlcNAc. Science **291:** 2376–2378

22 Varki A. (1998) Factors controlling the glycosylation potential of the Golgi apparatus. Trends Cell Biol. **8:** 34–40

23 Mazella S. M., Hooper L. V. and Baenziger J. U. (1996) Oligosaccharides containing β1,4-linked N-acetylgalactosamine, a paradigm for protein-specific glycosylation. J. Biol. Chem. **271:** 12117–12120

24 Nishikawa A., Nanda A., Gregory W., Frenz J. and Kornfeld S. (1999) Identification of amino acids that modulate mannose phosphorylation of mouse DNase secretory glycoprotein. J. Biol. Chem. **274:** 19309–19315

25 Krotkiewski H., Duk M., Syper D., Lis H., Sharon N. and Lisowska E. (1997) Blood group MN-dependent difference in galactosylation of O-glycans of glycophorin A is restricted to the GalNAc residues located on amino acid residues 2–4 of the glycophorin polypeptide chain. FEBS Lett. **406:** 296–300

26 Ohbayashi H., Takenaga H., Takenaga S., Shiraishi M., Kitajima T., Endo T. et al. (1992) Production of monoclonal antibodies against oligosaccharides coupled to protein. Carb. Res. **235:** 349–356

27 Schmitz B., Peter-Katalinic J., Egge H. and Schachner M. (1993) Monoclonal antibodies raised against membrane glycoproteins recognize N-linked oligomannosidic chains. Glycobiology **3:** 609–617

28 Ozawa H., Yamashita K., Sakuraba H., Itoh K., Kase R. and Tai T. (1997) Generation and characterization of mouse monoclonal antibodies specific for N-linked neutral oligosaccharides of glycoproteins. Arch. Biochem. Biophys. **342:** 48–57

29 Reid M. E and Lomas-Francis C. (1997) The Blood Group Antigen Facts Book, Academic Press, San Diego
30 Ishihara K., Kurihara M., Goso Y., Urata T. Ota H., Katsuyama T. et al. (1996) Peripheral α-linked N-acetylglucosamine on the carbohydrate moiety of mucin derived from mammalian gastric gland mucous cells: epitope recognized by a newly characterized monoclonal antibody. Biochem. J. **318:** 409–416
31 Joziasse D. H. and Oriol R. (1999) Xenotransplantation: the importance of the Galα1,3Gal epitope in hyperacute vascular rejection. Biochim. Biophys. Acta **1455:** 403–418
32 Yoshimura N., Sawada T., Furusawa M. and Fuchinoue S. (2001) Expression of xenoantigen transformed human cancer cells to be susceptible to antibody-mediated cell killing. Cancer Lett. **164:** 155–160
33 Staudacher E., Altmann F., Wilson I. B. H. and März L. (1999) Fucose in N-glycans: from plant to man. Biochim. Biophys. Acta **1473:** 216–236
34 Fötisch K., Altmann F., Haustein D. and Vieths S. (1999) Involvement of carbohydrate epitopes in the IgE response of celery-allergic patients. Int. J. Allergy Immunol. **120:** 30–42
35 Fabini G., Freilinger A., Altmann F. and Wilson I. B. H. (2001) Identification of core α1,3-fucosylated glycans and cloning of the requisite fucosyltransferase cDNA from *Drosophila melanogaster*: potential basis of the neural anti-horseradish peroxidase epitope. J. Biol. Chem. **276:** 28058–28067
36 Bardor M., Faye L. and Lerouge P. (1999) Analysis of N-glycosylation of recombinant glycoproteins produced in transgenic plants. Trends Plant Sci. **4:** 376–380
37 Kannagi R. and Hakomori S. (2001) A guide to monoclonal antibodies directed to glycotopes. Adv. Exp. Med. Biol. **491:** 587–630
38 Lisowska E. (2001) Antigenic properties of human glycophorins – un update. Adv. Exp. Med. Biol. **491:** 155–169
39 Lisowska E. (1995) Tn antigens and their significance in oncology. Acta Biochim. Polon. **42:** 11–18
40 Hanisch F.-G. and Baldus S. E. (1997) The Thomsen-Friedenreich (TF) antigen: a critical review on the structural, biosynthetic and histochemical aspects of a pancarcinoma-associated antigen. Histol. Histopathol. **12:** 263–281
41 Berger E. G. (1999) Tn syndrome. Biochim. Biophys. Acta **1455:** 255–268
42 Nakada H., Inoue M., Numata Y., Tanaka N., Fukanoshi I., Fukui S. et al. (1993) Epitopic structure of Tn glycophorin A for anti-Tn antibody (MSL-128). Proc. Natl. Acad. Sci. USA **90:** 2495–2499
43 Reddish M. A., Jackson L., Koganty R. R., Qiu D., Hong W. and Longenecker B. M. (1997) Specificities of anti-sialyl-Tn and anti-Tn monoclonal antibodies generated using novel clustered synthetic glycopeptide epitopes. Glycoconjugate J. **14:** 549–560
44 Duk M., Steuden I., Duś D., Radzikowski C. and Lisowska E. (1992) Application of chemically desialylated and degalactosylated human glycophorin for induction and characterization of anti-Tn monoclonal antibodies. Glycoconjugate J. **9:** 148–153
45 Duk M., Sticher U., Brossmer R. and Lisowska E.: (1994) The differences in significance of α2,3-Gal-linked and α2,6-GalNAc-linked sialic acid residues in blood group M- and N-related epitopes recognized by various monoclonal antibodies. Glycobiology, **4:** 175–181
46 Reid M. E., Lisowska E. and Blanchard D. (1997) Coordinator's report: glycophorin/band 3 and associated antigens. Transfus. Clin. Biol. **4:** 57–64
47 Imperiali B. and O'Connor S. E. (1999) Effect of N-linked glycosylation on glycopeptide and glycoprotein structure. Curr. Opin. Chem. Biol. **3:** 643–649
48 Qasba P. K. (2000) Involvement of sugar chains in protein-protein interactions. Carbohydr. Polymers **41:** 293–309
49 Seitz O. (2000) Glycopeptide synthesis and the effects of glycosylation on protein structure and activity. Eur. J. Chem. Biol. **1:** 214–246
50 Vliegenthart J. F. G. (2001) Intramolecular carbohydrate-protein interaction. Adv. Exp. Med. Biol. **491:** 133–140
51 Munk K., Pritzer E., Kretzschmar E., Gutte B., Garten W. and Klenk H. D. (1992) Carbohydrate masking of an antigenic epitope of influenza virus haemagglutinin independent of oligosaccharide size. Glycobiology **2:** 233–240
52 Matsuura H., Takio, K., Titani K., Greene T., Levery S. B. and Salyan M. E. K. (1988) The oncofetal structure of human fibronectin defined by monoclonal antibody FDC-6. J. Biol. Chem. **263:** 3314–3322
53 Petrakou E., Murray A. and Price M. R. (1998) Epitope mapping of anti-MUC1 mucin protein core monoclonal antibodies. Tumor Biol. **19 (suppl 1):** 21–29
54 Spencer D. I. R., Price M. R., Tendler S. J. B., De Matteis C. I., Stadie C. and Hanisch F.-G. (1996) Effect of glycosylation of a synthetic MUC1 mucin-core-related peptide on recognition by anti-mucin antibodies. Cancer Lett. **100:** 11-15
55 Spencer D. I. R., Missailidis S., Denton G., Murray A., Brady K. Matteis C. I . D. et al. (1999) Structure activity studies of the anti-MUC1 monoclonal antibody C595 and synthetic MUC1 mucin-core-related peptides and glycopeptides. Biospectroscopy **5:** 79–91
56 Karsten U., Diotel C., Klich G., Paulsen H., Goletz S., Muller S. et al. (1998) Enhanced binding of antibodies to the DTR motif of MUC1 tandem repeat peptide is mediated by site-specific glycosylation. Cancer Res. **58:** 2541–2549
57 Reddish, M. A., Suresh, M. R., Koganty R. R., Fortier S., Baronic L. and Longenecker B. M. (1998) Analysis of the role of type 1 core O-glycans in the binding of anti MUC1 antibodies by cytofluorometry and synthetic peptide/glycopeptide binding inhibition studies. Tumor Biol. **19 (suppl 1):** 57–66
58 Dupradeau F.-Y., Stroud M. R., Boivin D., Li L., Hakomori S., Singhal A. K. et al. (1994) Solid-phase synthesis and immunoreactivity of penta-O-(N-acetyl-α-D-galactosaminyl)-MUC1 eicosapeptide, a glycosylated counterpart of highly immunogenic tandem repeat sequence of carcinoma-associated mucin. Bioorg. Med. Chem. Lett. **4:** 1813–1818
59 Mensdorf-Pouilly S. von, Petrakou E., Kenemans P, Uffelen K. van, Verstraeten A. A., Snijdewint F. G. M. et al. (2000) Reactivity of natural and induced human antibodies to MUC1 mucin with MUC1 peptides and N-acetylgalactosamine (GalNAc) peptides. Int. J. Cancer **86:** 702–712
60 Waśniowska K., Duk M., Czerwiński M., Steuden I., Duś D., Radzikowski C. et al. (1992) Analysis of peptidic epitopes recognized by the three monoclonal antibodies specific for the same region of glycophorin A but showing different properties. Mol. Immunol. **29:** 783–791
61 Waśniowska K., Jaśkiewicz E., Czerwiński M., Syper D. and Lisowska E. (1997) Mapping of peptidic epitopes of glycophorins A (GPA) and C (GPC) with peptides synthesized on plastic pins (Pepscan analysis). Transfus. Clin. Biol. **4:** 73–75
62 Rasamoelisolo M., Czerwiński M., Bruneau V., Lisowska E. and Blanchard D. (1997) Fine characterization of a series of new monoclonal antibodies directed against glycophorin A. Vox Sang. **72:** 185–191
63 Rasamoelisolo M., Czerwiński M., Willem C. and Blanchard D. (1998) Shared epitopes of glycoprotein A and protein 4.1 defined by antibody NaM10-3C10. Hybridoma **17:** 283–288
64 Loirat M. J., Czerwiński M., Duk M. and Blanchard D. (1999) The murine monoclonal antibody NaM26-4C6 identifies a common structure on band 3 and glycophorin C. Transfus. Med. **9:** 69–79
65 Duk M., Ugorski M. and Lisowska E. (1997) β-Elimination of O-glycans from glycoproteins transferred to Immobilon P membranes: method and some applications. Anal. Biochem. **253:** 98–102

66 Cyster J. G., Shotton D. M. and Williams A. N. (1991) The dimensions of the T lymphocyte glycoprotein leukosialin and identification of linear protein epitopes that can be modified by glycosylation. EMBO J. **10**: 893-902.
67 Jaśkiewicz E., Czerwiński M., Syper D. and Lisowska E. (1994) Anti-M monoclonal antibodies cross-reacting with variant M^g antigen: an example of modulation of antigenic properties of peptide by its glycosylation. Blood **84:** 2340–2345
68 Carbonne F. R. and Gleeson P. A. (1997) Carbohydrates and antigen recognition by T cells. Glycobiology **7:** 725–730
69 Harding C. V., Kihlberg J., Elofsson L., Magnusson G. and Unaue E. R. (1993) Glycopeptides bind MHC molecules and elicit specific T cell responses. J. Immunol. **151:** 2419–2425
70 Deck M. B., Sjölin P., Unaue E. R. and Kihlberg J. (1999) MHC-restricted, glycopeptide-specific T cells show specificity for both carbohydrate and peptide residues. J. Immunol. **162:** 4740–4744
71 Jensen T., Hansen P., Galli-Stampino L., Mouritsen S., Frische K., Meinjohanns E. et al. (1997) Carbohydrate and peptide specificity of MHC class II-restricted T cell hybridomas raised against an O-glycosylated self peptide. J. Immunol. **158:** 3769–3778
72 Michaëlsson E., Broddefalk J., Engström Å., Kihlberg J. and Holmdahl R. (1996) Antigen processing and presentation of naturally glycosylated protein elicits major histocompatibility complex class II restricted, carbohydrate specific T cells. Eur. J. Immunol. **26:** 1906–1910
73 Haurum J. S., Tan L., Arsequell G., Frodsham P., Lellouch A. C., Moss P. A. H. et al. (1995) Peptide anchor residue glycosylation: effect on class I major histocompatibility complex binding and cytotoxic T lymphocyte recognition. Eur. J. Immunol. **25:** 3270–3276
74 Glithero A., Tormo J., Haurum J.S., Adsequell G, Valencia G., Edwards J. et al. (1999) Crystal structures of two H-$2D^b$/-glycopeptide complexes suggest a molecular basis for CTL crossreactivity. Immunity **10:** 63–74
75 Abdel-Motal U. M., Berg L., Rosen A., Bengtsson M., Thorpe J. C., Kihlberg J. et al. (1996) Immunization with glycosylated K^b-binding peptides generates carbohydrate-specific, unrestricted cytotoxic T cells. Eur. J. Immunol. **26:** 544–551
76 Speir J. A., Abdel-Motal U. M., Jondal M. and Wilson I. A. (1999) Crystal structure of an MHC class I presented glycopeptide that generates carbohydrate-specific CTL. Immunity **10:** 51–61
77 Corthay A., Bäcklund J., Broddefalk J., Michaëlsson E., Goldschmidt T. J., Kihlberg J. et al. (1998) Epitope glycosylation plays a critical role for T cell recognition of type II collagen in collagen-induced arthritis. Eur. J. Immunol. **28:** 2590–2590
78 Dudler T., Altmann F., Carballido J. M. and Blaser K. (1995) Carbohydrate-dependent, HLA class II-restricted, human T cell response to the bee venom allergen phospholipase A2 in allergic patients. Eur. J. Immunol. **25:** 538–542
79 Dustin M. L., McCourt D. W. and Kornfeld S. (1996) A mannose 6-phosphate-containing N-linked glycopeptide derived from lysosomal acid lipase is bound to MHC class II in B lymphoblastoid cell line. J. Immunol. **156:** 1841–1847
80 Androlewicz M. J. (1996) An N-glycosylated tyrosinase epitope associates with newly synthesized MHC class I molecules in melanoma cells. Human Immunol. **51:** 81–88
81 Sandmaier B. M., Oparin D. V., Holmberg L. A., Reddish M. A., MacLean G. D. and Longenecker B. M. (1999) Evidence of a cellular immune response against sialyl-Tn in breast and ovarian cancer patients after high-dose chemotherapy, stem cell rescue, and immunization with theratope STn-KLH cancer vaccine. J. Immunother. **22:** 54–66