Review

The trail of my studies on glycoproteins
from enterokinase to tumor markers

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Abstract: This review describes the results of the author's studies on glycoproteins which have been carried out for more than 50 years. Starting from the elucidation of basic structures of glycoproteins, i.e. the structure of the linkage between an amino acid and a sugar and the occurrence of the β-mannosidic linkage as the common structure of glycoproteins, the author became interested in the cell membrane glycoproteins focused on the comparison of cancer cells *versus* normal cells. These studies were then extended to the establishment of sugar-directed and cancer-associated monoclonal antibodies. Some of the monoclonal antibodies are useful for cancer diagnosis.

Keywords: mucin, cell membrane, glycopeptide, glycosylamine, monoclonal antibody, cancer diagnosis

Introduction

Glycoproteins are widely distributed in living organisms and show considerable diversity in structure and function. I have been involved in the study of the structures and biological functions of glycoproteins since 1953, and feel privileged to be able to review the results of my studies, thanks to many colleagues. This review comprises three sections. Section I describes the basic structure of glycoproteins, *i.e.* the structure of the linkage between an amino acid and a sugar. As to sugars, the occurrence of the β-mannosidic linkage was discovered as the common structure of glycoproteins. Section II describes the characterization of cell membrane glycoproteins, and Section III describes sugar-directed and cancer-associated monoclonal antibodies.

Section I. The basic structures of glycoproteins

(i) Isolation of a glycopeptide from ovalbumin.   During the period of 1953 to 1957, I had the chance to study at Karolinska Institutet in Stockholm, Sweden. Professor Eric Jorpes suggested that I study "enterokinase", which had been reported by a

Russian physiologist, I.V.Pavrov, to be present in the duodenal contents, activating proteases secreted from the pancreas. Trypsinogen was supposed to be the substrate of enterokinase, but its function and chemical entity were entirely unknown. After laborious attempts to purify the enzyme, I finally obtained a nearly homogeneous enterokinase preparation. The mechanism underlying the activation of trypsinogen by enterokinase was revealed and this discovery is now cited in all textbooks on biochemistry. However, I was more interested in the finding that the sugar content of the enterokinase preparation amounted to 45%. There was no terminology for glycoproteins at that time. Such preparations were designated as mucoproteins or sugar–protein complexes. I had a feeling that the polypeptide moiety should be covalently linked to the sugar moiety, an intuition based on the purification procedures for enterokinase.

After looking for glycoproteins that are easier to prepare than enterokinase, I decided to use ovalbumin since this protein is easy to prepare on a large scale and had been shown by Prof. Neuberger to contain a few percent of sugar. The term "glycoproteins" was accepted universally when a book entitled "Glycoproteins", edited by Dr. Gottschalk, was published in 1972.[1]

An advantage that I had at that time was that pronase, a potent proteinase with a broad specificity,

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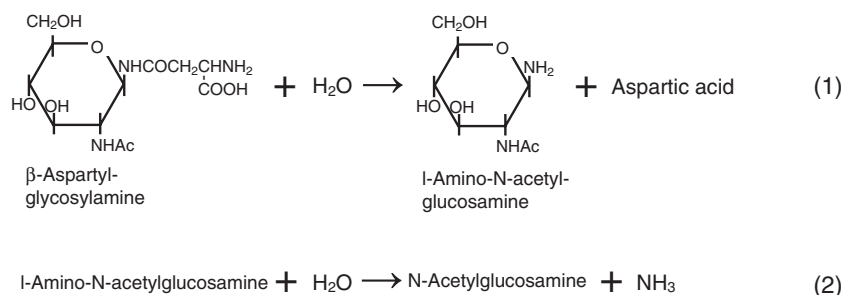
Fig. 1. Enzymatic cleavage of aspartylglycosylamine.

was only available in Japan. Using pronase, ovalbumin was digested extensively and finally a glycopeptide fraction was obtained that contained only asparatic acid as an amino acid and additionally ammonia in an equimolar amount, as judged after hydrolysis. The sugar components were identified as N-acetylglucosamine and mannose.[2] This was no longer glycopeptide, but it should be called glycosyl asparagine (or aspartic acid). The glycopeptide was then dinitrophenylated and subjected to partial acid hydrolysis (2 M HCl, 100°C, 20 min.). The hydrolysate contained several fragments that could be separated by paper electrophoresis. One of the fragments was composed of DNP-aspartic acid, ammonia and glucosamine in an equimolar ratio. This information prompted the Neuberger's group to synthesize aspartylglycosylamine (shown in Fig. 1). The British group had previously synthesized the aspartylglucosylamine (glucosamine being replaced by glucose), and found that the acid hydrolysis rates were nearly the same for the synthetic and natural linkages between asparagine and the sugar component. I also synthesized aspartylglycosylamine and tried to confirm the identity of the synthetic and natural aspartylglycosylamines. In this experiment, however, the yield of aspartylglycosylamine from the natural glycosyl asparagine through partial acid hydrolysis was only about 20% of the theoretical value although the identity was unanimously proved through the use of chromatographic techniques and infra-red spectroscopy.[3] The unequivocal confirmation of the linkage was obtained after the discovery of an enzyme that is specific to this linkage.

(ii) Discovery of an enzyme hydrolyzing the linkage between asparagine and N-acetylglucosamine.   Aspartylglycosylamine is apparently a derivative of asparagine in which one of the amide hydrogens is substituted by N-acetylglucosamine. This analogy would suggest that asparaginase may be able to hydrolyze the amide linkage in asparatyl-glycosylamine. Asparaginase from any source is known to be useful for the clinical treatment of leukemia patients since the leukemia viruses require asparagine for their growth. In the animal kingdom, asparaginase was known to be most abundant in guinea pig serum. Some amount of guinea pig sera was fractionated on an ion exchange column, and the non-identity of asparaginase and the new enzyme was clearly shown. The new enzyme was named "aspartyl-glycosylamine amide hydrolase", and its mode of action, substrate specificity and subcellular distribution were examined.

As shown in Fig. 1, the enzyme, simply designated as amidase, hydrolyzes the amide linkage producing aspartic acid and glycosylamine (1-amino-N-acetylglucosamine). Glycosylamine was slowly hydrolyzed non-enzymatically to produce ammonia and N-acetylglucosamine. This non-enzymatic reaction proceeded at a higher rate as the pH value fell.[4] Large scale purification of the enzyme was carried out from hog liver, which was found to contain a relatively large amount of the enzyme, a homogeneous preparation being obtained. It became clear that a single enzyme is involved in the cleavage of aspartylglycosylamine, verifying the reaction mechanism shown in Fig. 1.[5]

Quantitative isolation of aspartylglycosylamine from glycosyl asparagine was attempted using both chemical and enzymatic methods. The chemical method used was Smith degradation. Peeling off of the mannose and N-acetylglucosamine residues by repeating the procedures, comprising oxidation with periodate, reduction and mild acid hydrolysis, led to the isolation of aspartylglycosylamine nearly quantitatively. For enzymatic methods, α-mannosidase, β-mannosidase and β-N-acetylglucosaminidase were used, which also led to the isolation of aspartyl-glycosylamine quantitatively. Of the glycosidases we used, β-mannosidase was newly prepared from a snail (*Achatina fulica*), and it enabled us to discover the

occurrence of the β-form of the innermost mannose residue in the sugar moiety of ovalbumin.[6] The β-form mannose residue was then ubiquitously detected in all the glycoproteins so far investigated.

Several aspartylglycosylamines have been synthesized in which natural N-acetylglucosamine was replaced by mannose, galactose, and N-acetylgalactosamine, respectively. These synthetic aspartylglycosylamines were hydrolyzed with purified amidase at rates that are not very different from that with the natural aspartylglycosylamine.[7] However, these new types of linkage between asparagine and sugars other than N-acetylglucosamine have not been detected in any glycoproteins so far.

Amidase seems to require the free α-amino and α-carboxyl groups of asparagine for its activity, but exhibits a broad specificity as regards the sugar moiety. Monosaccharides and even oligosaccharides are utilized. Amidase splits off aspartic acid from the glycosyl asparagine from ovalbumin and some glycosyl asparagine from serum glycoproteins (*e.g.* orosomucoid). Thus, amidase provides decisive evidence for the aspartylglycosylamine linkage in glycoproteins. Amidase is, however, not useful for the release of the sugar moiety from the polypeptide moiety in native glycoproteins. Recently, a new enzyme named N-glycanase has become available for release of the sugar moieties from undigested polypeptide moieties.[8]

Amidase was found to be a lysosomal enzyme,[9] which suggested the possibility of a new genetic disease caused by a deficiency of this enzyme. In fact, the first report of this possibility appeared in the UK, and then hundreds of patients with this disease were found in Finland.[10] The disease was named "aspartylglycosylaminuria" and characterized by the daily excretion of about 300 mg of aspartylglycosylamine in the urine. Some of the aspartylglycosylamine possessed one or two moles of mannose attached to β-N-acetylglucosamine, probably due to the addition of a mannose residue during excretion.[11]

(iii) Classification of glycoproteins. Glycoproteins are divided into two groups based on the nature of the sugars and their modes of linkage to proteins. One group is referred to as serum-type glycoproteins, which consist predominantly of mannose-containing sugar chains linked N-glycosidically *via* N-acetylglucosamine to asparagine in the polypeptide. The other group is referred to as mucin-type glycoproteins, which consist predominantly of mannose-free sugar units O-glycosidically linked *via* N-acetylgalactosamine to serine and/or threonine in the

polypeptide.[12] These two groups of glycoproteins can be distinguished further based on structural characteristics. In mucin-type glycoproteins, the sugar units (chains) are densely linked to the polypeptide, in which serine and threonine are the major amino acids, which makes the glycoproteins rather resistant to proteinase digestion. In serum-type glycoproteins, however, sugar-linking asparagine is rather sparsely distributed in the polypeptide, which makes the glycoproteins susceptible to proteinases. Thus, extensive digestion of these glycoproteins with pronase produces glycopeptides of different sizes that were separated by gel filtration on Sephadex G-50, namely G-50I from mucin-type glycoproteins and G-50II from serum-type glycoproteins, respectively. G-50I is eluted from the column earlier than G-50II. These glycopeptides will be dealt with in Sections II and III.

Section II. Characterization of membrane glycoproteins

We have been particularly interested in cell surface glycoproteins because they determine cell behaviour. Comparison of cancer *versus* normal cells in humans is an attractive approach. Prior to this approach, we investigated the cell membranes of rat liver and hepatomas. Due to the limited space, the results are summarized as follows.

1. All the subcellular membranes examined contained certain amounts of sugar. Plasma membranes contained more sialic acid than intracellular membranes. No mucin-type glycoproteins are present in liver or regenerating liver of rats.[13]–[15]

2. Plasma membranes of high purity were prepared from hepatoma cells. The membranes contained more than 30 protein components, several of which were glycoproteins. A major glycoprotein was isolated that contained about 40% sugar composed of both O-glycosidically and N-glycosidically linked sugar units.[16] The structures of nine O-glycosidic sugar units derived from the major glycoproteins were determined. The smallest unit was N-acetylgalactosamine (GalNAc) and the largest one was GlcNAc-containing hexasaccharide. Some of them were novel sugar units.[17]

3. The plasma membranes from hepatomas contained proteoglycans. Heparan sulphate and chondroitin sulphate were identified for AH 66 and AH 130, respectively, as their sugar chains.[18]

Section III. Sugar-directed and cancer-associated monoclonal antibodies

Many sugar chain-directed monoclonal antibodies are regarded as effective agents for detecting tumor markers.[19] Using the LS 180 and SW 1116 cell lines, which were separated from human colorectal cancer tissues and which were provided by the Type Culture Collection, USA, we have tried to establish hybridoma clones producing sugar-directed monoclonal antibodies recognizing tumor markers more effectively. Unfortunately, we did not have reliable human cancer cell lines in Japan at that time.

Glycoproteins embedded in cell surface membranes are often potent antigens. We adopted a means of immunizing mice with whole cells that led to the production of many antibodies directed to antigenic molecules on the cell surface. However, it is tedious to select hybridoma clones that produce sugar-directed and cancer-associated antibodies from among large numbers of hybridomas by means of cell-binding assaying of the antibodies.[19] We have invented a new procedure for selecting hybridomas in this regard.[20]

(i) From LS 180 cells. The cell membranes of LS 180 cells were prepared by treating the cells with a buffer containing 1% Triton X-100. This treatment does not result in membranes of high purity, but assures the release of glycoproteins and glycolipids from the cells. After the removal of lipids, the remaining sample was exhaustively digested with pronase to produce G-50I and G-50II. The reason why we used glycopeptides for the detection of antibodies instead of sugar chains released from polypeptides is that some antibodies might recognize not only sugar chains but also those attached to a limited region of peptides.

To detect antibodies directed to G-50I, immuno-assay plates were coated with these glycopeptides with the aid of glutaraldehyde and polylysine. For G-50II, the glycopeptides were acylated with palmitic acid to increase the coating efficiency. Coating with glycolipids was carried out using a conventional procedure.

In the experiments involving LS 180 cells, the culture fluid produced by hybridomas in 12 out of 7X96 wells showed significant binding to G-50I and/or G-50II plates, and one of them showed binding to only G-50I plates. Cloning of the hybridomas by the limited dilution method led to the establishment of more than ten clones.[20]

(ii) MLS 102 antibody. The epitopic structure for one of the antibodies, designated as MLS 102, was determined. MLS 102 reacted with a wide variety of mucin-type glycoproteins, which included ovine (OSM), bovine (BSM), and porsine (PSM) submaxillary mucins.[21] OSM exhibited exceedingly high reactivity compared to other mucin-type glycoproteins, even more than that of G-50I prepared from LS180 cells. The sugar units constituting OSM, BSM and PSM had already been investigated to some extent. In OSM, $Sia\alpha2\rightarrow6GalNAc\alpha\rightarrow Ser/Thr$ in the polypeptide chain had been identified as the main structure of the sugar attachment motif. In BSM and PSM, some longer sugar chains had been detected in addition to the OSM disaccharide.

Further studies revealed that $Sia\alpha2\rightarrow6GalNAc$(or Gal) was the complete antigen for MLS 102 although its antigenic reactivity was only one thousandth of that of OSM. Sialic acid and $Sia\alpha2\rightarrow3Gal$ were incomplete antigens, inhibiting the antigen–antibody reaction only partially even at very high concentrations. N-Glycolylsialic acid and trisaccharides in which galactose is attached to GalNAc of the antigenic disaccharide were completely inactive. The big difference in antigenic activity between OSM and the released disaccharide may be explained according to the immunologic concept of **avidity**. This contrasts the affinity between a monovalent antigen and an antibody.[22]

MLS 102 did not react with umbilical cord tissue, amniotic fluid, saliva, seminal fluid, meconium or human milk, but reacted with the mucosal epithelium of the esophagus and stomach of humans. The use of MLS 102 as a tumor marker antibody awaits further investigation.

The antigens derived from LS 180 cells were purified by immunoaffinity column chromatography. Sugar chains were released from the purified antigens by alkaline borohydride treatment. The sugar chains of the MLS 102 antigen were composed of 0-linked $Sia\alpha2\rightarrow6GalNAc$ (56%) and 0-linked GalNAc (25%) residues. Thus, the antigen apparently possesses an OSM-like structure as shown in Fig. 2. An 0-linked $Sia\alpha2\rightarrow6GalNAc$ residue is not a specific sugar chain in the cancer-associated antigen, being also found in normal colonic mucins. However, the small amount and probably limited distribution of the disaccharide in the polypeptide chain are not enough for reaction with MLS 102 type antibodies.

Other antibodies, MLS 103–MLS 105, did not react with mono- and disaccharides, but reacted with much longer sugar chains (Fig. 2). These

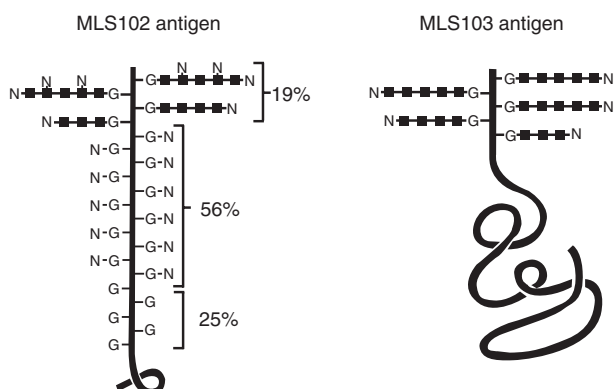MLS102 antigen   MLS103 antigen

Fig. 2.   Proposed gross structures of the MLS 102 and MLS 103 antigens. Dark bands, nonglycosylated regions of the polypeptide chain. G, GalNAc. N, NeuAc. ■, any monosaccharide constituting linear or branched oligosaccharide chains.

antibodies reacted with cancer cells as well as normal mucosal epithelium. The differences in size and structure of sugar chains between cancer-associated and -unassociated antigens may support the idea that the malignant transformation of cells is accompanied by aberrant and incomplete glycosylation.[23),24)]

(iii) MLS 128 (anti-Tn) antibody.   The next antibody we investigated was MLS 128, which was assigned as an anti-Tn antibody based on its capacity to agglutinate Tn erythrocytes. Tn antigen was discovered in 1959 as an antigen on erythrocytes of a patient with hemolytic anemia,[25)] and is regarded as a tumor-associated antigen.[26),27)] Since then it has been believed that GalNAc-Ser/Thr is included in Tn antigen,[28)] but its exact epitopic structure is unknown. Two anti-Tn monoclonal antibodies are known. One is NCC-LU-35 which was raised against human lung cancer cells,[29)] and the other is CA 3239, which was raised against glycophorin A from Tn erythrocytes.[30)] These antibodies and MLS 128 inhibited the reactions with Tn erythrocytes, G-50I from LS 180 cells and OSM each other completely. All the anti-Tn antibodies reacted with asialo G-50I and asialo OSM to the same extent as with the intact antigens. These findings suggest that all known anti-Tn antibodies recognize the same epitope. Digestion of asialo OSM with pronase reduced the binding capacity of asialo OSM as to all the anti-Tn antibodies.[31)] Digestion of asialo OSM with trypsin, V8 protease and thermolysin, followed by fractionation of the digests, led to the isolation of a glycopeptide with strong immunoreactivity. The glycopeptide had the sequence of Leu–Ser*–Thr*–
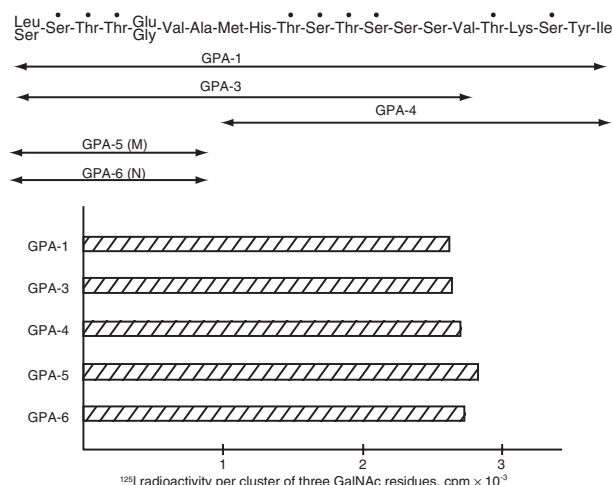

Fig. 3.   Structures and immunoreactivities of the glycopeptides derived from glycophorin A. GPA, glycopeptides derived from glycophorin A. The reactivity which was determined using ^{125}I-labeled protein A and is expressed relative to the clusters of three consecutive GalNAc-Ser/Thr. ● indicates O-glycosylation sites. The sequence indicates from the N terminus to Ile-21 comprising the MM and NN types of glycophorin.

Thr*–Gln–Leu–Pro–Gly (asterisks indicate that a GalNAc residue is linked).[32)]

Similar studies were carried out on glycophorin A from human erythrocytes. We could use a glycoprotease from *Pasteurella haemolytica* A1 that is capable of hydrolyzing sialylated mucin-type glycoproteins.[33)] On treatment of the glycoprotease digest with sialidase and β-galactosidase, Tn antigenicity was induced since $Sia\alpha2\rightarrow3Gal\beta1\rightarrow3(Sia\alpha2\rightarrow6)GalNAc$-Ser/Thr is one of the major sugar chains of glycophorin A. Fractionation of the digest by HPLC led to the isolation of five glycopeptides with potential Tn antigenicity, as shown in Fig. 3. The reactivities of the three- and four-consecutive-residue sequences of GalNAc-Ser/Thr were equal.[34)]

We found that a major glycoprotein, leukosialin (CD 43), of a T-lymphoid cell line, Jurkat, expresses Tn antigen.[35)] On the basis of its amino acid sequences and sugar structure,[36),37)] leukosialin seems to contain seven cluster structures, each consisting of three or four consecutive GalNAc-Ser/Thr residues. gp120, a coat protein of human immunoreactive virus, also expresses Tn antigen.[38)] Although the antigenic site has not been determined, possible consecutive sites to which GalNAc is bound could be found in the deduced amino acid sequence.[39)] These glycoproteins and Tn antigens all appear to play significant biological roles.

(iv) From SW1116 cells. Similar experiments involving SW 1116 cells established 14 monoclonal antibodies, designated as MSW 111–MSW 124.[40] They were classified into three types, Types A–C. Twelve of the 14 antibodies were Type A ones that reacted with G-50I and glycolipids, but did not react with their asialo forms. All the A type antibodies were found to recognize the same epitope since on the binding of these antibodies to G-50I they inhibited each other completely. The type B antibody, MSW 111, reacted with G-50I, asialo G-50I, G-50II and glycolipids. The type C antibody, MSW 124, did not react with GP-I, but did with GP-II and glycolipids. The antibodies belonging to Types B and C were not investigated further since they reacted with normal tissues strongly.

(v) MSW 113 antibody. MSW 113, one of the A Type antibodies was chosen for further studies since the growth rate and antibody productivity of the hybridoma producing this antibody were highest among the hybridoma clones producing other A Type antibodies.

Since MSW 113 reacted with both mucin-type glycoproteins and glycolipids, the epitope should be the sugar moiety of these glycoconjugates. Candidate oligosaccharides with known structures were examined as to their reactivity with MSW 113, which suggested that sialyl-Le^a type sugar chains comprise one group of epitopes.

Human colostrum is known to contain a number of oligosaccharides and some of them carry determinants for blood group antigens including Le^a. This background information prompted us to look for epitopic oligosaccharide(s) in human colostrum. From an immunoaffinity column containing MSW 113, immunoreactive oligosaccharides were obtained in the eluate.[41] By fractionating oligosaccharides in the eluate on a column of TSK-GEL NH$_2$, 14 oligosaccharides could be isolated.[42]

Determination of the structures of these oligosaccharides was performed by ^{1}H-NMR and FAB-MS. Most of the oligosaccharides recognized by MLS 113 had the sialyl-Le^a structure, nine of them having novel structures.[43]–[46] Some representative oligosaccharides are shown in Fig. 4.

In addition, four oligosaccharides of known structures, A–D, were identified.[42] A was sialyl-lacto-N-tetraose (CA 50), which is known as antigen recognized by the C-50 antibody,[42] and is a useful tumor marker for pancreatic cancer. B was an antigen for the FH 9 antibody and is frequently detected in liver and pancreatic cancer.[47] C was an antigen for the FH 7 antibody.[48] D was disialyl-mono-fucosyl lacto-N-hexaose.[49] It should be noted that none of oligosaccharides A–D had the sialyl-Le^a structure, yet they reacted with MSW 113.[42]

(vi) Comparison of MSW 113 with NS 19-9. While many monoclonal antibodies recognizing tumor antigens have been produced, antibodies towards CEA and CA 19-9 are the most popular and are utilized mainly for diagnosis of gastrointestinal cancer. CEA is a glycoprotein with antigenic sites residing in the polypeptide moiety. CA 19-9 recognized by the NS 19-9 antibody is an oligosaccharide, sialyl-Le^a hexasaccharide, which is one of the major antigenic oligosaccharides as to MSW 113. We have compared the levels of antigens in sera of various cancer patients using NS 19-9 and MSW 113. In gastrointestinal cancer, these two antibodies revealed abnormal levels of the antigens of nearly the same extent. However, the patients with liver, gall bladder and pancreatic cancer who appeared normal on NS 19-9 screening showed distinctly abnormal values with MLS 113. Furthermore, immunostaining of stomach and pancreatic cancer tissues gave more intensive images with MSW 113 than with NS 19-9.

Figure 4 shows the reactivities of MSW 113 and NS 19-9 with oligosaccharides having the sialyl-Le^a structure isolated from human colostrum. The reactivities were determined as the inhibition of the binding of these two antibodies to G-50I from SW 1116 cells. While the reactivities of all the oligosaccharides were nearly the same for MSW 113, the values were divergent for NS 19-9. Even with sialyl-Le^a hexasaccharide, the reactivity of NS 19-9 was only about one-sixth of that of MSW 113. NS 19-9 seems to react poorly with branched oligosaccharides such as Fractions 2–4, and with oligosaccharides lacking the reducing terminal lactose structure such as Fractions 5 and 6. The reactivities of oligosaccharides A–D derived from colostrum with NS 19-9 were very poor, being nearly unreactive, and a concentration of more than 3 mM being required to detect the reactivity. These results clearly indicate that antibody MSW 113 reacts with not only sialyl-Le^a oligosaccharides but also non-Le^a ones, while NS 19-9 is reactive only with sialyl-Le^a oligosaccharides. These differences between the two antibodies show that MSW 113 should be more useful than NS 19-9 as an antibody recognizing tumor markers.

To summarize the binding experiments, we present an illustration of the epitope recognition by MSW 113 and NS 19-9, in Fig. 5.

Fraction	50% Inhibition(μM)	
	MSW 113	NS 19-9

1 Neu5Acα2 → 3Galβ1 → 3GlcNAcβ1 → 3Galβ1 → 4Glc 1.86 11.1
                   4
                   ↑
                Fucα1

2 Galβ1 → 4GlcNAcβ1 ↘
                     6 Galβ1 → 4Glc 1.25 610
  Neu5Acα2 → 3Galβ1 → 3GlcNAcβ1 ↗ 3
                  4
                 ↑
              Fucα1

3 Fucα1
          ↓
         3
  Galβ1 → 4GlcNAcβ1 ↘
                 6 Galβ1 → 4Glc 1.25 610
  Neu5Acα2 → 3Galβ1 → 3GlcNAcβ1 ↗ 3
               4
             ↑
         Fucα1

4 Neu5Acα2 → 6Galβ1 → 4GlcNAcβ1 ↘
                 6 Galβ1 → 4Glc 1.25 610
  Neu5Acα2 → 3Galβ1 → 3GlcNAcβ1 ↗ 3
               4
            ↑
         Fucα1

5 Neu5Acα2 → 3Galβ1 → 3GlcNAc 1.88 1310
                4
              ↑
          Fucα1

6 Neu5Acα2 → 3Galβ1 → 3GlcNAcβ1 → 3Gal 1.88 42.5
                   4
                 ↑
             Fucα1

Fig. 4. Reactivities with MSW 113 and NS 19-9 of the oligosaccharides with the sialyl-Le[a] structure in human milk. The reactivities were determined as the inhibition of the binding of NSW 113 and NS 19-9 to G-50I derived from SW 1116 cells. Figures express the amounts (μM) of inhibitors to give 50% inhibition.
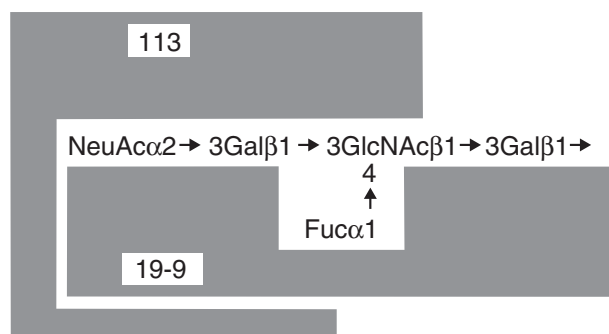

Fig. 5. Illustration of the epitope recognition of MSW 113 and NS 19-9.

(vii) Characterization of mucin-type glycoproteins and oligosaccharides produced by cancer cells recognized by MSW 113. Western blot analyses of the antigens recognized by MSW 113 that appeared in culture medium of SW 1116 cells, and sera from patients with pancreatic or stomach cancer showed that the antigens are very large molecules with molecular sizes in the range of 100–200 KDa.

Oligosaccharides were released by alkaline borohydride treatment from the cell lysates and culture media of LS 180 and SW 1116 cells that had been metabolically labeled with [³H] glucosamine. The oligosaccharides were fractionated by affinity chro-

matography on an immobilized MSW 113 column. The amounts of the immunoreactive oligosaccharides in the eluate from the immunoaffinity column, expressed on the basis of radioactivity, were 20%, 10%, 7% and 4%, respectively, of the totals for SW 1116 cells, LS 180 cells, SW 1116 culture medium and LS 180 culture medium, respectively. The immuno-reactive oligosaccharides were submitted to gel filtration on Sephadex G-50. Surprisingly, the radio-activity of all samples was distributed in an apparent molecular weight range of 2,000 to 10,000.[50] By treating the oligosaccharides with endo-β-galacto-sidase of *Escherichia freundii*,[51] about 30% of the radioactivity was released as oligosaccharides with molecular weights of 1,000–1,500. These results indicate that the oligo- or polysaccharides recognized by MSW 113 from human colorectal cancer cells are complex in structure, and that some of them contain the poly-N-acetyllactosamine structure. There has been a report in which the authors claimed that many of the sialyl-Le^a oligosaccharides on the glycoproteins from SW 1116 culture medium are extremely large and structurally complex N-linked poly-N-acetyllactosamines.[52] In our study, however, the oligosaccharides recognized by MSW 113 were found not to contain mannose, indicating that all the oligosaccharides are O-linked sugar chains, as they are in human seminal plasma and amniotic fluid.[53]

Summary

In this review, I have summarized the works which I and my colleagues accomplished for a period of about 50 years since 1953. Starting from the establishment of the basic structures of glyco-proteins, we have investigated the structural and immunological properties of the sugar moieties of cell membrane glycoproteins. Using a new procedure for selecting hybridomas, monoclonal antibodies recog-nizing the sugar moieties of cell membrane glyco-proteins could readily be established. Some of these antibodies seem to be eligible for the use in cancer diagnosis.

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Profile

Ikuo Yamashina was born in 1926 in Tokyo and graduated from the Faculty of Science, The University of Tokyo in 1948. He studied biochemistry, especially biochemistry of sulfated polysaccharides under the direction of Prof. T. Soda. In 1953, he went to Sweden to study at Karolinska Institute under the direction of Prof. E. Jorpes who is an expert in studies on heparin. However, his first work was the purification of enterokinase. The finding that the purified enterokinase was a glycoprotein with a high carbohydrate content prompted him to study glycoproteins. He was appointed as an associate Professor at the Faculty of Science of Kanazawa University in 1957. then moved to the Faculty of Pharmaceutical Sciences of Kyoto University in 1963. He continued his studies on glycoproteins, but the studies were focused on glycoproteins on cell membranes. Characterizations of membrane glycoproteins then led to the establishment of sugar specific monoclonal antibodies. Several novel antibodies were established and some of them seemed to be eligible for the use in cancer diagnosis. He retired from Kyoto University, and between 1989 and 1997 he was Dean of Faculty of Engineering of Kyoto Sangyo University. He is an honorary member of the Japanese Biochemical Society. He was a member of the Science Council of Japan in 1989–1998.