The complexity of membrane-bound glycans in health and disease and the beneficial properties of glyconutrients

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Abstract

The surface of all free living cells and all multicellular cell types are covered with a dense and complex array of sugars mostly attached to proteins and lipids. These specific sugars are referred to as glycans and the biological role of these sugars includes cell-cell, cell-matrix, cell-molecule interactions, and interactions between other organisms. The chemistry of carbohydrates has been studied well since the first part of the 20th century without understanding the complexity of the glycans. The development of new technologies was the beginning for exploring a new field of molecular biology. Glycobiology is one of the more rapidly growing fields in natural science with a broad relevance to biotechnology, biomedicine and the nutritional supplement industry. Improved methods for analysis of these sugar structures are revealing remarkable complexity and diversity. While glycobiology contributes to the understanding of human health and disease, recent scientific research claims that certain plant extracts referred to as glyconutrients, can exert a wide range of effects on human health. Some plant-derived polysaccharides are among the best known and most potent immunomodulatory substances, impacting both humoral and cellular immune responses. Glyconutrient researchers imply a link between the research of prominent glycobiologists and the health benefits of glyconutrients. These actual or implied therapeutic claims have raised questions, some of which have been brought to the attention of glycobiologists. Glycobiologists claims that these plant polysaccharides are not digestible to monosaccharides by humans. Anaerobic bacteria in the colon can convert them to metabolic waste products such as butyric acid or other short-chain fatty acids, but not to monosaccharides available to the host. The question that arises is whether branched plant polysaccharides are effectively digested to provide biologically concentrations of individual monosaccharides that reach human tissue. On the other hand humans biosynthesize the different monosaccharides the body needs raising the question of whether dietary glycans are required. A wealth of findings suggests that there is a link between glycobiology and glyconutrients, and many publications support the conclusion that dietary glycans are key components in supporting optimal human health.

Glycan structure and synthesis

Monosaccharides are carbohydrates in the form of ketoses or aldoses and cannot be hydrolyzed into a simpler form. The empirical formula is $(CH_2O)_n$ where n is three or more, and they can be either aldehydes or ketones with two or more hydroxyl groups. The backbone is unbranched carbon chains in which all the carbons are linked by single bonds. The hydroxyl groups are attached to carbon atoms which may be a chiral center and that gives rise to many stereoisomers found in nature. Monosaccharides are colorless, crystalline solids, freely soluble in water and insoluble in nonpolar solvents. In aqueous solution all monosaccharides with 5 or more carbon atoms occur as cyclic structures. The carbonyl group has formed a covalent bond with the oxygen of a hydroxyl group along the chain. This reaction between alcohol and aldehydes or ketones results in the formation of hemiacetals and hemiketals. For molecules with chiral centers the configurations are carried out by using x-ray crystallography. Fischer projection formulas and Haworth perspective formulas describes their three dimensional structures. If the non-H group is on the right in the Fisher projection, the overall configuration is D. If the non-H group is on the left side, the overall configuration is L. Monosaccharides have greater combinations than nucleotides and amino acids. If we compare the disaccharide glucose to a dipeptide we can illustrate the complexity. The former can produce 11 different disaccharides, but the latter can only produce a single dipeptide. 4 different hexoses can produce 35560 unique tetrasaccharides, but 4 different amino acids may produce 24 different tetrapeptides. The structures and functions of carbohydrates are well defined in carbohydrate chemistry. The glycans which define the biological functions of sugars attached to proteins and lipids are far more complex.

Glycans

Glycobiology is the study of the structure, biosynthesis, biology, and evolution of saccharides that are widely distributed in nature, and the proteins that recognize them. All cells and numerous macromolecules in nature carry an array of covalently attached sugars or sugar chains, which are generically referred to as glycans. Sometimes, these glycans can also be freestanding entities. Because many glycans are on the outer surface of cellular and secreted macromolecules, they are in a position to modulate or mediate a wide variety of events in cell–cell, cell–matrix, and cell–molecule interactions critical to the development and function of a complex multicellular organism. They can also act as mediators in the interactions

between different organisms (e.g., between host and a parasite or a symbiont). In addition, simple, rapidly turning over, protein-bound glycans are abundant within the nucleus and cytoplasm, where they can serve as regulatory switches. The biological roles of glycans can be divided into two broad categories: (1) the structural and modulatory properties of glycans and (2) the specific recognition of glycans by other molecules—most commonly, glycan-binding proteins (GBPs).

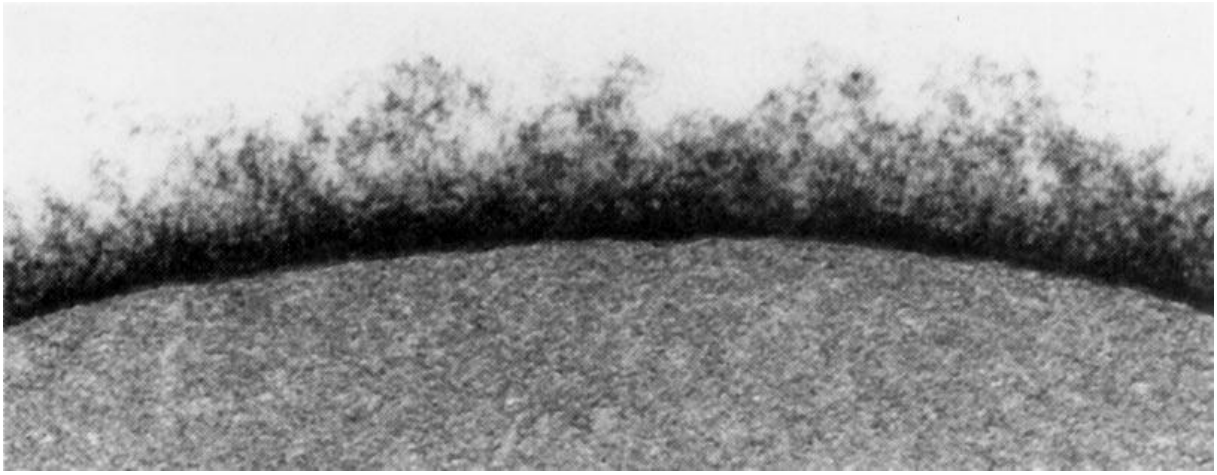

Fig.1 The erythrocyte glycocalyx. All cells in the human body are covered with glycans.

Glycans are generally complex heteropolymers rather than repeating homopolymers such as glycogen and amylose. They can be built from the same building blocks that serve as energy stores, such as glucose, but they also include other monosaccharide units. Over the years, many theories have been advanced regarding the roles of glycans. Although there is evidence to support all of these theories, exceptions to each can also be found. In general the biological roles of glycans can be divided into five broad intrinsic and extrinsic functions:

INTRINSIC FUNCTIONS OF GLYCANS
PROVIDING STRUCTURAL COMPONENTS • Cell walls • Extracellular matrix
MODIFYING PROTEIN PROPERTIES • Solubility • Stability

EXTRINSIC FUNCTIONS OF GLYCANS
DIRECTING TRAFFICKING OF GLYCOCONJUGATES • Intracellular • Extracellular
MEDIATING AND MODULATING CELL ADHESION • Cell-cell interactions • Cell-matrix interactions
MEDIATING AND MODULATING SIGNALLING • Intracellular • Extracellular

Table.1 General functions of glycans

Several hundred monosaccharides are found in nature but only nine of these are common in animal glycans:

- ***Pentoses***- Five-carbon neutral sugars (e.g. D-xylose [xyl]).
- ***Hexoses***- six-carbon neutral sugars (e.g. D-glucose [Glc], D-galactose [Gal] and D-mannose [Man]).
- ***Hexosamines***- Hexoses with an amino group at the 2-position, which can be either free or, more commonly, N-acylated (e.g. N-acetyl-D-glucosamine [GlcNac] and N-acetyl-galactosamine [GalNac]).
- ***Deoxyhexoses***- Six-carbon neutral sugars without the hydroxyl group at the 6-position (e.g. L-fucose [Fuc]).
- ***Uronic acids***- Hexoses with a negatively charged carboxylate at the 6-position(e.g. D-glucoronic acid [GlcA]).
- ***Sialic acids***- Family of nine-carbon acidic sugars (generic abbreviation is Sia) of which the most common is N-acetylneuraminic acid [Neu5Ac].

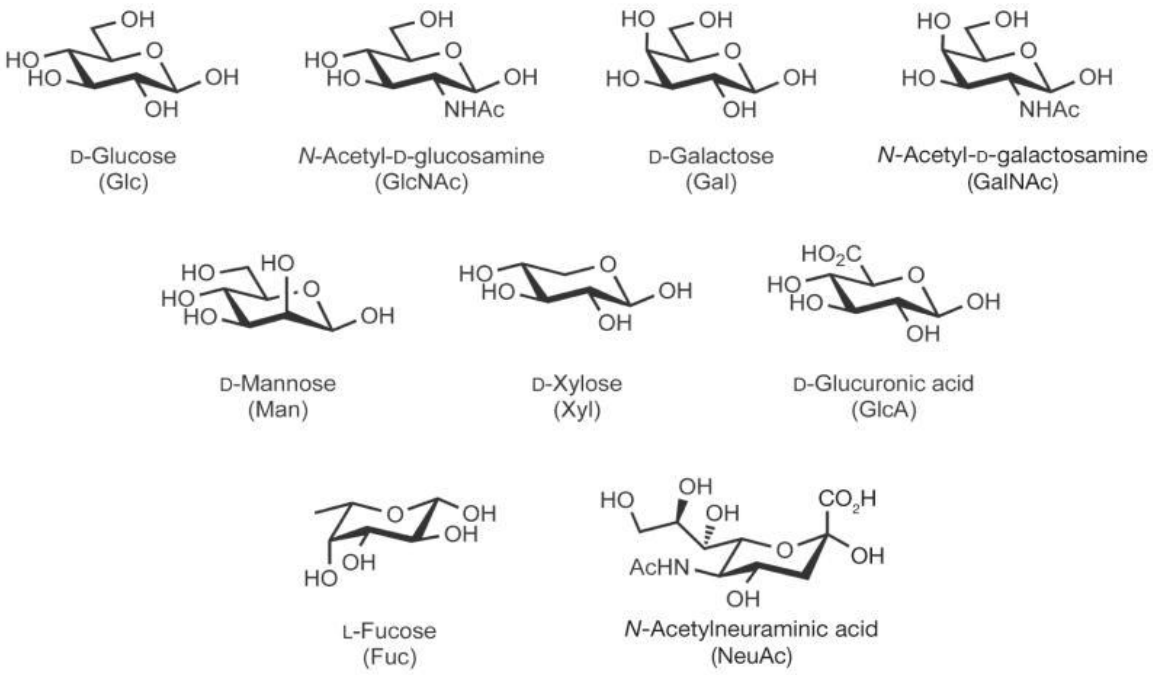

Fig.2 The 9 common monosaccharides found in animal glycans.

Formation and breaking of glycosidic linkages

The condensation reaction between two monosaccharides results in the formation of a disaccharide. The reducing end of a monosaccharide reacts with a hydroxyl group of a second monosaccharide and are joined together by a glycosidic linkage that can be found in two stereoisometric forms, alpha and beta. Glycosidic linkages are the fundamental linkage in all oligosaccharides. They can be formed with any hydroxylated compound and they can be formed between sugars and amino acids to form glycoproteins. No oxidation or reduction can take place at the anomeric centre that is involved in a glycosidic bond but like acetals they can be hydrolyzed in dilute acid.

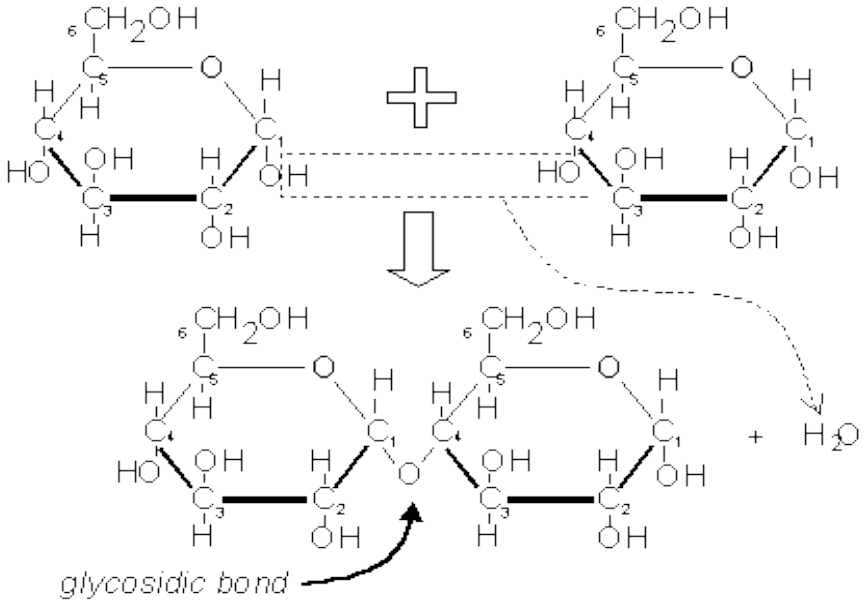

Fig.3 Monosaccharides are joined together by a glycosidic bond to build oligosaccharides and this linkage is found in all monosaccharides joined together. The glycosidic bond is the most flexible part of the structure. Oligosaccharides have a polarity that is defined by their reducing and non-reducing ends. In a reducing disaccharide the reducing end bears a free anomeric center which is not engaged in a glycosidic bond and retains the chemical activity.

The formation of a disaccharide is an energetically unfavorable process. A nucleotide sugar can serve as a sugar donor where the energy needed is a hydrolysis of two phosphate anhydride bonds. Glycosyltransferases catalyses the transfer of the sugar from the donor to the acceptor. A glycolsyltransferase has specificity for a nucleotide sugar donor and an

acceptor. The full name (e.g. β1,4-galactosyltransferase) indicates the nature of the donor, acceptor and the bond formed. A glycosyltransferase has a strict donor and acceptor specificity and can only add one type of sugar in a linkage. On the other hand there are often different glycosyltransferases with similar specificities. One example in humans is that there are six sialyltransferases that can each add sialic acid in 2-3 linkages in galactose. Glycans are linked to other biomolecules, such as lipids or amino acids within polypeptides, through glycosidic linkages to form glycoconjugates. Glycans are often referred to as the glycon of a glycoconjugate and the non-carbohydrate component is the aglycone.

Breaking of glycosidic linkages is a hydrolysis reaction and does not require energy. Glycosidases catalyze the reaction and are highly specific. As an example sialidase only catalyze the release of the monosaccharide sialic acid. Glycosidases can also be linkage specific and as an example some sialidases only hydrolyze α 2-3 linkage of NeuAc while others do not discriminate between α 2-3 and α 2-6 linked NeuAc.

Understanding structure-function relationship for glycans can be more difficult than for other biopolymers. Amino acids and nucleotides are linked in only one fashion during the formation of polypeptides and nucleic acids. There is no stereochemical or regiochemical diversity in these polymers. The multiple monosaccharide building blocks can be linked to various stereochemistries and regiochemistries and the resulting oligosaccharides can be assembled on protein or lipid scaffolds. The function of the protein and the glycan portions of many glycoproteins can be independent of each other. In other cases, the independent function of protein core and glycan decoration may be manifest in a different way. When a particular glycan is attached to a protein or lipid it may mediate adhesion or anti –adhesion events independently of the core protein or lipid to which it is attached. Sequences of protein dictate their three dimensional structures and these structures determine their functions. Principles relating structure to function in the case of oligosaccharides have not been easy to establish. Because oligosaccharides can be branched it is often inappropriate to use the term sequence. To avoid confusion, the term conformation is more commonly used to refer to the arrangement of the oligosaccharides. Analysis of the conformations shows difficulties but there is a reasonable understanding of the N-linked glycans. Increasing the database of known conformations is necessary for understanding all biological roles of glycans.

Glycan synthesis

Nine common sugars found in mammalian cells can be combined in a myriad number of ways to form complex carbohydrate structures. The glycan repertoire (glycome) of a given cell or organism is thus many orders of magnitude more complex than the genome or the proteome. Genomic DNA sequences dictate the structure of glycoconjugates. The sugar structures are not encoded directly in the DNA sequences but are determined by transcription and translation of genes to generate glycosyltransferases.

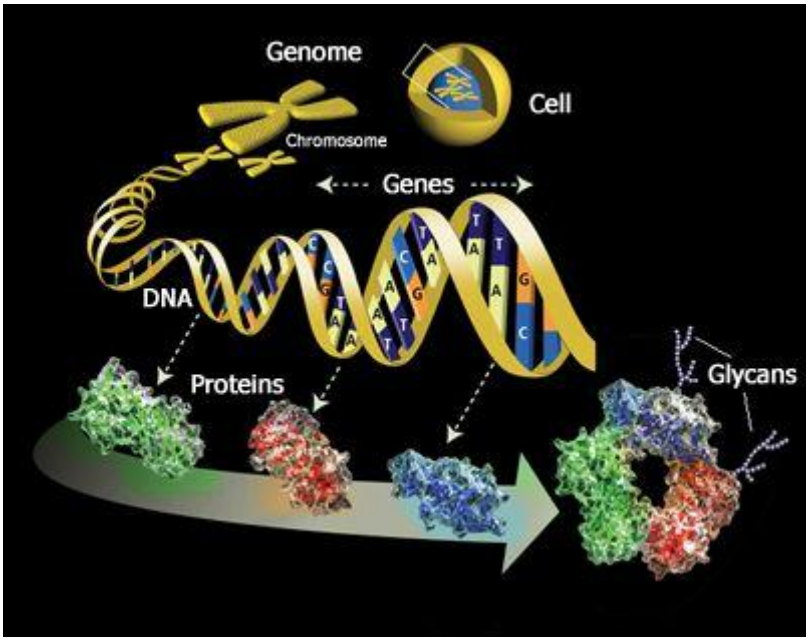

Fig.4 Glycan synthesis

Glycan synthesis requires the monosaccharides to be activated to a high-energy donor form. This process requires nucleoside triphosphates (such as UTP or GTP) and a glycosyl-1-P (monosaccharide with a phosphate at the anomeric carbon). Several variations are used, but regardless of the monosaccharide, all must be either activated by a kinase or generated from a previously synthesized activated nucleotide sugar. The nucleotide sugar–specific transporters carry the activated donors into the Golgi.

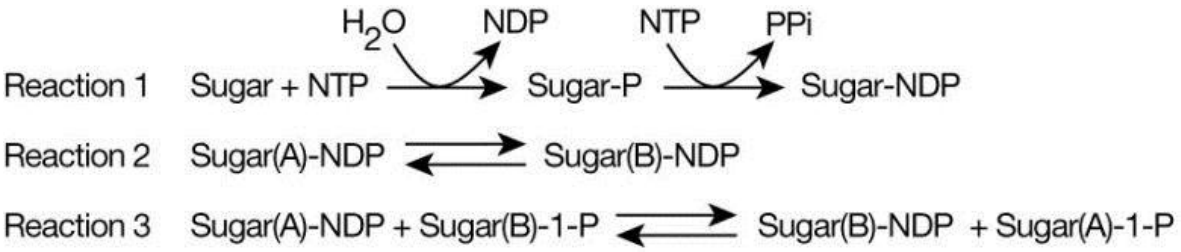

Fig.5 Reaction 1- activated by a kinase, reaction 2 and 3- generated from a previously synthesized activated nucleotide sugar.

Glucose and fructose are the major monosaccharides in which all other monosaccharides needed for biosynthesis can be derived from. Monosaccharides are imported into the cell, salvaged from degraded glycans, or derived from other sugars within the cell. Nucleotide sugar- specific transporters carry the activated donors into the Golgi. Most of the glycan synthesis takes place in the ER. They then make their way via an intermediate compartment through multiple stacks of the Golgi apparatus. From the trans-Golgi network they are distributed to various destinations. Certain types of glycoconjugates are synthesized and reside within the cytoplasm and nucleus. Hyaluron and chitin assemble at the plasma membrane, with direct extrusion into the extracellular matrix. Some glycan chains are made on the cytoplasmic face of intracellular membranes and flipped across to the other side, but most are added to the growing chain on the inside of the ER or the Golgi. It is clear that a variety of interacting and competing factors determine the final outcome of the reactions. The one enzyme- one linkage rule suggests that it will be possible to describe the structure-function of glycans in a particular cell. An important tool in understanding the synthesis of glycoconjugates has been the generation of knockout mice in which glycosyltransferases have been eliminated.

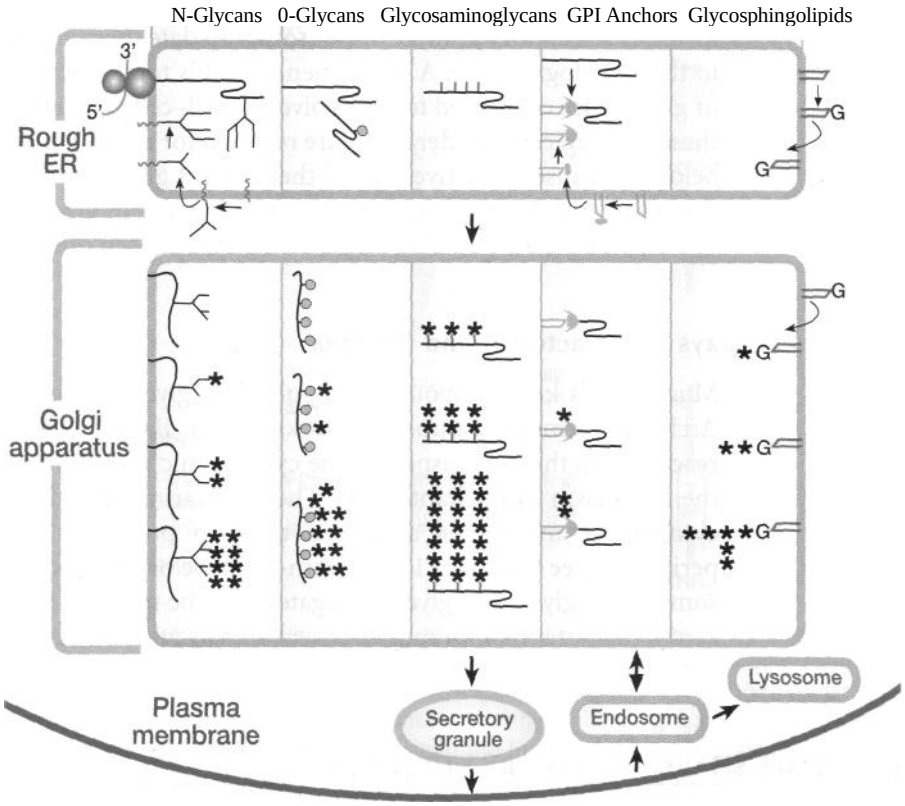

Fig.6 Initiation and maturation of the major types of eukaryotic glycoconjugates in relation to sub-cellular trafficking in the ER-Golgi-plasma membrane pathway. This illustration outlines the different mechanisms and topology for initiation, trimming, and elongation of the major glycan classes in animal cells. *Asterisks* represent the addition of outer sugars to glycans in the Golgi apparatus. N-glycans and gly-cosylphosphatidylinositol (CPI) anchors are initiated by the en-bloc transfer of a large preformed precursor glycan to a newly synthesized glycoprotein. O-glycans and sulfated glycosaminoglycans are initiated by the addition of a single monosaccharide, followed by extension.

Turnover and recycling of glycans

Most glycoconjugate turnover occurs by endocytosis and degradation in the lysosomes. Endoglycosidases can cleave glycans internally, producing substrates for exoglycosidases in the lysosome. Once broken down, the monosaccharides are exported from the lysosome into the cytoplasma to be reused. Glycans derived from the cytoplasma and the nucleus may be more dynamic and rapidly turned over than those derived from the ER-Golgi pathway.

Intracellular sources of monosaccharides

Monosaccharides can be salvaged from lysosomal degraded glycoconjugates. Salvaged neutral hexoses (glucose, mannose, galactose, N-acetylated amino sugars and acidic hexoses) exit the lysosome through neutral carriers to be reused. The neutral carriers also transport fucose and xylose. Uronic acids cannot be reused and are degraded via the pentose phosphate pathway. It has been demonstrated that up to 80% of labeled glycans are reused to new

glycoprotein synthesis. Salvage pathways have received relative little attention; still their contribution to glycosylation may be quite substantial.

Glycosylation

Glycosylation referees to the enzymatic process that attaches glycans to proteins and lipids, and is determined by the expression and relative activities of glycosyltransferases in particular tissues. Non-enzymic attachment of sugars to proteins can also occur, and is referred to as glycation. Most glycosylation reactions occur in the Golgi, at least 1% of the genome is involved, and precursor activation and interconversions occur mostly in the cytoplasm. There are two distinct types of protein glycosylation: N-linked glycosylation, which involves the addition of an oligosaccharide to the amino group (NH_2) of asparagine; and O-linked glycosylation, which involves the addition of an oligosaccharide to the hydroxyl group (OH) of serine and threonine. Changes in glycosylation are common in several diseases such as malignancy, and as almost all surface proteins are glycosylated, this can dramatically affect the behavior of tumor cells.

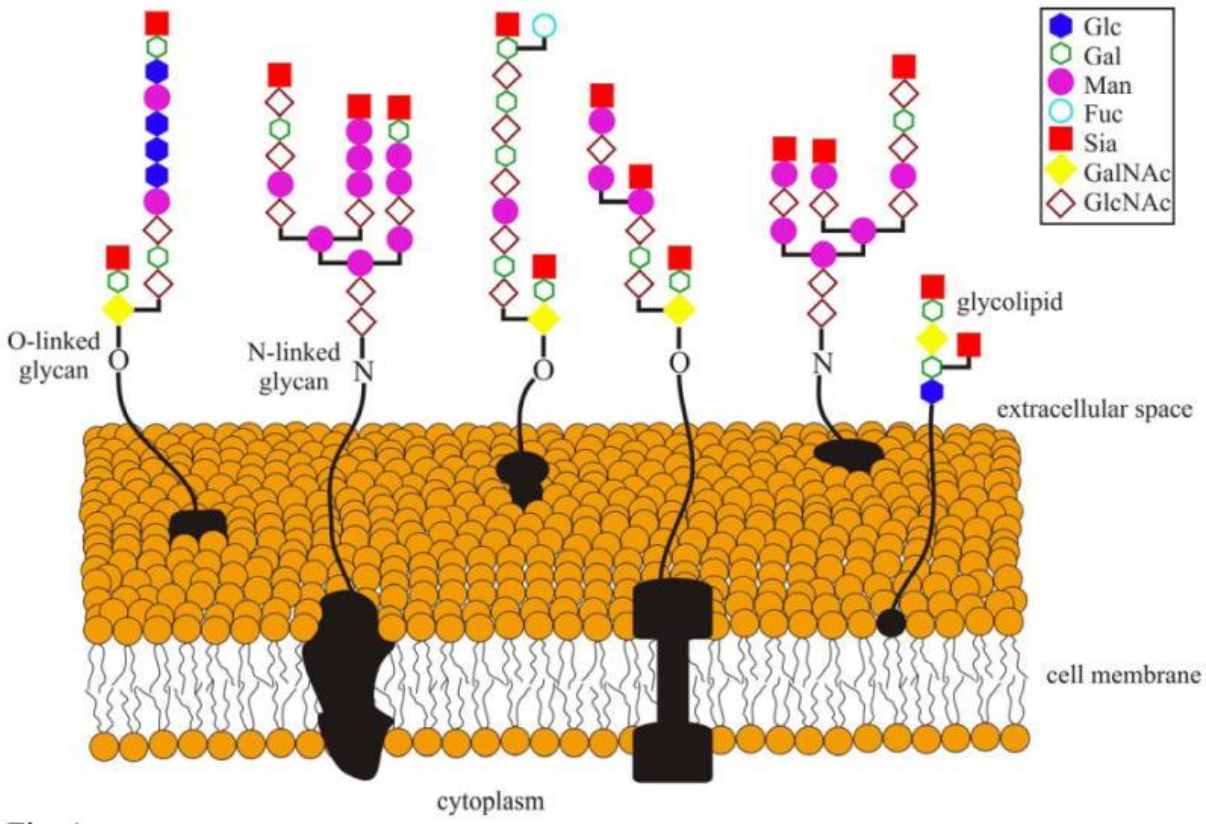

Fig.7 Sialic acids (Sia) are usually found at the terminal residue of O-linked and N-linked glycans of glycoproteins and glycolipids.

N-linked glycosylation

The N-linked glycosylation pathway is the best understood route to protein glycosylation. N-linked glycans are covalently attached to protein at asparagine residues by an N-glycosidic bond.

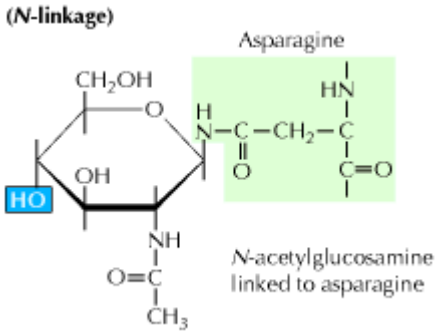

Fig.8 GlcNAcbeta1-Asn is the most common N-linked glycans. Always in beta configuration.

Five N-linked glycans have been reported of which N-acetylglucosamine attached to asparagines is the most common. The arrangement is similar to a glycosidic linkage, except that the anomeric carbon is bonded to the amide nitrogen. N-linked glycosylation occurs simultaneously with translation. A core oligosaccharide is assembled and attached to a lipid carrier on the outer side of the lipid bilayer, which is then translocated across the ER membrane and added to asparagine residues of a nascent polypeptide. After the addition of the core oligosaccharide, sugars are cleaved in the ER, and then, in some instances, further modified in the Golgi complex.

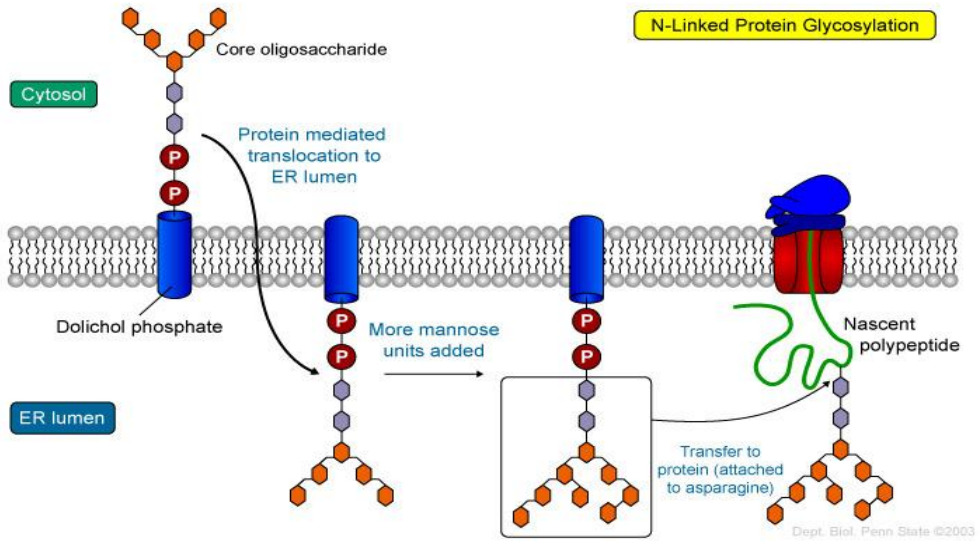

Fig.9 The mechanism of N-linked protein glycosylation. The core oligosaccharide is assembled and attached to dolichol phosphate (a lipid carrier) on the outer ER membrane. This lipid-oligosaccharide complex is flipped across the lipid bilayer by proteins referred to as flippases. Then, the oligosaccharide is elongated by specific enzymes. Finally, it is transferred to the nascent polypeptide while it is being translated.

There are three major classes of N-linked glycans: complex, hybrid, and high mannose. Each type shares a common pentasaccharide, $Man_3GlcNAc_2$, but they differ in their outer branches.

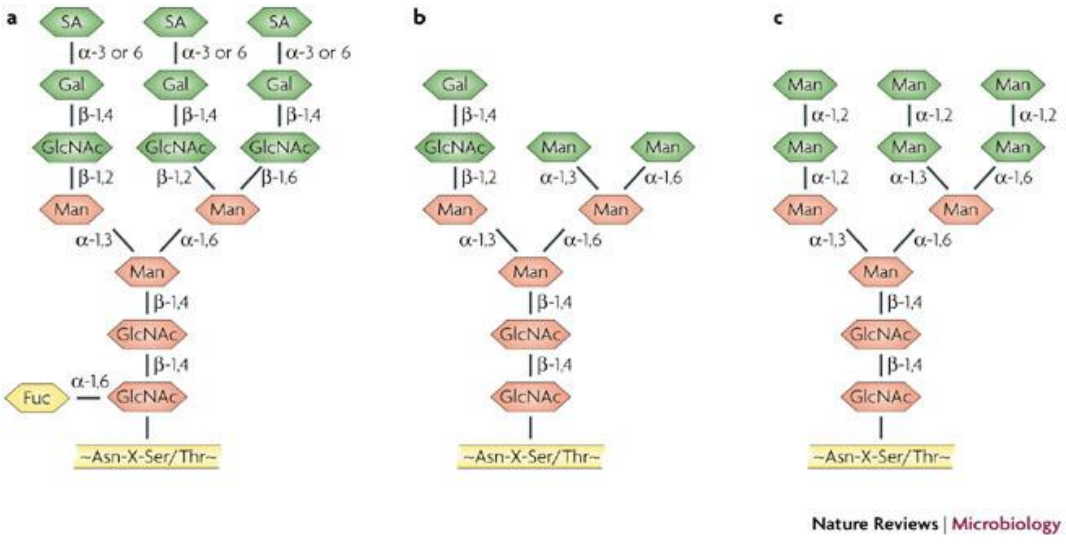

Fig.10 Examples of the structural composition of high-mannose-type N-glycans. A) Tri-antennary complex-type N-glycans. B) Hybrid-type N-glycans. C) High-mannose-type N-glycans.

Why it is important to understand these N-glycan pathways is because they affect many properties of glycoproteins such as their conformation, solubility, antigenicity, and recognition by glycan-binding proteins. N-glycans are used as tags to localize a glycoprotein and to follow its movement. The study of cells and organisms mutated in a specific gene that affects N-glycosylation has been a major source of information regarding the function of N-glycans. Determining the function of N-glycans may be accomplished by the use of inhibitors of N-linked glycosylation such as tunicamycin. There is evidence that complex N-glycans and glycosaminoglycans have critical roles in the development and organization of the nervous system and that mutations in enzymes for synthesis of N-linked glycans cause congenital disorders of glycosylation (CDGs).

O-linked glycosylation

O-linked glycosylation is a modification of glycoproteins that is most likely catalyzed in the Golgi apparatus. O-glycans have a great diversity in both function and structure and the full extent have not been established. The groups of O-linked glycans are built on different protein-glycan linkages, in which GalNAc, fucose, GlcNAc, mannose, xylose, or galactose can be attached to serine, threonine, or hydroxyl-lysine residues. Some of the terminal structures of O-glycans are similar or identical to those of N-glycans and enzymes involved in synthesis of these structures may be shared. Thus, there may be functional overlap between these types of glycosylation. One group of O-glycans, mucins, is glycoproteins that are heavily O-glycosylated. They are covalently α-linked via a GalNac moiety to the –OH of serine or threonine by an O-glycosidic bond, and the structures are named mucin O-glycans or O-GalNAc glycans.

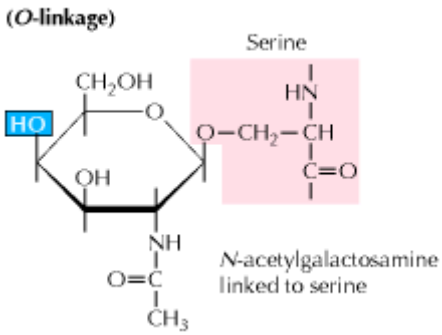

Fig.11 O-linked glycosylation. O-linked glycosylation occurs through the stepwise addition of monosaccharides in either the ER or Golgi complex. Most O-linked oligosaccharides are short, containing only four sugars.

Disorders of glycosylation

Inherited disorders in glycan biosynthesis were identified in the early 1980s based on clinical symptoms and deficiencies in multiple plasma glycoproteins. These human diseases are biochemically and clinically heterogenous and usually affect multiple organ systems. Defects have been found in the activation, presentation, and transport of sugar precursors, in the glycosidases and glycosyltransferases involved in glycan synthesis and processing, and in proteins that control the traffic of components of the glycosylation machinery within the cell.

CDGs are a rapidly growing disease family with about 40 diseases reported and the large majority of these are diseases of protein hypoglycosylation. The congenital disorders of glycosylation (CDG) were originally called carbohydrate-deficient glycoprotein syndromes and are a subset of genetic defects affecting primarily N-glycan assembly. The broad clinical features involve many organ systems but especially the development of certain regions of the brain and functions of the gastrointestinal, hepatic, visual, and immune systems, indicating the importance of normal glycosylation in their functions. The variability of clinical features makes it difficult for physicians to recognize CDG patients. The syndrome is biochemically divided into 4 types, based on results of the transferrin isoelectric focusing test. Transferrin usually has 2 complex-type N-linked oligosaccharide chains, each with 2 sialic acids giving 4 negative charges. A few chains have 3 sialic acids, yielding transferrin molecules with 5 and 6 negative charges. Any genetic or physiologic condition that reduces the number of sugar chains on the proteins or changes the structure of the sugar chains so that they carry fewer sialic acids will change the isoelectric point and the IEF pattern. Most known cases of CDGs result in loss of an entire sugar chain. A few patients make incomplete chains. Both give rise to partially carbohydrate-deficient transferrin with an altered IEF pattern. In some instances, patients have been successfully treated by oral administration of simple sugars. It has been demonstrated that the ingestion of a standardized mixture of plant-derived polysaccharides can result in significant serum protein glycomodifications in normal healthy individuals.

Defects in N-linked glycosylation

One type of CDG results from reduction in the pool of dolichol-linked high-mannose precursors that serve as donors in the initial step of N-glycosylation. The most common defect (CDG-Ia) is in phosphomannomutase, the enzyme that normally catalyses isomerization of mannose 6-phosphate to mannose 1-phosphate. The resulting hypoglycosylation leads to the

range of clinical symptoms, including developmental defects and loss of muscle tone. A bypass route to synthesis of GDP-mannose is available, because mannose can be phosphorylated directly to generate mannose 6-phosphate. High levels of mannose in the diet or as a nutritional supplement are sufficient to restore normal levels of dolichol donor and thus prevent hypoglycosylation of serum glycoproteins.

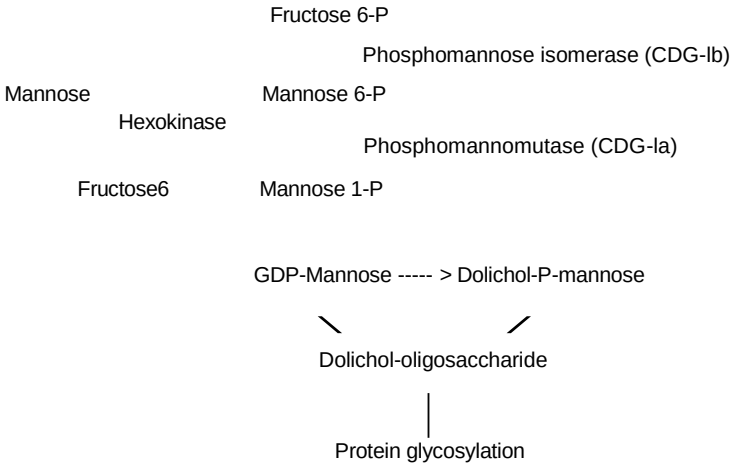

Fig.12 Pathway for mannose incorporation into glycoproteins. Mutations in phosphomannose isomerase or phosphomannomutase lead to CDG types Ib and Ia, respectively. CDG-Ib can be treated with mannose because hexokinase allows the missing enzyme to be bypassed.

Phosphomannose isomerase (PMI) deficiency is the cause of another type of CDG. CDG type Ib is a gastrointestinal disorder characterized by protein-losing enteropathy. PMI is a key enzyme in the metabolism of mannose and it has been demonstrated that oral administration of mannose corrects the hypoglycosylation of serum proteins. Studies have proved that mannose therapy improved the general condition and digestive symptoms in all reported patients but one, and the clinical response to mannose was observed within two weeks. Normal serum mannose level in fasting controls is from 46-65 mM but patients with CDG Ib have less than 10 mM of serum mannose before treatment. The dose of mannose recommended is 0.2 g/kg/4h at the beginning of the treatment. Following this, patients are treated with 4 uptakes of mannose and the doses and frequency depend on the plasma mannose measurements. Unbound mannose can be determined in blood samples collected on EDTA in an assay derived from the method of Etchison and Freeze in specialized laboratories. Oral administration of mannose is well tolerated and no side effects have been reported. Mannose therapy normalized hypoglycemia and vomiting and the general status of

patients dramatically improved. In the long-term treatment, neurological development has been normal and therapy with mannose has transformed lethal CDG Ib into a treatable disease. It should be pointed out that liver disease persists in the most severe patients while their other symptoms disappeared.

Defects in O-linked glycosylation

Changes in glycosylation are observed in nearly all malignancies, including breast cancers. The membrane-bound MUC1 which is expressed by luminal mammary epithelial cells is highly upregulated in breast carcinomas and has often been used as a model to study the changes in O-linked glycosylation that occurs in breast cancer. It has been demonstrated that over-expression of a glycosyltransferase involved in mucin-type O-linked glycosylation can promote tumorigenesis. In breast carcinomas, the O-linked glycans are frequently truncated, often as a result of premature sialylation. The sialyltransferase ST3Gal-1 adds sialic acid to the galactose residue of core 1 O-glycans and this enzyme is overexpressed in breast cancer resulting in the expression of sialylated core 1 glycans.

Detection, purification and structural analysis of glycans

The primary structure of a glycan is defined not only by the nature and order of constituent monosaccharides, but also by the configuration and position of glycosidic linkages and the nature and location of nonglycan substituents. For a typical mammalian glycoprotein, the aim is often to identify the correct structure from a range of known or predictable candidate structures. Choice of methodology is often dictated by the amount and purity of material available and its source, and include direct chemical reactions with constituent monosaccharides, metabolic labeling with either radioactive or chemically reactive monosaccharides, and detection with specific lectins or antibodies. After total hydrolysis of a glycan into its monosaccharide constituents, colorimetric reactions can be used to determine the total amount of hexoses, hexuronic acid, or hexosamine in the sample. These approaches only require common reagents and a spectrophotometer, but determination of total glycan content may not always be accurate because of variations in the sensitivities of different linkages to hydrolysis, variations in the degradation of individual saccharides, or lack of specificity and/or sensitivity in the assay. Quantitative monosaccharide analysis using gas-liquid chromatography involves the following steps: cleavage of all glycosidic linkages (typically by acid hydrolysis), fractionation of the resulting monosaccharides, detection, and quantification.

Method	Use
Periodic acid- Schiff reagent	Detects glycoproteins as pink bands after electrophoretic separation.
Incubation of cultured cells with a radioactive sugar	Leads to detection of glycoproteins as radioactive bands after electrophoretic separation.
Treatment with appropriate endo- or exoglycosidases or phospholipases	Resultant shifts in electrophoretic migration help distinguish among proteins with N-glycan, O-glycan, or GPI linkages and also between high mannose and complex N-glycans.
Sepharose-lectin column chromatography	To purify glycoproteins or glycopeptides that binds the particular lectin used.
Compositional analysis following acid hydrolysis	Identifies sugars that the glycoprotein contains and their stoichiometry.
Mass spectroscopy	Provides information on molecular mass, composition, sequence, and sometimes branching of a glycan chain.
NMR spectroscopy	To identify specific sugars, their sequence, linkages, and the anomeric nature of glycosidic linkages.
Methylation (linkage) analysis	To determine linkages between sugars.
Amino acid or cDNA sequencing	Determination of amino acid sequences.

Table.2 Some important methods used to study glycoproteins.

Glycan-binding proteins

Plant lectins

Lectins were first discovered in plants. In 1888, Stillmark found that extracts of castor bean seeds contained a protein that could agglutinate animal red blood cells. A number of plant seeds were soon after found to contain such agglutinins. The interest in them began to wane until the second world war when blood typing for blood transfusion showed that some lectins where found to be specific for various ABO blood types. These agglutinins where thus renamed “lectins”, a terme derived from the Latin word “legere,” meaning to” select.”

Plant lectins are some of the best-understood examples of carbohydrate-recognition systems and they are powerful tools for detecting glycans. Lectins in seeds and fruits defend plants against predators and pests. Toxic reactions induced by lectins cause gastrointestinal distress, which can condition animals to avoid eating particular plants. Plant lectins have been known and studied for far longer than animal lectins. The first lectin described, concanavalin A, was isolated from jack beans. The ability of plant lectins to stimulate cell-surface receptors also accounts for toxicity associated with eating uncooked beans of many plant species, because the undenatured lectins interact with intestinal epithelial cells. One group of plant lectins consist of toxins, such as ricin from castor beans. The ability of ricin and its homologues to bind sugars provides a mechanism for delivering toxins to the interior of the cells. The active subunits of the toxins are glycohydrolases that remove a critical base from the RNA component of ribosomes. The resulting inhibition of protein synthesis leads to cytotoxicity. For ricin to reach its substrate in the cytosol, it must be endocytosed, transported through the endomembrane system to reach the compartment from which it is translocated into the cytosol, and there avoid degradation making it possible for a few molecules to inactivate a large proportion of the ribosomes and hence kill the cell. Ricin binds to the cell surface through the lectin activity of its sugarbinding B chain. Two inhibitory chemical compounds have been identified, Retro-1 and Retro-2. These molecules do not act on the toxins themselves. Rather, they inhibit retrograde transport between early endosomes and the TGN.

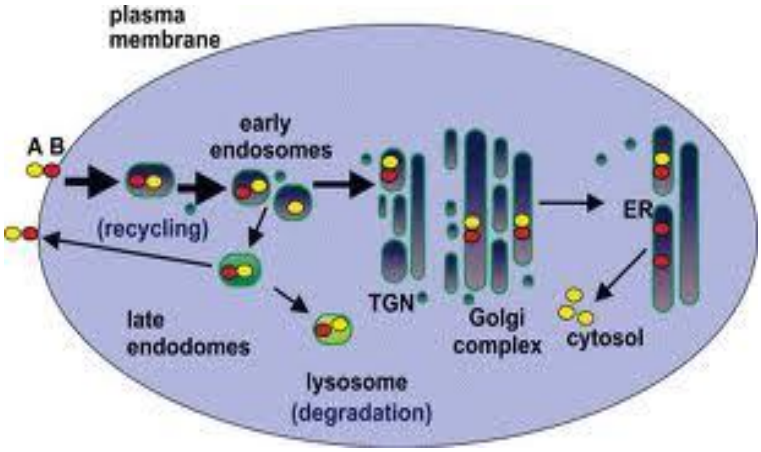

Fig.13 The toxic lectin, ricin, acts on ribosomes to inhibit protein synthesis and are among the most toxic substances known.

Glycan-binding proteins (GBPs)

Binding of glycans to proteins represents the major way in which the information contained in glycans structure is recognized, deciphered, and put into biological action. Glycans interact with many types of proteins by binding to enzymes, antibodies and GBPs. Glycans can mediate a wide variety of biological roles and many of their roles are mediated via recognition by GBPs. Nature appears to have taken full advantage of the vast diversity of glycans expressed in organisms by evolving protein modules to recognize discrete glycans that mediate specific physiological or pathological process. GBPs bind to only a limited number of glycans or even a single glycan among the thousands that are produced by a cell. There are no living organisms in which GBPs have not been found.

There are two major classes of GBPs- Lectins and glycosaminoglycan-binding proteins. There are other animal proteins that recognize glycans in a lectin-like manner and do not appear to fall into these classes. (e.g., various cytokines). Hyaluron (HA) - binding proteins (hyaloadherins) fall in between these two classes. The reason is that some of the hyaloadherins have shared evolutionary origins and recognition involves internal regions and HA, which is a nonsulfated glycosaminoglycan.

	Lectins	**Glycosaminoglycan-binding proteins**
Shared evolutionary origins	Yes (within each group)	No
Shared structural features	Yes (within each group)	No
Defining AA residues involved in binding	Often typical for each group	Patch of basic amino acid residues
Types of glycans recognized	N-glycans, O-glycans, glycosphingolipids (a few also recognize sulfated glucosaminoglycans)	Different types of sulfated glucosaminoglycans
Location of cognate residues within glycans	Typically in sequences at outer ends of glycan chains	Typically in sequences internal to an extended sulfated glucosaminoglycan chain
Specificity for glycans recognized	Stereospecificity high for specific glycan structures	Often recognize a range of related sulfated glucosaminoglycan structures
Single-site binding affinity	Often low; highavidity generated by multivalency	Often moderate to high
Valency of binding sites	Multivalency common (either within native structure or by clustering)	Often monovalent
Subgroups	C-type lectins, galectins, P-type lectins, I-type lectins, L-type lectins, R-type Lectins etc.	Heparan sulfate-binding proteins, chondroitin sulfate-binding proteins,dermatan sulfate-binding proteins
Types of glycans recognized within each group	Can be similar (e.g., galectins) or variable (e.g., C-type Lectins)	Classification itself is based on type of glycosaminoglycan chain recognized

Table.3 Comparison of the two major classes of glycan-binding proteins.

Lectins

Lectins serve as receptors for specific glycans. Some of them recognize foreign cell surfaces and mediate or modulate immune responses to pathogens while others bind to endogenous carbohydrates and mediate adhesion or signaling events at the cell surface. Classification of Lectins is based on the structure of CRDs. Carbohydrate-recognition domains are responsible for the recognition functions of lectins. Different CRD families assume very different protein folds but there are common themes in the way that these diverse proteins bind sugars selectively. Lectins tend to recognize specific terminal aspects of glycans chains by fitting them into shallow, but relatively well-defined, binding pockets.

Mannose-binding C-type lectins

C-type lectins are Ca^{2+}- dependent glycan-binding proteins that share primary and secondary structural homology in their CRDs. The CRD has two highly conserved disulfide bonds and up to four sites for binding Ca^{2+}, with site occupancy depending on the lectin. There are at least 17 groups of proteins with CTLDs, which are distinguished by their domain architecture. Most of these groups have a single CTLD, but the macrophage mannose receptor has eight of these domains, and a cysteine-rich domain. These proteins have a C-type lectin fold which has been found in more than 1000 proteins and it is not restricted to binding sugars. The C-type lectin fold is a rigid scaffold with highly variable protein sequences that are found in all organisms. The large family of C-type lectins includes collectins, selectins, endocytic receptors and proteoglycans. Some of these proteins are secreted and some of them are transmembrane proteins. They often ologomerize into homodimers, homotrimers, and high-ordered oligomers, which increase their avidity for multivalent ligands. C-type lectins differ significantly in the types of glycans they recognize. These proteins function as adhesion and signaling receptors in many immune functions such as inflammation and immunity to tumor and virally infected cells.

Galectins in innate and adaptive immunity

Galectins are a family of glycan-binding proteins that function, either intracellularly or extracellularly, as key biological mediators capable of monitoring changes occurring on the cell surface during fundamental process such as cellular communication, inflammation, development and differentiation. To date, 15 members of the galectin family have been identified in vertebrates. A main feature of any given galectin is represented by its ability to recognize nonreducing terminal or internal galactosyl residues, no divalent cation requirement for binding, a shared primary structure motif, and a unique structural fold. Galectins have a distinct affinity for β-galactosides, and on the basis of their structure they have been classified into three main groups; Proto-type, chimera-type, and tandem-repeat type.

GALECTINS	INNATE IMMUNITY	ADAPTIVE IMMUNITY
Galectin-1 (prototype)	Induces tolerogenic DCs	Induces apoptosis of T cells
	Suppresses acute inflammation	
	Inhibits neutrophil transmigration and promotes phosphatidylserine exposure	Inhibits pro-inflammatory cytokine secretion
	Inhibits mast cell degranulation	
	Promotes DCs migration	Selectively deletes Th 1 and Th 17 cells
	Contributes to alternative activation of macrophages	Favors differentiation toward plasma cells
Galectin-3 (chimera-type)	Promotes acute inflammation	Induces apoptosis of T cells (Extracellular)
	Potentiates eosinophil migration	Protects T cells from apoptosis (Intracellular)
	Promotes neutrophil transmigration and degranulation	Promotes pro-inflammatory cytokine secretion
	Favors mast cell degranulation	Favors Th2 responses (Extracellular)
	Inhibits IL-12 production from DCs	Favors Th1 responses (Intracellular)
	Mediates alternative activation of macrophages	Favors differentiation toward memory B cells
Galectin-9 (tandem-repeat type)	Modulates acute allergic inflammation	Promotes death of fully-activated T cells
	Promotes maduration of DCs and IL-12 secretion	Inhibits development of Th17 cells
	Promotes tissue inflammation through interaction with TIM-3 on macrophages	Induces apoptosis of Th1 cells through interaction with TIM-3

Table.4 Galectin functions. Typical function in innate and adaptive immunity of the most widely studied members of the galectin family.

Glycans and their diverse roles in physiological systems

Reproductive biology

Both man and female reproductive processes are affected by glycans and glycans-binding proteins. Fertilization has been studied extensively in sea urchins, fish, frogs, and mammals. Sea urchin and mouse fertilization are the best-characterized systems. Other roles of glycans include the significance of glycans recognition in sperm interactions with the lining of the fallopian tube and functions of glycans in the process of implantation of the early embryo. Fertilization is a multistep process starting with binding of sperm to the zona pellucida that surrounds the egg. One of the three major glycoproteins of the zona, ZP3, contains the sperm-

binding site. Female mice lacking ZP3 are infertile, but replacement of the mouse protein with human ZP3 restores their fertility. Genetic modifications of glycosylation in mice have also been revealed examples of male infertility caused by glycans structural perturbations.

Embryology and development

Gene- knockout experiments in mice have demonstrated a critical role in embryogenesis for all most all major classes of glycans (except the mucin O-glycan pathway), as well as for certain classes of monosaccharides such as sialic acids. For example, major modifications of glycosaminoglycans cause developmental abnormalities, most likely because of their roles in modulating growth factor function and their proposed roles in setting up morphogen gradients.

Musculoskeletal biology

Glycans appear to have a critical role in the interactions of extracellular matrix molecules like laminin with glycan chains on a α-dystroglycan, which is the key component of muscle. Multiple defects in the pathway for assembly of these O-mannose-linked glycans are known to be associated with muscular dystrophies of various kinds, both in humans and mice. The process of formation and ossification of cartilage into bone intimately involves a variety of glycosaminoglycans, including hyaluronan, heparan and chondroitin sulfate, and keratin sulfate.

Cardiovascular Physiology

Gene knockouts of hyaluronan synthase indicate that hyaluronan has a critical role in the development of the heart. There is considerable evidence that glycosaminoglycans have a role in modulating angiogenesis, partly by virtue of their ability to bind a variety of growth factors. The high density of sialic acids at the luminal surface of endothelial cells and the presence of glycosaminoglycans within the basement membrane are thought to contribute to the structural integrity of the vessel wall. Evidence suggests that sialic acids have unclear roles in modulating calcium fluxes in cardiac muscle cells.

Airway and pulmonary physiology

The lining epithelia of the upper and lower airways are coated with a dense complex layer of glycoproteins, glycolipids and also secreted soluble mucin molecules. Both membrane-bound and soluble glycoconjugates have roles in the effective functioning of the airways, in hydration of the surfaces, and in protection against external agents, both physical and microbial. Embryonic stem cells that lack complex N-glycans do not form part of the

organized layer of bronchial epithelium. Normal N-glycans are important for healthy lung function, and mice lacking the core α- 1,6 fucose of N-glycans develop emphysema-like symptoms due to overexpression of matrix metalloproteinase that degrade in the lung tissue. This is apparently caused by misregulation of the transforming growth factor-β1 signaling pathway, most likely through its misglycosylated receptor.

Endocrinology

There is abundant evidence that O-GlcNAc has a role in modulating the actions of insulin and in explaining some of the effects of hyperglycemia on a variety of systems. Mice deficient in their ability to make triantennery N-glycans develop the characteristics of type 2 diabetes, especially when they are fed a high-fat diet. This appears to result from altered glycosylation of the GLUT2 glucose transporter in the pancreatic islet cells. The improper glycosylation leads to accelerated endocytosis of the transporter, leaving an insufficient amount on the surface to perform its critical role in the ultimate action of insulin.

Gastroenterology

The importance of glycans in physical protection against luminal contents is likely even greater in this instance due to the microbial contents of the gut. The glycosphingoloipids of gastrointestinal epithelial cells are highly concentrated at the outer leaflet of the apical domain, such that they may even outnumber phospholipids as the dominant component of this leaflet. There is also abundant evidence for the involvement of glycans in the interactions of pathogens and symbionts with the gastrointestinal epithelium, ranging from interaction of Helicobacter species with the stomach mucosa to the symbiotic relationships of anaerobic bacteria in the colon, which selectively bind to Gal(alfa 1-4)Gal sequences found in the internal regions of glycosphingolipids. Also of interest is the fact that *Helicobacter Pylori* infection is rarely found in the duodenum where certain unusual alfa1-4GlcNAc-terminated O-linked mucins are expressed. This glycan apparently act as a natural antibiotic against *Helicobacter Pylori* infections by inhibiting the biosynthesis of Glcα-O-cholesterol. There is also evidence for extensive “glycans-foraging” by various organisms in the gastrointestinal tract, as part of their complex relationship with the host. Heparan sulfate in the basement membrane also serves a critical role as a permeability barrier, preventing protein loss into the gut.

Hepatology

The majority of proteins secreted by the liver are heavily glycosylated. Thus hepatocytes have been an excellent system for studying the organization and function of the Golgi apparatus. Various cell types of the liver also express a variety of receptor systems that mediate clearance, based on recognition of specific glycans on circulating molecules. These receptor systems appear to cooperate to remove unwanted molecules from the circulation. There is also emerging evidence for a role of glycosaminoglycans in controlling lipoprotein clearance in the liver via sequestration of lipoprotein in the space of Disse, which is located between the fenestrated endothelium and the hepatocytes, and by affecting endocytosis.

Nephrology

Mucin-like molecules and glycosaminoglycans have an important role in providing a barrier function of the luminal surface of the ureter and bladder. There is extensive evidence that heparan sulfate glycosaminoglycans and sialic acid residues on podocalyxin are involved in assuring the optimal filtering function of the glomerular basement membrane. Reduced branching of complex N-glycans causes kidney pathology that may result from an autoimmune response.

Skin Biology

Glucosylceramide and related glycosphingoloipids and adducts appear to have a critical role in maintaining the barrier function of the skin. A lack of O-fucose glycans on Notch receptors results in skin lesions due to changes in hair cell differentiation.

Oral Biology

Glycosaminoglycans have a critical role in the development, organization, and structure of the gums and teeth. Interaction of oral commensal organisms with the host epithelium can involve recognition of glycans. Mucins produced by the salivary glands may have protective effects in the oral cavity, preventing bacterial biofilm formation on teeth.

Hematology

The trafficking of leukocytes throughout the body is regulated by glycans recognition. Variable glycosylation of red blood cells is responsible for explaining many of the intraspecies blood group differences that affect the practice of blood transfusion. Nearly all blood proteins are N-glycosylated, which is important for maintaining their stability in the circulation. Patients with impaired N-glycosylation often have insufficient levels of coagulation factors such as antithrombin-III and proteins C and S.

Neurobiology

The unusual polysialic acid structure attached to the neural cell-adhesion molecule appears to modulate the plasticity of the nervous system with respect to neural changes during embryogenesis in adult life. Neural cells are highly enriched in sialic-acid-containing glycolipids, and alterations in these glycans affect neurological function. There are two instances where specific glycans appear to inhibit nerve regeneration after injury. First, recognition certain sialyated glycolipids by myelin associated glycoprotein appears to send a negative signal against neuronal sprouting following injury. Similar inhibitory effects appear to be mediated by the glycosaminoglycans chondroitin sulfate. In both instances, targeted degradation of the glycans in vivo can stimulate growth and repair, supporting the hypothesis that these glycans normally act to block regeneration.

Glycans in bacterial, parasitic and viral infections

Bacterial infection

The innate immune system developed early in evolution as the first line of defense of eukaryotes against infection by microorganisms. A key prerequisite of this system is the ability to distinguish self from infectious nonself. In higher eukaryotes, this has been accomplished by the evolution of a range of receptors that recognize conserved molecular patterns on pathogens that are not found in the host. The receptors on the host cells are referred to as pattern-recognition receptors (PRRs). Pathogen-associated molecular patterns (PAMPs) are glycans found on the surface of bacteria that are not produced by the host. PAMPs are recognized by the innate immune system and stimulate inflammatory response to clear bacteria. Examples of PAMPs are the lipopolysaccharides (LPS) of Gram-negative bacteria and the peptidoglycans of Gram-positive bacteria. All of these glycans can potentially interact with host-cell lectins and these interactions can aid in infection and colonization. LPS contains a lipid A moiety, which is embedded in the outer membrane, and two carbohydrate components that extend outward. Soluble LPS released by invading bacteria, and particularly its lipid A content, interacts with the opsonic receptor CD14 and the membrane protein Toll-like receptor 4 (TLR4) to initiate the immune signaling process. In a similar way, the polysaccharide capsule that covers the bacterial surface contains glycans that may be recognized by mammalian cell lectins. Effective killing of bacteria by phagocytes such as

neutrophils or macrophages requires opsonization, a process in which the bacterial surface is tagged with complement proteins or specific antibodies. Phagocytes express receptors for activated compliment or antibody Fc domains, which allow host defense cells to bind, engulf, and kill the bacteria. The ability to generate good antibody response is diminished at extremes of age. Infants and elderly are particularly prone to invasive infection with encapsulated pathogens. Certain bacteria avoid antibody defenses through molecular mimicry of common host glycan structures, masquerading as "self" to avoid immune recognition. An example is the leading pathogen, group A *Streptococcus* (GAS), which express a nonimmunogenic capsule of hyaluronan, identical to the nonsulfated glycosaminoglycans so abundant in host skin and cartilage.

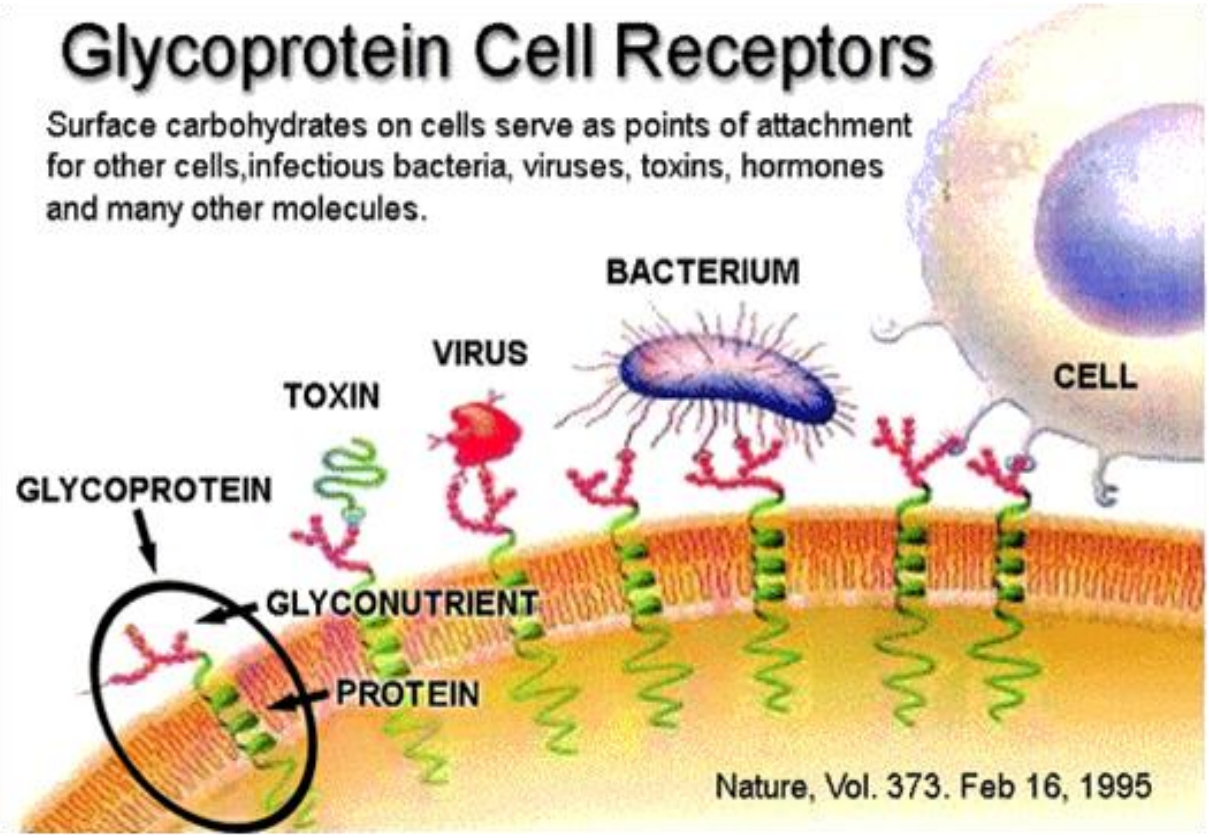

Fig.14 Glycan-receptor interactions play crucial roles in microbial pattern recognition as well as in the regulatory signals that govern the normal activities of immune cells.

Surface capsules

A challenge posed to host immunity by certain pathogens is the great diversity of capsular structures, which is reflected in the different compositions and linkages of repeating sugar units that are produced by different strains of the same bacterial species. Often, these structures are immunologically distinct, allowing classification of different capsule "serotype" strains. For example, there are more than 90 different serotypes of *Streptococcus pneumonia*, which is a leading cause of bacterial pneumonia, sepsis and meningitis. Antibodies generated by the host against the capsule of one serotype strain typically do not provide cross-protective immunity. Genetic exchange of capsule biosynthetic genes among serotypes strains of an individual species can lead to capsule switching in vivo, which provides another means of pathogen escape from protective immunity. Capsule- deficient mutants are rapidly cleared from the bloodstream by opsonophagocytosis and they are unable to establish systemic infections.

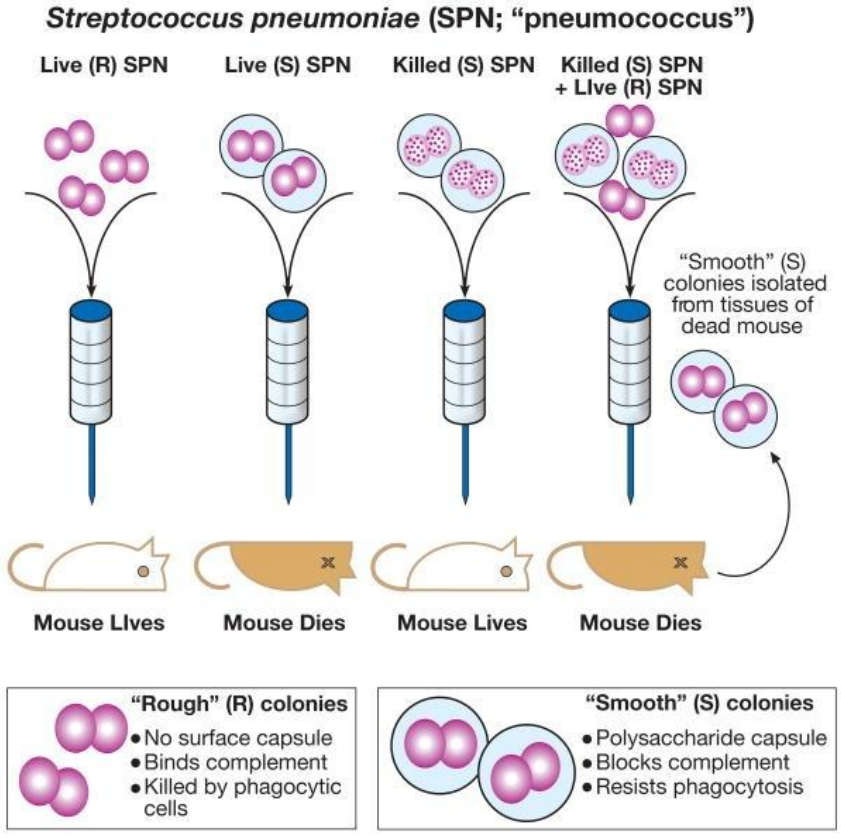

Fig.15 SPN strains can be identified with either a rough or a smooth phenotype, the latter being due to expression of a thick polysaccharide capsule on their surface. In 1928, Frederick Griffith found that (R) SPN strains did not produce disease, but when mixed with live (R) bacteria, the mouse died, and the recovered bacteria expressed the (S) phenotype. Thus, the live (R) strain had been transformed to (S) strains by a factor present in the (S) strain later proved to be DNA.

Mechanisms of adherence

The first step in the pathogenesis of many infectious diseases is the adherence to skin or mucosal surfaces. Most microorganisms express more than one type of adherence factors and most of the microbial adhesins are lectins. These adhesins bind directly to cell surface glycoproteins, glycosphingoloipids, or glycosaminoglycans. Such adhesion can be part of an infection process leading to a disease (pathogenic) or it can be a normal mechanism for coexistence (symbiotic or commensal). In other cases, the bacteria express adhesins that bind matrix glycoproteins or mucin, providing a form of attachment to the mucosal surface. In a number of cases, the key adhesive factor is an assembly of protein subunits that project from the bacterial surface in hair-like threads known as pili or fimbriae.

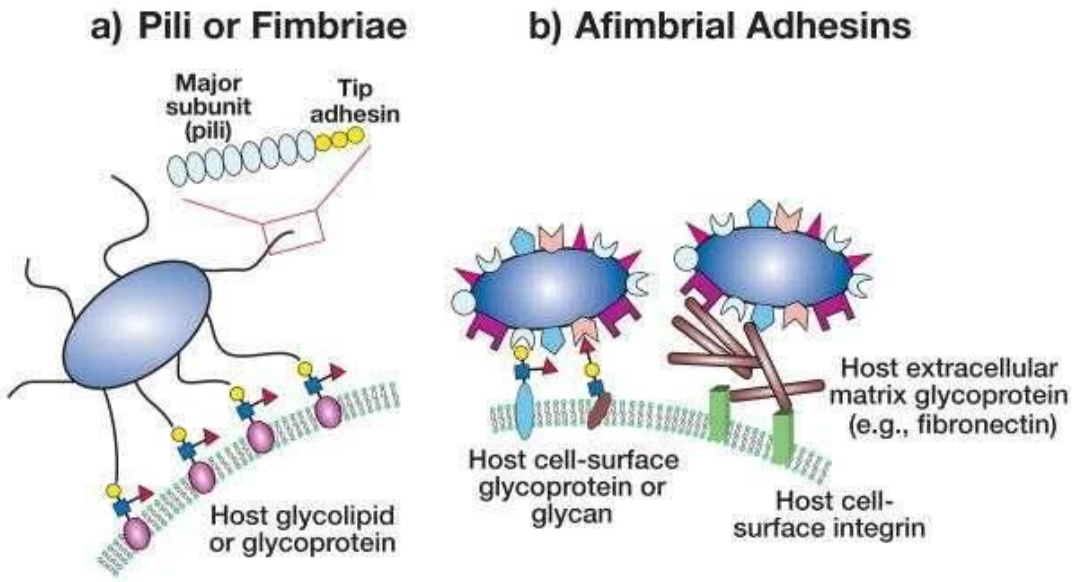

Fig.16 Bacterial lectins occur commonly in the form of elongated, submicroscopic, multisubunit protein appendages, known as fimbriae (hairs) or pili (threads). Pili or fimbriae are made up of a repeating structural subunit and a protein at their tip that mediates recognition of a specific host-cell glycan motif. Afimbrial adhesions are integral bacterial cell wall proteins or glycoproteins that directly engage host-cell receptors to promote colonization.

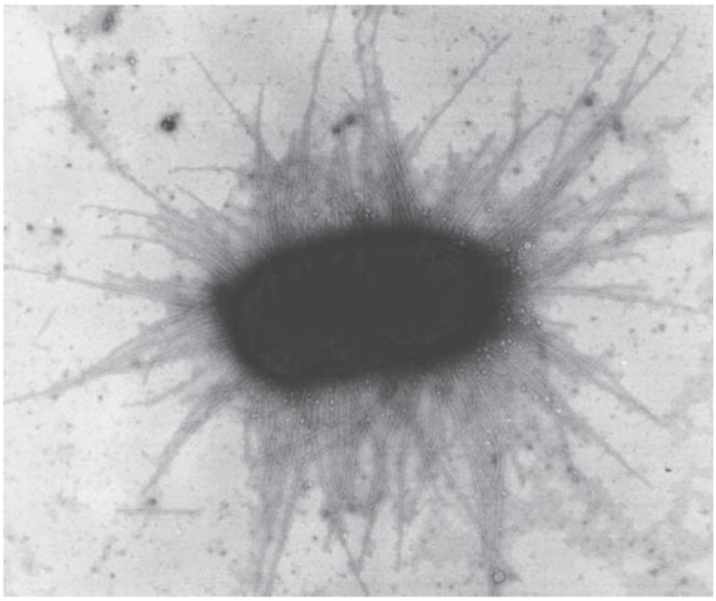

Fig.17 *E.coli* expresses multiple pili as indicated by the fine filaments surrounding the cells. Fimbriated bacteria express 100-400 of these appendages, which typically have a diameter of 5-7 nm and can extend hundreds of nanometers in length.

Biofilm formation

Biofilm formation is another mechanism that promotes bacterial attachment to host surfaces. For example dental plague represents an oral biofilm in which dense, mushroom-like clumps of bacteria pop up from the surface of the tooth enamel, interspersed with bacteria-free channels filled with extracellular polysaccharide (EPS) produced by the bacteria that can serve as diffusion channels. Bacteria within biofilms communicate with one another through soluble signaling molecules in a process known as "quorum sensing" to optimize gene expression for survival. In biofilms, bacteria live under nutrient limitation and in a dormant state in which defense molecules produced by the immune system and pharmacologic antibiotics are less effective. Moreover, the EPS matrix can bind and inactivate these same agents, contributing to the persistence of the biofilm and difficulty in medical treatment of biofilm infections, such as those that arise on catheters and other medical devices. EPS types are usually polyanionic but in rare cases, polycationic, like *Staphylococcus epidermis* strains that produce biofilms on catheters.

A number of secreted bacterial toxins also bind to glycans. The disease symptoms associated with many bacterial infections, such as diarrhea and fluid loss, can largely be attributed to the action of secreted exotoxins that initiate their action by binding to glycosphingolipid receptors

on host cells to alter the function of host cells. The best studied example is the toxin from *Vibrio cholera.*

Macrophage receptors in bacterial recognition

Macrophages are professional phagocytes and the time frame from the phagocytosis of a microbe to the maturation of its phagosome is short. Macrophages have evolved a restricted number of phagocytic receptors, PRRs, which recognize carbohydrates on the surface of microbes. Such receptors include the C-type lectins mannose receptor (MR), and DC-specific intracellular adhesion molecule 3 grabbing nonintergrin (DC-SIGN), among others. There are four known members of the MR family in humans and they are among the few mammalian glycan-binding proteins that have two separate lectin motifs, C -type and R-type in the same molecule. This group is also unusual in that it is the only known lectin group in mammals with more than two C-type lectin domains in the same molecule. Only CTLDs 4 and 5 of the MR have been shown to bind glycans in a Ca^{2+}- dependent manner and to bind mannose, N-acetylglucosamine and fucose. The MR family includes the MR, the phospholipase A2 (PLA2) receptor, DEC-205/MR6-gp200, and Endo 180/urokinase plasminogen activator receptor-associated protein. The MR has important roles in the innate and adaptive immune system. It is expressed at high levels on hepatic endothelial cells, Kupffer cells, immature dendritic cells, other endothelial and epithelial cells, and macrophages. The MR functions in adaptive immunity through its ability to deliver antigens to major histocompatibility (MHC) class II compartments and through its cleavage and release as a soluble protein into blood. The MR is also an important part of the innate immune system were it facilitates the phagocytosis of mannose-rich pathogens. The MR is the only member of the MR family that can function both in clathrin-dependent endocytosis and in the phagocytosis of nonopsinized microbes and large ligands. The MR can bind many different microorganisms such as *Candida Albicans, Pneumocystis carinii, Leishmania donovani, Klebsiella pneumoniae, and Mycobacterium tuberculosis.*

Mycobacterial interaction with innate receptors in macrophages

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), is a major health problem, with 10 million new cases diagnosed each year, causing the death of over 1.5 million victims. MTB was discovered in 1882 by Robert Koch (Nobel Laureate in 1905) and is responsible for more human deaths than any other single pathogen today. The combination with HIV co-

infection, which dramatically compromises host resistance to TB, leads to high disease prevalence in affected endemic populations. From the estimated 2 billion individuals that have been initially infected with *M.tuberculosis*, only 5% to 10% develop symptomatic TB. *Mycobacterium tuberculosis* has co-evolved with humans for centuries. It infects via the airborne route and is a prototypic highly adapted intracellular pathogen of macrophages. The reason why some infected individuals develop active disease while others do not may be due to the role of inborn variability in susceptibility. The first interaction between MTB and the human host takes place in the lung. When MTB bacilli reach the alveolar space, resident alveolar macrophages (AMs) within the surfactant monolayer along with recruited monocytes, neutrophils and lymphocytes represent the array of immune cells that participate in host defense. In a normal healthy individual, AMs represent more than 90% of the cells in the bronchoalveolar lavage fluid. Many studies have demonstrated that resident AMs can phagocytose large numbers of microbes through both opsonic and non-opsonic receptors. The mechanism through which the immune response to MTB is initiated is the recognition of mycobacteria as invading pathogens, followed by activation of innate host defense response, and the subsequent initiation of adaptive immune responses. Initiation of the innate immune response starts with recognition of microbial PAMPs performed by germline encoded receptors expressed mainly on immune cells PRRs. The PAMPs of MTB are sensed by specific PRRs, which in turn trigger production of proinflammatory cytokines and chemokines, phagocytosis and killing of the mycobacteria, and antigen presentation.

MTB is a slow-growing intracellular pathogen that can survive inside the macrophage of the host. MTB is an acid-fast bacterium and the cell wall mainly consists of hydrophobic mycolic acids. Due to this acidic layer, the entry of nutrients is impaired, which causes slow growth of mycobacteria, but it also increases cellular resistance to degradation through lysosomal enzymes.

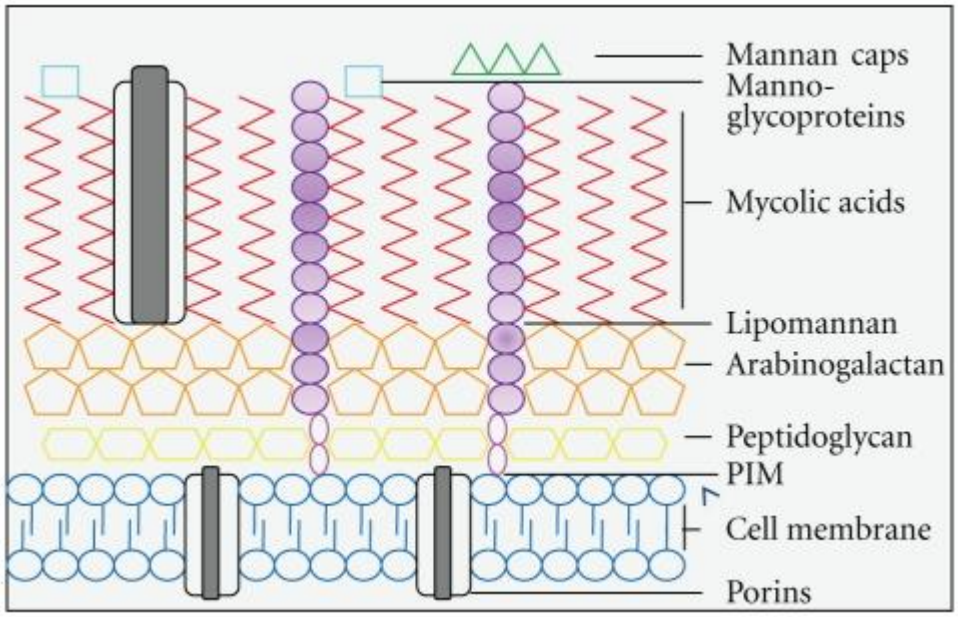

Fig.18 A Schematic representation of the major components of the *Mycobacterium tuberculosis* cell wall. Mycolic acids are distributed as a thick layer mostly at the external portions of the cell wall, while the internal layers of mycobacteria consist mostly of arabinogalactan, phosphalidyl-*myo*-inositol mannosides (PIMs), and peptidoglycans. Next to the mycolic acid layer, other components include mannose-containing biomolecules including mannose-capped lipoarabinomannan (Man-LAM), the related lipomannan (LM), and mannoglycoproteins. Mannan and arabinomannan are present on the surface and form the outer capsule of this bacterium. Man-LAM, LM, and PIMs all share a conserved mannosyl-phosphatidyl-*myo*-inositol (MPI) domain that presumably anchors the structures into the plasma membrane.

Mycobacterium tuberculosis has developed multiple strategies to enhance its entry and intracellular survival by engaging a defined set of phagocytic receptors. MTB survives in the macrophages in part by limiting phagosome-lysosome (P-L) fusion. *Mycobacterium tuberculosis* mannose-capped lipoarabinomannan (ManLAM) blocks phagosome maturation. Studies report that engagement of the MR by ManLAM during the phagocytic process is a key step in limiting P-L fusion. This engagement directs MTB to its initial phagosomal niche, thereby enhancing survival in human macrophages. ManLAM are one of the most abundant mannose-containing macromolecules of the MTB cell envelope. ManLAM is an extremely heterogeneous lipoglycan with a defined tripartite structure: a carbohydrate core (*i.e.* D-mannan and D- arabinan), a mannosyl-phophatidyl-*myo*-inositol (MPI)-anchor and various mannose-capping motifs. Questions regarding the nature of ManLAM within the host and how it directly participates in regulating vesicular fusion remain unanswered. But resent studies indicates that Intracellular processing of ManLAM may be a critical step in directing the outcome of *M. tuberculosis* infection. Apart from ManLAM, the outermost layer of *M.*

tuberculosis also contains other mannose-containing biomolecules. E.g.; LM and PIMs which regulate cytokine, oxidant and T cell responses. *M. tuberculosis* mannosylated biomolecules like ManLAM, LM, and PIMs are key microbial virulence determinants, and great efforts are being made by several laboratories to resolve their biosynthetic pathway.

Viral infections

Most viral receptors attach to cell-surface glycans for entry and the subsequent intracellular replication steps in the viral life cycle. The best understood examples involve binding to terminal sialic acid residues on cell-surface glycoproteins and glycolipids such as the influenza virus hemagglutinin. The affinity of this reaction is relatively low, like that of other glycan-binding proteins with their glycan ligands, but the avidity for cell membranes increase because of oligomerization of the hemagglutinin into trimers and the high density of glycan receptors present on the host cell. Influenza virus has been studied in detail and the subtypes are designated by a nomenclature that is based on their surface glycoproteins- namely, haemagglutinin (H) and neuraminidase (N). The establishment of a productive influenza virus infection is dependent on both of the two glycoproteins. The first influenza viruses to be isolated in the 1930s were designated H1N1 based on serological reactions. Several antigenic shifts have resulted in other strains with the H3N2 as the most prevalent in recent years. Haemagglutinin and other lectins were detected based on their ability to cross-link red blood cells, and haemagglutinin is involved in entering a cell. The haemagglutinin trimer is an elongated molecule with three sialic acid-binding sites at the ends of the molecule that point away from the viral membrane. The binding sites interacts primarily with the terminal sialic acid residues linked to galactose. Key interaction in the binding site include the packing of the N-acetyl side chain against a tryptophan at the bottom of the binding site as well as polar and cooperative hydrogen bonds to the glycerol side chain and carboxyl group. The second molecule on the surface of influenza virus, the tetrameric neuraminidase molecule forms smaller spikes on the surface, and is required for efficient release of newly made virus particles from an infected cell. Its role is to remove sialic acid (the viral receptor) residues from the surface of the producing cell so that the virus is not retained by the hemagglutinin. Without this step, the newly forming virus particles would immediately rebind to their receptor and not be efficiently released into the extracellular space. Thus, the establishment of a productive influenza virus infection is dependent upon both of the glycoproteins, hemagglutinin and neuraminidase The process by which influenza virus enters cells may have further levels of complexity that also depend on protein-glycan interactions. In influenza

infection of macrophages, the viruses undergo additional lectin-like interaction with host mannose receptors after the initial sialic acid binding. In addition, experiments in cell lines deficient in terminal N-linked glycosylation showed deficient cell entry, even though the initial sialic-acid-dependent interactions occurred normally. Neuraminidase inhibitors for the treatment of influenza virus infection appear to provide some relief in the early stages of viral infection. It is difficult to make low-molecular-weight inhibitors of the haemagglutinin-receptor interaction and large ligands are difficult to design as orally delivered therapeutics.

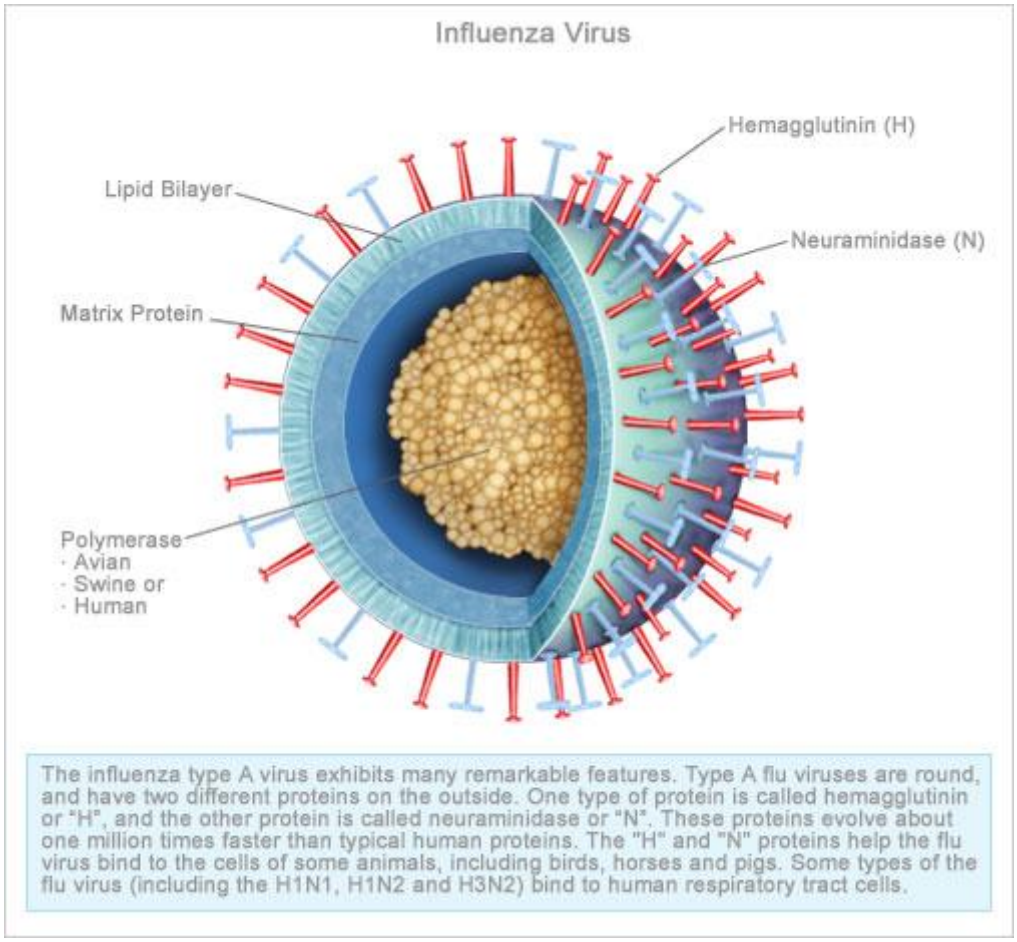

Fig.19 The influenza type A virus

A number of viruses use heparan sulfate proteoglycans as adhesion receptors such as HSV and HIV. Herpes simplex viruses-1 and -2 are human pathogens capable of infection and spread in a number of human cell types. Several lines of evidence identified HS as the critical initial receptor for HSV infection. HSV glycoproteins B (gB) and C (gC) have been showed to be involved in the initial attachment phase through the interaction of positively charged

residues with negatively charged HS of cell-surface proteoglycans. Subsequently, a higher-affinity binding of viral protein gD to a member of the tumor necrosis factor- nerve growth factor (TNF/NGF) receptor family promotes membrane fusion.

Human immunodeficiency virus is a retrovirus and the etiologic agent of the acquired immunodeficiency syndrome (AIDS). HIV surface glycoprotein gp120 binds sequentially to the CD4 receptor on T cells, macrophages, dendritic cells, and microglial cells and then to a coreceptor such as chemokine receptor CCR4 or CXCR4. The latter interaction triggers a conformational change in gp120, which exposes gp41, the HIV factor capable of initiating membrane fusion. The differences in chemokine coreceptors present on cells can also explain how different strains of HIV may infect cells selectively.

Parasitic infection

Parasites affect millions of people worldwide and cause tremendous suffering and death, especially in less-developed countries. Research into parasite glycobiology is important due to the fact that several million people die each year from parasitic diseases such as malaria caused by Plasmodium species. However, parasite glycobiology can be frustrating because of the difficulty in obtaining sufficient amounts of material for study. *In vitro* experimentation is also difficult because most of the parasites require animal hosts for survival and cannot be grown independently. In addition, many parasites have specific primary and intermediate hosts, thus making it difficult to study all stages of the life cycle.

Many parasitic protozoans (single-celled organisms) and helminths (parasitic worms) synthesize unusual glycan structures and GBPs that are often antigenic and involved in host invasion and parasitism. The survival strategies of protozoans involve the participation of glycoconjugates that form a protective barrier against hostile forces.

Malaria in humans is caused by Plasmodium species and several major species infect humans, with *P.falciparum* being the most virulent. These parasites lead a complicated life cycle, alternating between a sexual stage within the female *Anopheles* mosquito vector and an asexual stage within mammalian tissues (hepatocytes and erythrocytes) and the bloodstream. Cell-cell interactions between the parasite and host are critical for the successful completion of each stage.

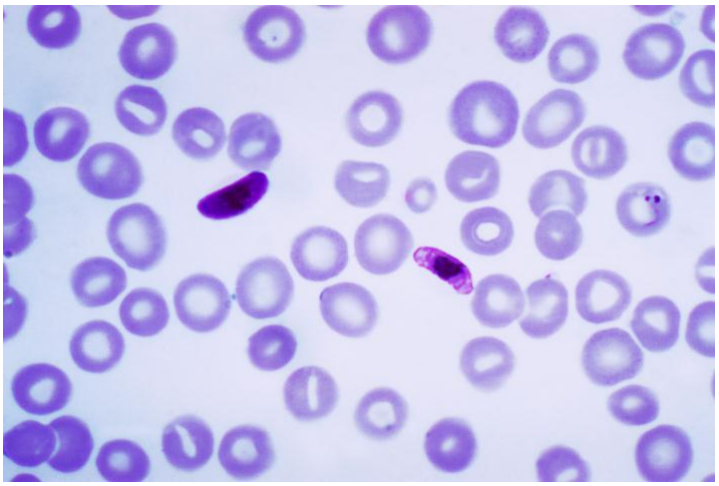

Fig.20 Blood smear with *Plasmodium falciparum.*

After inoculation, the sporozoites`s major circumsporozoite protein interacts with the liver`s heparan sulfate (HS) enabling invasion of hepatocytes. Upon exit from the liver, merozoites can use multiple ligand-receptor interactions to invade host erythrocytes, which vary in their dependency on sialic acid residues on the surface. The proteins on Plasmodium merozoites that mediate invasion fall into two broad superfamilies, the Duffy-binding-like (DBL) and the reticulocyte-binding-like (RBL) proteins. The merozoite protein EBA-175 (erythro-binding antigen-175) plays an important role in invasion. EBA-175 recognizes clusters of sialyated O-glycans attached to erythrocyte glycophorin A, particularly within a 30-amino-acid region that carries 11 O-glycans. The glycophorins are the major sialic-acid-containing glycoproteins on erythrocytes, and individuals lacking glycophorin A or B are refractory to invasion. Some strains of *P.falciparum* can reversibly switch from sialic-acid-dependent to sialic-acid-independent invasion, which depends on parasite ligand use and involves the expression of a *P.falciparum* RBL-like homolog 4 (PfRh4). The ability to switch receptor usage for erythrocyte invasion has important implications for vaccine design against malaria parasites. GIPs released from eruption of merozoites are believed to be prominent virulence factors that contribute to malaria pathogenesis. The presence of N- and O-linked glycans in Plasmodia has been controversial. Several Plasmodium proteins are believed to be nonglycosylated *in vivo,* even though potential N-glycosylation sites are present. Recent studies show that Plasmodium can synthesize dolichol-P-P-GlcNAc2, and that Plasmodium may contain N-glycans, but little is known about O-glycosylation.

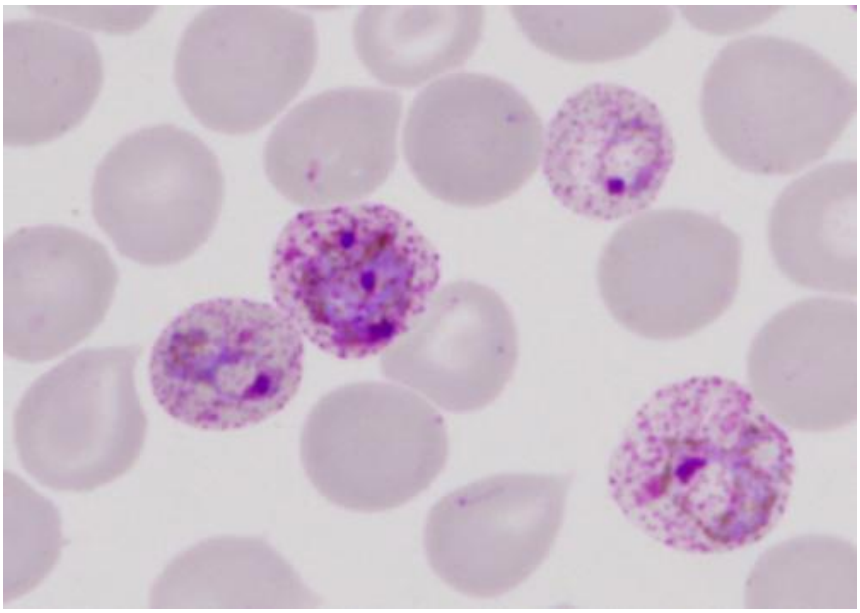

Fig.21 Blood infected with Plasmodium vivax malaria parasites. Four infected red blood cells are shown stained bright purple in contrast to grey uninfected cells.

Most West Africans and many African Americans have complete resistance to *P. vivax* because their RBCs lack the Duffy blood group, which is required for the attachment of *P. vivax* to RBCs. The binding site for *P.vivax* has been mapped to a 35-amino-acid segment of the extracellular region at the amino terminus of the Duffy antigen. The development of Plasmodium in RBCs is retarded in patients with hemoglobin S, hemoglobin C, thalassemia, G6PD deficiency, or elliptocytosis.

Parasite	Stage	Protein	Specificity
Plasmodium falciparum	merozoite	EBA-175	Neu5Acα2-3Gal/glycophorin A
	merozoite	EBA-140	sialic acid/glyco phorin B?
	merozoite	EBA-180	sialic acid
	sporozoite	circumsporozoite protein	heparan sulfate
Trypanosoma cruzi	trypomastigote	*trans*-sialidase	Neu5Acα2-3Gal
	trypomastigote	penetrin	heparan sulfate
Entamoeba histolytica	trophozoite	Gal/GalNAc lectin	Gal/GalNAc
Entamoeba invadens (a reptilian pathogen)	cyst	cyst wall protein (Jacob lectin)	chitin
Giardia lamblia	trophozoite	taglin (α-1 giardin)	Man-6-phosphate heparan sulfate
Cryptosporidium parvum	sporozoite	Gal/GalNAc lectin	Gal/GalNAc
	sporozoite	Cpa135 protein	?
Acanthamoeba keratitis	trophozoite	136-kD mannose-binding protein	mannose
Toxocara canis	larval	TES-32	?
Haemonchus contortus	gut-localized	galectin	β-galactosides

Table.5 Some major parasites and their glycan-recognizing proteins

Biological properties and nutritional values of some natural plant polysaccharides

William Coley`s saccharide vaccine

A surgeon at Memorial Sloan-Kettering Cancer Center made the first scientific association between the immune system and saccharides. In 1890, twenty-eight-year-old Harvard Medical School graduate William Coley, saw that most cancer therapies failed; most of the patients died. But, one patient who was severely afflicted with sarcoma did recuperate. Hospitalized and near death in the fall of 1884, he experienced two outbreaks of a severe skin infection, erysipelas. Caused by a strep bacterium, the infections resulted in high fever and roused his immune system. The tumor began to shrink and the patient recovered completely. Coley began a series of experiments on sarcoma patients with live cultures of the strep bacterium. Coley eventually procured an exceptionally potent strain that produced high fever and skin infection. The patient`s tumor vanished and Coley published his first paper. After culturing the bacteria, Coley killed the colonies or filtered them out, his theory being that the toxins the cultured bacteria had produced were at least partly responsible for the tumor shrinkage. In due course, Coley added the toxins from a second bacterium, which today is called *Serratia marcescens.* That combination proved remarkably success. Coley`s toxins- also known as fever therapy, was never really accepted in scientific circles although it was the only recognized systemic treatment for cancer other than surgery. About 8 years after Coley`s death in 1936, a scientist at the National Cancer Institute (NCI) discovered that a lipopolysaccharide was the biologically active substance in the toxins of *Serratia marcescens*, one of the components of Coley`s vaccine. In this case, the lipopolysaccharides resided in the cell walls of the *Serratia marcescens* bacteria. The fat in the lipopolysaccharide could cause toxicity problems, however; scientists eventually pared the fat from the sugar, leaving a nontoxic compound with salutary immune-system effects. These compounds incite the body to stimulate the immune cells and produce cytokines. Coley`s vaccine yielded impressive results, but in 1965, the American Cancer Society added Coley`s toxins to its list of unproven cancer drugs. However, scientists in several countries continued the research on vaccine therapy. Bacterial vaccines have been most effective for sarcomas, lymphomas, and melanomas.

Several saccharides have been tested on sarcomas. Japanese researchers at Tokyo`s National Cancer Center Hospital demonstrated that extracts of sarcodon and reishi mushrooms are also

effective against sarcomas in animals. Other researchers suggest that polysaccharide extracts of reishi and murill mushrooms as well as Aloe Vera are effective against sarcomas. When scientists tested an aloe polysaccharide on melanoma and sarcoma tumors in mice, they found that it affected cures only in fibrosarcoma tumors. However, the growth rate of other tumors slowed down in the treated mice, unlike the controls.

The importance of nutrition for optimal health

"All that mankind needs for good health and healing is provided in nature … the challenge for science is to find it." Paracelsus, Father of Pharmacology c. 1493-1541.

Chemicals, free radicals, radiation, medication and pathogens can induce changes or destruction of the genetic code, thereby resulting in aberrant cells with abnormal structure and function. The Father of Western Medicine, Hippocrates, said: *"Let your food be your medicine and your medicine be your food."* This statement resonate the importance of proper nutrition for optimal health. Food nourishes and promotes the growth, multiplication, division and renewal of all cells in the human body and nothing is better than getting nutrients from real food. Unfortunately most people do not obtain the proper nutrients from modern diets. Millions of deaths worldwide are attributable to low fruit and vegetable intake, and high levels of processed foods, and by processing foods we deplete nutrients. The cultivation of different strains of crops is based on market demand, which is determined not so much by nutritional value as by taste, shelf life and productivity, and by processing foods. Adequate

intake of fruits, vegetables and unprocessed foods, reduces the risk for a number of diseases. Sometimes, however, if nutrient-rich foods are not available, supplements are necessary to obtain optimal health. One of the most powerful arguments against taking supplements is that many of the formulations on the market have no clinical studies to support their claims and tend to mix compounds into a tablet or powder without testing their efficiency. Only when the nutritional supplement industry use validated assays and solid clinical studies to ensure the content and the efficiency of the product, will consumers experience beneficial health effects.

The current drug treatment is becoming less effective due to drug-resistant infectious agents and many of the current treatments of viruses, bacteria and parasites are innately toxic to humans. This has caused an increasing interest in medical plants with antiviral and antibacterial properties. The saccharide fractions of plants are some of the most important of these research areas. Several plant polysaccharides have been demonstrated to stimulate the immune system and bind to bacteria, viruses and cells to prevent adhesion, penetration, absorption, and infection. Several plants used by various indigenous peoples are the potential source of new remedies.

Glyconutrients - exogenous sources of biological active saccharides

Polysaccharide-rich fungi and plants have been employed for centuries by cultures around the world for their dietary and medicinal benefits. Often thought to merely support normal bowel function and blood glucose and lipid levels, certain polysaccharides have attracted growing scientific interest for their ability to exert marked effects on immune system function, inflammation and cancers. Many of these chemically and structurally diverse polysaccharides have been shown to beneficially affect one or more targeted cellular functions in vitro, but much of the in vivo literature consists of studies in which polysaccharides were injected. Polysaccharides that elicit effects in vitro or by injection may be ineffective or have different effects when taken orally. Previously scientists thought that plant polysaccharides could not be digested and absorbed into the blood. Recent research has proven that a small amount of oligosaccharides and monosaccharides are digested and absorbed in a time release manner with the help of enzymes, probiotics and friendly colonic bacteria. The biological role of glyconutrients is one of the most complex and at times controversial areas in nutritional science.

Food Sources of Monosaccharides		
Monosaccharide sugar	**Disaccharides, oligosaccharides, Polysaccharides containing the monosaccharide sugar**	**Some common food sources of disaccharides, oligosaccharides, and polysaccharides**
Mannose	Plant gums	Some seeds and plant saps, cacti and aloe
	Glucomannans	Most plants
	Galactomannans	Many plants
	Hexosanes	Carrot, beet, cauliflower, broccoli, kale, Lettuce, parsley, rhubarb, Brussels sprouts, red cabbage, asparagus
Fucose	Plant gum	Flaxseed gum, algae
N-acetylglucosamine	Plant gums	Some seeds and plant saps
	Chitosan	Some fungi, tempeh, some algae
	Chitin	Some fungi and yeasts
	Oligosaccharides	Milk
Glucosamine	Chitin	Some, tempeh, some algae
	Citosan	Some fungi and yeasts
N-acetylgalactosamine	Oligosaccharides	Milk
N-acetylneuraminic acid	Oligosaccharides	Milk
Arabinose	Arabinoxylans	Wheat, rye, oat, barley
	Arabinogalactans	Carrots, tomatoes, radishes, pears, corn, wheat, red wine, larch, seed gums, acacia, legumes
	Plant gums	Some seeds and plant saps
Xylose	Arabinoxylans	Husks of many grains, including wheat, rye, oat, barley
	Galactomannans	Many plant cells
	Plant gums	Some seeds and plant saps
	Pentosans	Carrot, beet, cauliflower, broccoli, kale, Lettuce, parsley, rhubarb, Brussels sprouts, red cabbage, asparagus, wheat
	Xylans	Some seeds
	Glucuronoxylans	All land plants
Rhamnose	Plant gums	Some seeds and plant saps

Food sources of monosaccharides		
Monosaccharide sugar	**Disaccharides, oligosaccharides, polysaccharides containing the monosaccharide sugar**	**Some common food sources of disaccharides, oligosaccharides, and polysaccharides**
Galactose	Lactose	Milk
	Pectin	Most plants
	Hexosans	Carrot, beet, cauliflower, broccoli, kale, Lettuce, parsley, rhubarb, Brussels sprouts, red cabbage, asparagus
	Plant gums	Some seeds and plant saps
	Arabinogalactans	Carrots, tomatoes, radishes, pears, corn, wheat, red wine, larch, seed gums, acacia, legumes
	Galactans	Some algae
	Galactomannans	Many plants
Fructose	Sucrose	Most fruits and vegetables, cane and beet juices, honey, sugar, sorghum, pineapple, carrot
	Plant gums	Some seeds and plant saps
	Fructooligosaccharides	Wheat bran, onion, leek, artichoke, asparagus, banana, garlic
	Some hexosanes	Carrot beets, cauliflower, broccoli, kale, lettuce, parsley, rhubarb, Brussels sprouts, red cabbage, asparagus
	Glucofructans	Grasses
Glucose	Sucrose	Most fruits and vegetables, cane and beet sugar, sorghum, dales, raisins, honey, pineapple, honey
	Starch	Corn, rice, wheat, potatoes
	Lactose	Milk
	Maltose	Germinating grains and malt
	Glucose syrup	Processed foods
	Gums	Some seeds and plant saps
	Trehalose	Mushrooms, yeast
	Beta-glucan	Mushrooms, yeast, bran
	Glycogen	Animal liver and muscle, corn, some fungi
	Cellulose	Most plants
	Many hemicelluloses	Most plants

Table.6 Food sources of monosaccharides

Biological activities of plant-polysaccharides

There is now a surge of research by various groups into the biological activities and potential beneficial effects of dietary saccharide biopolymers. These biopolymers represent a broad range of structurally diverse non- or low digestible, dietary soluble fibers that have been derived from different species of flora and have been demonstrated to affect beneficially one or more target cellular or body functions. The physiological effects attributed to these saccharides can be divided into six main categories:

1. Beneficial effects on colonic microflora and gastrointestinal physiology.
2. Immunomodulatory effects.
3. Anti-angiogenetic and antitumor effects.
4. Altered lipid metabolism.
5. Improved bioavailability of essential minerals.
6. Other beneficial health effects such as enhanced production of growth factors involved in re-epithelization and wound healing.

Many of the dietary saccharide polymers studied exhibiting multifunctional physic-chemical and physiological characteristics, are now being redefined as secondary metabolites or biological response modifiers (BRMs). Although many of the physiological traits attributed to dietary polysaccharides may be secondary, evidence from various pharmacodynamic and pharmacokinetic studies also indicate microflora-independent immunomodulatory effects. This immunomodulatory effect is instigated by the binding of certain BRM polysaccharides to specific receptors in immune cells in the gut-associated lymphoid tissues, and results in the intracellular activation of signal transducers and transcription factors that are associated with various effector functions of the immune response. Evidence supports the direct binding of BRM polysaccharides to pattern recognition receptors including toll-like receptors, non-toll pattern recognition receptors, complement receptor type 3 and certain transmembrane lectins. Recognition by these receptors can result in intracellular signaling cascades which can in turn result in subsequent activation or inactivation of a wide spectrum of target genes involved in the regulation of a variety of cellular responses, such as expression of various cell-surface receptors and cytokine production. One of the primary immunomodulatory effects of BRM polysaccharides is to promote or alter various leukocyte activities, in particular those of macrophages and immune-regulatory Gamma delta T cells via changes in cytokine expression. This in turn can impact both the innate and adaptive arms of the immune response and thus result in activation or dampening of these responses. Dietary polysaccharide induced immunomodulatory activities of note include:

- Increased macrophage cytotoxic and phagocytic activities.
- Altered pro- and anti-inflammatory and Th1-Th2 balance.
- Altered expression of certain adhesion molecules.

These activities support the notion that certain dietary plant polysaccharides may have significant immunomodulatory potential.

There is also growing evidence that dietary polysaccharides may have beneficial effects on brain function via the digestive tract due to the activation of parasympathetic nerve fibers, hormonal signaling, or additional brain-gut axis pathways. Six randomized, double-blind, placebo-controlled clinical studies were identified in which consumption of a blend of plant-derived polysaccharides showed positive effects on cognitive function and mood in healthy adults. Numerous animal and *in vitro* studies have demonstrated the ability of individual saccharide compounds to modify behavior, enhance synaptic plasticity, and provide neuroprotective effects. The mechanisms by which exogenous saccharides can influence brain function are not well understood and additional controlled clinical studies are necessary.

62 publications reported statistically significant effects of orally ingested glucans, pectins, heteroglycans, glucomannans, fucoidans, galactomannans, arabinogalactans and mixed polysaccharide products in rodents. 15 controlled human studies reported that oral glucans, arabinogalactans, heteroglycans, and fucoidans exerted significant effects. Although some studies investigated anti-inflammatory effects, most studies investigated the ability of oral polysaccharides to stimulate the immune system. These studies, as well as safety and toxicity studies, suggest that these polysaccharide products appear to be largely well-tolerated. Taken as a whole, the oral polysaccharide literature is highly heterogenous and is not sufficient to support broad product structure/function generalizations. Numerous dietary polysaccharides, particularly glucans, appear to elicit diverse immunomodulatory effects in numerous animal tissues, including the blood, GI tract and spleen. Glucan extracts from the *Trametes versicolor* mushroom improved survival and immune function in human RCTs of cancer patients; glucans, arabinogalactans and fucoidans elicited immunomodulatory effects in controlled studies of healthy adults and patients with canker sores and seasonal allergies.

Some natural plant-polysaccharide products may enhance the performance of the human immune system. However, source and method of preparation can have significant impact on efficiency. Products that are of similar chemical composition may differ significantly in terms of biological activity. Studies investigating the orally ingested commercial glyconutrient products, Advanced Ambrotose and Ambrotose Complex, showed that these standardized mixtures of plant-derived polysaccharide supplements are metabolized by human colonic bacteria. It has also been demonstrated that orally administration of this products can result in significant changes in the N-glycosylation status of serum glycoproteins in normal healthy individuals and support cognitive and gastrointestinal health. At present there is no recommended daily intake for saccharides. Among the several plant saccharides found in nature, except for alpha and beta glucans, the breakdown, absorption and biological role of mannose containing molecules are the best understood followed by fucose containing fucoidans and arabinose and galactose containing arabinogalactans.

Category	Product	Metabol-ized by human gut bacteria?	Study type	Fate (method: tissues detected)
Arabinogalactans	*Larix* spp.	yes	*in vitro*	NA
Fucoidans	*Undaria pinnatifida*	no	*in vitro*	Ab: human plasma
Galactomannans	*Cyamopsis tetragonolobus* (partially hydrolyzed guar gum)	yes	*in vivo*	NA
	Cyamopsis tetragonolobus (guar gum)	yes	*in vitro*	NA
Glucans	*Hordeum vulgare*	NA	*in vivo*	Fluorescein-labeled: mouse Mø in the spleen, bone marrow, lymph nodes
	Laminaria digitata (laminarin)	yes	*in vitro*	NA
	Sclerotium rofsii (scleroglucan) glucan phosphate, *Laminaria* spp. (laminarin)	NA	*in vivo*	Alexa Fluor 488-labeled: mouse intestinal epithelial cells, plasma, GALT
	Saccharomyces cervisiae (particulate)	NA	*in vivo*	Fluorescein-labeled: mouse macrophage in the spleen, bone marrow, lymph nodes
	Trametes versicolor (PSK)	NA	*in vivo*	^{14}C-labeled: rat and rabbit serum; mouse GI tract, bone marrow, salivary glands, liver, brain, spleen, pancreas
Mannans	*Aloe barbadensis* (aloemannan)	yes	*in vitro*	FITC-labeled: mouse, GI tract
	Aloe barbadensis (gel powder)	yes	*in vitro*	NA
	Aloe barbadensis (acemannan)	NA	*in vivo*	^{14}C-labeled: dog systemic, particularly liver, bone marrow, gut, kidney, thymus, spleen
Mixed polysaccharide products	Ambrotose complex®, Advanced Ambrotose® powder	yes	*in vitro*	NA
Pectins	NA	yes	*in vitro*	NA
	Buplerum falcatum (bupleuran 2IIc)	NA	*in vivo*	Ab bound: mouse Peyer's patch, liver

Table.7 Fate of some polysaccharide products following oral intake

Beneficial properties of glucomannan, sulfated polysaccharides and arabinogalactan

Aloe Vera and its components

Aloe is a succulent plant. Succulents are xerophytes, which are adapted to living in areas of low water availability and are characterized by possessing a large water storage tissue, found inside the outer green rind. *Aloe Vera* L (*Aloe barbadensis* Miller), a member of the lily family, is the most widely used of the 360 species. Only four aloe species, *A. barbadensis* Miller, *Aloe perryi* Baker, *Aloe ferox*, and *Aloe arborescence*, have been used medically. Each leaf consists of two major components. The yellow sap, which is found in the outer green rind, is a stimulant laxative called aloin. Aloin has been shown to affect Na/K pump and Cl- channels of the colonic membrane, preventing reabsorption of water in the large intestine and potentially causing potassium depletion. In 2002, FDA issued a final ruling, stating that aloin in over-the-counter stimulant laxative products is not recognized as safe and effective and cannot be sold.

Fig.22 ***Aloe Barbadensis***

The inner leaf (parenchyma) is comprised of cellular organelles, cell walls, and the clear gel portion. Cellular organelles and leucoplasts are rich in galactose. The cell walls, which comprise the insoluble pulp, are composed mainly of galacturonic acid. The remaining clear liquid gel (> 0,5%) is an acetylated mannose-rich polymer called glucomannan, which is produced by the leucoplasts. The approximate ratio of monosaccharides found in the glucomannan gel fraction is 31 beta (1-4)-linked mannoses, 1 beta (1-4)-linked glucose, and 1 alpha (1-6)-linked galactose. In addition, acetates are randomly found on mannose residues at the 2, 3, or 6 positions. In the early 1980s the acetylated polymannose glucomannan was identified as the active component in aloe inner gel. Glucomannan is considered safe and effective, accounting for a wide variety of immune stimulating and healing effects in several studies.

Carbohydrate	Ratio
Rhamnose	1
Fucose	1
Arabinose	2
Xylose	5
Galactose	8
Galacturonic acid	26
Glucose	31
Mannose	51

Table.8 Monosaccharide ratios in aloe parenchyma. These ratios can vary depending on the age and growing conditions of the plant. Only the monosaccharides found in glucomannan have been shown to be absorbed into the blood. The other monosaccharides from cell walls, organelles and fibers have not been shown to be broken down and absorbed.

Commercially available aloe products

Glucomannan have been a commercially interest for many producers of plant derived polysaccharides claiming dozens of undocumented health effects. Improper manufacturing processes used by many manufacturers can produce products with little or no glucomannan. The active mannose content is partially and sometimes fully removed by manufactures during the processing phase. Glucomannan must be present in a sufficient amount to experience any

health benefits. When manufacturers refer to their aloe product as stabilized, they mean that enough preservatives have been added to control microbial growth. However, this does not mean that any of the natural components have been stabilized and are present in the products. This has been demonstrated in several studies. Many of the preservatives added to Aloe products for bacterial control are toxic to skin fibroblasts, thus outweighing the benefits. There are hot or cold processes in order to preserve aloe products. The cold-processed aloe requires more chemical preservatives because enzymes, mannanases and cellulases, were found rapidly digest and reduce the glucomannan content. The hot process can take several hours for the gel to reach pasteurization temperature, in order to inactivate the beta-mannases. As the temperature gradually increases, mannanase activity also increases until it is denatured. The hot process therefore has a tendency to decrease the glucomannan content. Dried aloe products do not require preservatives until they are put in a liquid state. Then bacteria can grow above acceptable levels and digest all available glucomannan. Only when the Aloe industry use validated assays to ensure the glucomannan content and therefore the efficacy of the commercial aloe product, will consumers experience the health benefits attributed to fresh Aloe. For glucomannan to be effective, both its size and amount must be controlled. A manufacturing method for isolating glucomannan from Aloe has been developed that includes filtering out the cell walls, fiber and organelles. The clear gel containing glucomannan is precipitated with alcohol, freeze-dried and ground to the desired size. Glucomannan polymers have been isolated with an average size greater than 5 million Da and mechanically sheared into an average molecular weight of 1-2 million Da for topical use in the treatment of all types of wounds in humans and animals. The United States Department of Agriculture (USDA) has approved the use of injectable Acemannan Immunostimulant as an aid in the treatment of fibrosarcomas in dogs and cats. Injectable Acemannan has also been approved as a vaccine adjuvant for Marek`s disease, where it functions as an immunostimulant. It consists of long-chain polydispersed β-(1,4)-linked mannan polymers interspersed with O-acetyl groups. Acemannan Immunostimulant has been shown to increase TNF-α and IL-1 production in animals. A study was done in 9 dogs and 9 cats with histopathologically confirmed fibrosarcomas. Animals were treated with intraperitoneal and intralesional injections. Eleven of the 18 had surgery in addition to immunotherapy. Twelve of the 18 showed gross tumor necrosis. Twelve had tumors that increased in size - usually very rapidly (2-4 weeks). This rapid increase suggests an effect attributable to TNF-α. Lymphocytic infiltration and tumor encapsulation were also seen. Benefits of long-term treatment have not been clinically

established, however, no tumor regrowth was observed in 5 animals treated with monthly IP injections of 1 mg/kg for 6 months following surgery.

Breakdown and absorption of mannose

The inability of humans to break beta bonds is compensated by probiotics present in the intestine. Beta-linked mannose polysaccharides are digested by friendly bacteria within the mammalian gastrointestinal system. These bacteria synthesize the enzymes that can break the beta-bonds of glucomannan into mannose and mannose-containing oligosaccharides. Several published studies have demonstrated that intestinal bacteria break down glucomannan into monosaccharides, oligosaccharides, and short chain fatty acids. One in vitro study demonstrated that polymers greater than 1,000 kDa were digested differently than those with sizes from 10 to 800 kDa. The dietary supplement tested contained several different plant polysaccharides, ranging from 10 to more than 1,000 kDa, that were digested. They concluded that all sizes of polysaccharides are digested by probiotics. Intestinal epithelial cells through a Na^+ - dependent mannose-specific transporter system quickly absorb the released mannose molecules from the intestine into the blood. Once in the blood, mannose is processed in three ways;

1) It can be incorporated into cellular glycoproteins and glycolipids

2) It can be incorporated into mannose oligosaccharides for use by other cells

3) Excess can be converted to fructose-6-phosphate and used as energy

Several cell types are able to absorb mannose from the blood through mannose-specific transporters that are not affected by glucose. An in vivo study demonstrated that orally and intravenously administrated glucomannan was found mainly in the liver and spleen, but also in the pancreas, kidney, thymus, heart, and brain. Further evidence of this study is demonstrated by a decrease in phosphomannose isomerase activity, the enzyme that converts glucose into mannose, in the presence of dietary mannose. Comparing intravenously to the oral administration of glucomannan demonstrates that 11, 6% was absorbed through oral ingestion. Other studies demonstrate that the level of saccharide absorption into the blood, around 12 %, is consistent throughout species tested. Once inside the cell, mannose undergoes

various enzymatic conversions to produce GDP-mannose which serves as a precursor in glycoprotein synthesis. Dolichylpyrophosphate-linked $Man_5GlcNAc_2$ is formed in the cytosol and flipped into the interior of the ER by dolichol. Dolichol is also responsible for actively moving GDP-mannose and other monosaccharides into the ER to form the oligosaccharide chains needed to produce glycoproteins.

Mannose is required for N-glycosylation and glycophospholipid anchor synthesis. Generating mannose requires PMI which converts F6P to M6P. Two types of mannose-specific transporters are identified in mammalian cells. One is a sodium-dependent energy requiring transporter on the brush-border surface of Caco-2 cells and the other is a sodium-independent facilitated transporter on the basolateral surface of Caco-2 cells and in a variety of other cells.

Recent studies show that mannose is present in human and animal blood at a concentration of 40 to 120 mmol/L and is taken up by a mannose-specific transporter. This transporter is probably physiologically relevant because the Kuptake is about 50 mmol/L, and transport is only weakly inhibited by glucose. Cultured fibroblasts and human hepatoma cells given physiologic concentrations of glucose (5.5 mmol/L) and mannose (50 mmol/L) prefer to use mannose directly for glycoprotein synthesis (75% to 80%) over that generated within the cell from glucose by PMI despite a 50- to 100- fold higher concentration of glucose. It is unknown whether this preference is valid for all tissues. This finding indicates that glucose is not the preferred source of mannose for these cells and that mammals use mannose transporters to deliver mannose to the liver and other organs for glycoprotein biosynthesis.

The finding that mannose, but not glucose, corrects glycosylation in PMM-deficient fibroblasts was surprising. Mannose offers an attractive therapy for CDGs because it should be easy to administer and nontoxic. There is scant information on the bioavailability of mannose in food, but dietary mannose is probably insufficient to supply all glycosylation.

Identification of the efficient mannose transport system in the liver and intestine made it likely that oral mannose supplements could be absorbed and utilized. Mannose therapy was begun on the first patient with CDG Type Ib after a life-threatening upper intestinal bleeding episode. Mannose was given 3 to 5 times daily and blood mannose levels rose immediately, and soon after mannose was started, the AT-III levels returned to normal. Within 2 months, the protein-losing enteropathy was completely reversed. The IEF pattern of transferrin and

other misglycosylated serum glycoproteins became normal. Because mannose is rapidly metabolized, it will probably be needed throughout the patient's life.

Patients with carbohydrate-deficient glycoprotein syndrome type 1 (CDGs) underglycosylate many serum glycoproteins by failing to add entire N-linked oligosaccharide chain. The defect in the majority of these cases is a loss of phosphomannomutase (PMM) activity, which converts M6P to mannose-1-phosphate (M1P). Fibroblasts from these patients synthesize reduced amounts of truncated lipid-linked oligosaccharide precursor and incorporate less mannose into glycoproteins. Both underglycosylation and precursor truncation are corrected by supplementing the culture medium with mannose.

Mannose transport

Mannose is moved by three transport mechanisms: the mannose-specific transporter in the intestine, the mannose receptor, and the mannose-binding lectin. The mannose transport systems move mannose monosaccharides from the blood into the cells, even in the presence of a much higher concentration of glucose. Additionally, mannose mono-and oligosaccharides, and high mannose glycoproteins may enter and affect the cellular responses of various immunomodulatory cells. Macrophage mannose receptor and the mannose-binding protein are examples of proteins that are able to detect exogenous mannose and assist in their phagocytosis by various immunomodulatory cells.

1) **Mannose receptor**

Mannose receptors are transmembrane glycoproteins that are involved in Ca^{2+}-dependent specific adhesion. Mannose receptors play important roles in the innate and adaptive immune system and are found on antigen representing cells such as macrophages, lymphatic and hepatic endothelium, and mature dendritic cells. Mannose receptors can recognize, bind and internalize monosaccharides and soluble glycoproteins that have oligosaccharide chains terminating in mannose, fucose or N-acetylglucosamine. It has been demonstrated that macrophages and other effector cells with mannose receptors are more efficiently stimulated by larger quantities of mannose-containing compounds. The amounts of mannose present in the blood or wound site determines the potency of the immune cell response. It has been demonstrated that mannose receptors;

- Stimulate phagocytic activity
- Induce other cellular activities
- Induce NF-κB activation, which stimulates the production of inflammatory cytokines, and the production of mannose receptors and induces their shedding
- Have immunomodulatory activity that can initiate different responses, e.g. induce cyclooxygenase-2 expression and prostaglandin PGE_2 production in macrophages.
- Play a role in binding collagen
- Have been implicated in IL-4-induced macrophage fusion

Compounds that up-regulates the expression of mannose receptors include;

- PGE_2
- Tumor necrosis factor-alpha (TNF-α), IL-1β, and IL-4

Compounds that down-regulates mannose receptor expression;

- IFN-γ
- Macrophage activation and migration

2) Mannose-binding lectin

The mannose-binding lectin (MBL) is a Ca^{2+} –dependent plasma protein produced by the hepatocytes and is an integral part of the classical complement pathway and immune modulation. The MBL belongs to a family of proteins called collectins and is able to recognize and bind to mannose, fucose, and N-acetylglucosamine. Collectins have a carbohydrate-recognition domain, a hydrophobic region, a collagenous region, and a cysteine-rich N-terminal region. Several studies have demonstrated the activity of MBL;

- Stimulation of phagocytosis through an induced actin cytoskeletal rearrangement
- Modulation of inflammatory activity of monocytes and macrophages depending on receptor-cell interactions. The production of MBL depend on the amount of mannose present in the body
- Promotion of apoptosis of cancer cells
- Activation of the complement pathway by forming a complex with MASPs (s.92) which leads to lysis and death of the microbe or cell

The binding affinity of MBL is enhanced with increased mannose density in mannosylated proteins. Thus, the larger the mass of mannose present in the blood or wound site, the greater MBL`s affinity to bind and initiate macrophage interactions. A drawback to MBL-induced production of cytokines is that it does not discriminate between exogenous mannose and mannose found on the glycoproteins of human cells. Recognition, binding and activation of MBL by exposed mannose or other saccharides on structurally incorrect glycoproteins have been implicated in the development of some autoimmune diseases. Rheumatoid arthritis (RA) has even been linked with recognition of specific glycoforms, namely IgG-G0. It is thought that this glycoprotein leaves terminal N-acetylglucosamine residues accessible to MBL. Subsequently, this can trigger the complement pathway and induce the production of an autoimmune inflammatory response.

Macrophage activation and stimulation

Bacteria, fungi and viruses, can activate macrophages and induce immune responses such as foreign antigen recognition, microorganism capture and removal, and wound healing. Studies reveal that it is the saccharide component of most of these organisms and substances which stimulates macrophages. One example is the lipopolysaccharide structure of Gram-negative bacteria that is able to promote the secretion of cytokines, especially in macrophages, and initiating an immune response. This ability is not limited to microorganisms. Plants are composed of saccharide structures and many of these plant polysaccharides have been demonstrated to be powerful immune modulators. It has been demonstrated that glyconutrients can affect macrophages functions resulting in killing of several bacteria including *C. albicans, E. coli, S. aureus in vitro*. Due to the limitations of an *in vitro* system, the use of more animal models is necessary to investigate the effects of glyconutrients on macrophage functions *in vivo*.

The plant polysaccharide, polymannose, is able to induce an immune response by directly and indirectly activating macrophages through three different mechanisms;

1) **The antigen non-specific mechanism of activation**

This activation for the most part applies to tissue resident macrophages, which are some of the first immune cells to interact with the foreign antigen or microorganism. The interaction of mannose with TLR-4 and CD-14 initiates a cascade of intracellular reactions which activates transcription factors that promotes the production of several cytokines and radicals like reactive oxygen species (ROS) and nitric oxide (NO). The released cytokines stimulates an inflammatory immune response.

2) **Classical mode of activation of macrophages**

This activation involves stimulation by mannose and IFN-γ released by resident-tissue macrophages. Recruited monocytes and macrophages enter the infection site where they interact with IFN-γ released by the stimulated cells. Mannose polymers interact with the TLR-4 and CD-14 in order to complete the activation. This activation induce the production of cytokines, NO, and hydrogen peroxide (H_2O_2) which serve to create a deadly environment for the invading bacteria.

3) **Alternative activation of macrophages**

Mannose is able to initiate this response and activity through its immunomodulation of dendritic cells through interaction with dendritic TLR-4 and CD-14 receptors. This activation produces a cocktail of both pro-inflammatory and anti-inflammatory cytokines which serves to initiate a potent inflammatory response, and to activate and stimulate other tissue-resident and recruited macrophages, in combination with mannose.

Mannose in colonic health

Probiotics use the carbohydrate fermentation process for energy production, with short chain fatty acids; acetate, propionate, and butyrate, as a by-product. The fermentation process serves two purposes:

- The polysaccharides are partially digested- providing ATP for the bacteria and releasing SCFA as a by product
- Humans use SCFA for energy production and promotion of colonic health

Short chain fatty acids provide epithelial cells in the colon with 60-70% of their energy requirement. They have also been demonstrated to stimulate the growth of epithelial cells

lining the colon, jejunum, and ileum. Additionally, failure to produce sufficient amounts of butyric acid has been one of the proposed theories on the development of ulcerative colitis. Beneficial effects seen with short chain fatty acids enemas in colitis patients have supported this theory. There is also some research to support that propionate may be the hypocholesterolemic short-chain fatty acid.

Some actions of butyrate
Diminishes production of mediators of inflammation by macrophages
Suppresses secretion of IL-8
Inhibits VCAM-1 mediated leukocyte adhesion to endothelial cells
Inhibits expression of ICAM-1 and VCAM-1 in endothelial cells
Enhances activation of perixosomal proliferator activated receptor (PPAR)
Suppresses production of tumor necrosis factor-alpha

Table.9 Some actions of butyrate, a short-chain fatty acid derived from the metabolism of carbohydrates by the action of commensal bacteria.

Seaweeds and its components

Seaweeds or marine algae have long made up a key part of the Asian diet and is believed to provide many health benefits. Green, red and green algae are found throughout the world's oceans and seas and none is known to be poisonous. Among the several thousand species identified, the therapeutic potential of the fucoidans found only in brown algae has demonstrated a number of health effects. Fuciodans are a class of sulfated, fucose-rich polymers and the first isolation of these fucans was reported 90 years ago. The fucans has been known for some time to act as modulators of coagulation similar to the mammalian molecule heparin sulfate. Published research on fucoidans has increased three fold between 2000 and 2010 and these carbohydrates present numerous valuable bioactivities. In Korea, new mothers are given a diet that is rich in seaweed for the first month after birth because this diet is believed to provide many health benefits for mothers and their children. The relatively

longevity and health of Okinawan Japanese populations has been attributed in part to dietary algae in studies. These studies compared Okinawan descendants who were living in Brazil with Okinawans. The former have a higher risk of developing cardiovascular and other diseases. For a dietary intervention study, 3g decosahexaenoic acid, 5g of wakame powder, and 50 mg of isoflavonoids from soybean were given daily to immigrants, at high risk for developing diseases, in Brazil for 10 weeks. This combination reduced blood pressure and cholesterol levels, suppressed the urinary markers of bone resorption, and attenuated a tendency toward diabetes. Current research interest in fucoidan is global and the therapeutic potential of natural polysaccharides found in wakame and other seaweeds have been extensively studied and are well documented.

Brown algae

Brown algae consist mainly of water (90%) in the native state. Polysaccharides are the major component and comprise alginates, cellulose, and sulfated polysaccharides. Other components include proteins, free mannitol, minerals, peptides, fatty compounds, and various pigments. The long branched chain of sugars includes a substantial amount of fucose and in addition, wakame fucoidan contains a high portion of galactose. The type of fucoidan, its sulfation, molecular weight, and conformation of sugar residues varies with the species of seaweed. The fucose composition (as a percent of total sugars) of the fucoidans isolated from the Undaria and Laminaria is 57.11 and 80.43 percent respectively.

Fig.23 Wakame hanging out to dry in Sapporo

Breakdown and absorption of fucoidans

Although a number of research papers indicate biological effects after oral ingestion or systemic delivery, very little research has taken place on the uptake and fate of fucoidan. The expectation that large molecules are not well absorbed causes difficulties in understanding how apparently systemic effects occur. In a recently published study, a sulfated polysaccharide fucoidan extracted from *Saccharina japonica* was administered to normal and alloxan-diabetic rats/mice, and its effects on glycemia, insulin and serum lipid levels were evaluated. Fucoidan administered at 200 or 1200mg/kg body weight/day could significantly reduce the blood glucose level by 22% and 34%, respectively, in alloxan-induced diabetic rats. Serum insulin levels in diabetic mice were increased by the administration of fucoidan ($P<0.05$). The results of an oral glucose tolerance test (OGTT) revealed that fucoidan treatment had some effect on glucose disposal after 15 days of treatment. Perhaps systemic observations are partly a result of prebiotic effects. Recent research indicates that there are favorable changes in intestinal flora after ingestion of fucoidan, including increased Lactobacilli.

Researchers has developed an *in vivo* antibody-based method for detection of fucoidans in blood and urine after orally but not intravenously administration of fucoidans. In a recent study researchers raised a novel antibody against fucoidan and developed a sandwich ELISA method. The antibody was specific and did not react with other polysulfated polysaccharides. Ten healthy male volunteers were orally administered 1g fucoidan and the concentration in their serum and urine was measured after 0, 3, 6, and 9h after administration. The fucoidans were obtained from *C. okamuranus* and from *F. vesiculosus* and was not detected in the blood or urine of the volunteers before administration. In seven of the ten serum and plasma samples, the fucoidan concentration was significantly elevated after fucoidan administration. On the other hand, fucoidan was detected in any of the urine samples. There was no difference in fucoidan concentration in the serum and plasma and the results indicate that by ELISA, the fucoidan concentration was detectable after 3 h and mostly after 6 h after administration. Time and peak concentration profiles varied among individuals suggesting that the absorption rate of fucoidan in the intestine is different among individuals. The molecular weight of the serum fucoidan determined by HPCL gel filtration (66kDa) remained unchanged, whereas that of urine fucoidan was significantly reduced (1.8-3.1 kDa). A fucose transporter has been reported in several types of mammalian cells. Its Km is approximately 250 μM, which is probably much higher than the fucose concentration in blood. Much of what is taken up can be converted into GDP-Fuc and incorporated into glycoproteins, but its contribution to glycosylation as compared to synthesis from GDP-Man is not known.

Fucose biosynthesis and biological functions in mammals

L-fucose is a monosaccharide that is a common component of many N-linked and O-linked glycans and glycolipids produced by mammalian cells. Fucosylated glycans are synthesized by fucosyltransferases and thirteen fucosyltransferases genes have thus far been identified in the human genome. Among the most well-known fucosylated glycans are the ABO blood group antigens which may be important for host-microbe interactions. In mammals, fucose containing glycans have important roles in blood transfusion reactions, selectin-mediated leukocyte-endothelial adhesion, host-microbe interactions, and numerous ontogenic events, including signaling events by the Notch receptor family. Alterations in the expression of fucosylated oligosaccharides have also been observed in several pathological processes, including cancer and atherosclerosis. One of the best studied functions of fucose is its role as an essential component of the carbohydrate ligands for the selectin family of cell-adhesion

receptors. Increased expression of fucosylated glycans has also been reported on serum immunoglobulins in both juvenile and adult rheumatoid arthritis patients. It is not known if such changes are important to the pathogenesis of inflammatory arthritis or if they represent a secondary consequence due to up regulation of the fucosylation machinery in the context of autoimmunity.

Two prominent examples of altered glycosylation in cancer involve fucose-containing oligosaccharides. First, expression of A and B blood group antigens is lost in many tumor with concomitant increases in H and Lewis^{y} expression, changes that correlate with poor clinical prognosis. Second, up-regulation of sialyl Lewis^{x} and sialyl Lewis^{a} serve as ligands for the selectin molecules and thus may aid in hematogenous metastasis by direct binding of cancer cells to E- and P- selectin expressed by the endothelium.

Fucose deficiency is accompanied by a complex set of phenotypes both in humans with leukocyte adhesion deficiency type II (LAD II; also known as congenital disorder of glycosylation type IIc) and a recently generated strain of mice with a conditional defect in fucosylated glycan expression. Fucosylated glycans are constructed by fucosyltransferases, which require the substrate GDP-fucose. Two pathways for the synthesis of GDP-fucose operate in mammalian cells, the GDP-mannose dependent *de novo* pathway and the free fucose-dependent salvage pathway.

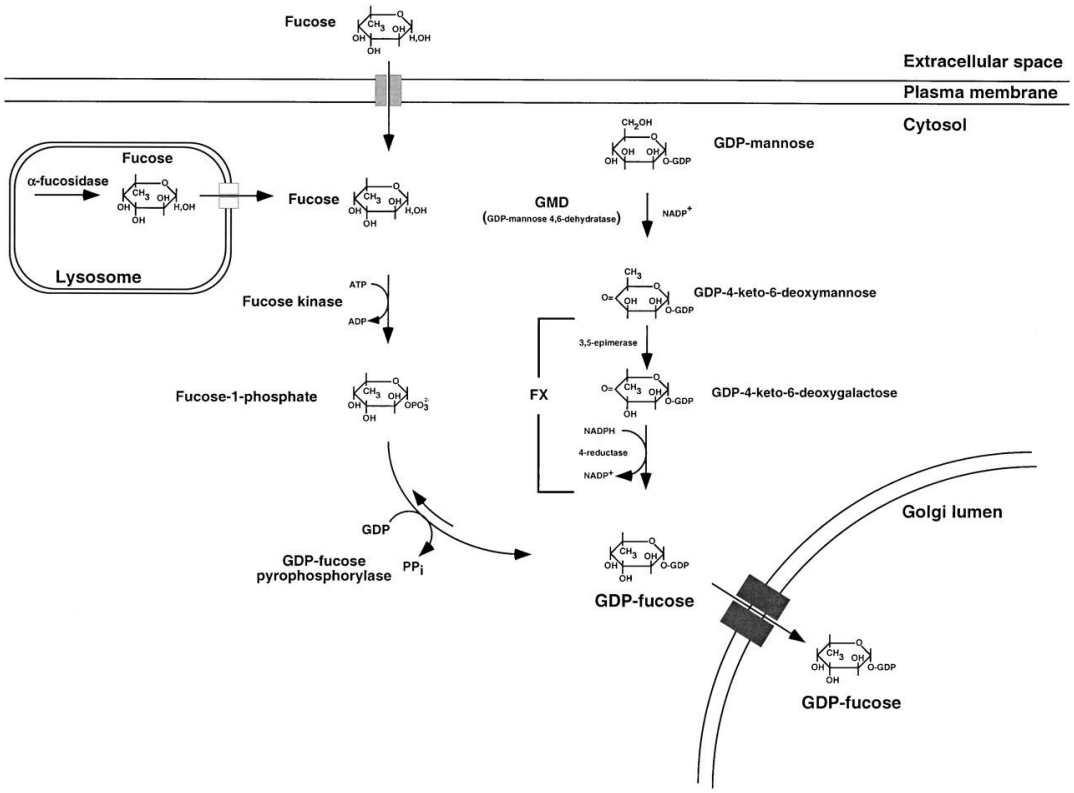

Fig.24 Biosynthesis of GDP-fucose. In mammalian cells, GDP-fucose is synthesized by two distinct pathways. The *de novo* pathway is characterized by conversion of GDP-mannose to GDP-4-keto-6-deoxymannose by GMD. This keto intermediate is then converted to GDP-fucose by an epimerase/reductase known as the FX protein. An alternative salvage pathway can yield GDP-fucose derived directly from fucose. The salvage pathway utilizes fucose that is transported into the cytosol from an extracellular origin or fucose that is liberated from catabolism of fucosylated glycans in the lysosome and then transported into the cytosol. The salvage pathway is enabled by fucose kinase and GDP-fucose pyrophosphorylase, with fucose-1-phosphate is the metabolic intermediate. GDP-fucose synthesized by these pathways is then transported into the lumen of the Golgi apparatus where it becomes available to the catalytic domains of fucosyltransferases that also localize to this membrane-delimited compartment within the secretory pathway.

Other pathways of fucose metabolism

Existence of an alternative salvage pathway for GDP-fucose biosynthesis was first inferred from studies in which radiolabeled fucose was administered to rats or provided in the culture medium of HeLa cells. These experiments demonstrated that fucose is directly incorporated into glycoproteins with little or no conversion to other sugars, thus indicating that fucose can be directly “activated” to GDP-fucose by means of a pathway independent of GDP-mannose. Free fucose derived from dietary sources or, in the case of cultured cells, from the culture medium, is transported across the plasma membrane into the cytosol. Relatively little is

known about the cell surface fucose transport system, but it appears to operate by facilitated diffusion and to be specific for fucose.

Free fucose that supplies the salvage pathway may also derive from lysosomal catabolism of glycoproteins and glycolipids by one or more fucosidase activities. Fucose liberated in the lysosomal compartment can be transported across the lysosomal membrane into the cytosol by a relatively uncharacterized transport system that appears to allow efflux of multiple neutral sugars by facilitated diffusion.

The available evidence indicates that the salvage pathway makes a minor contribution to cellular GDP-fucose pools under normal conditions. Although the amount of fucose in a typical modern human diet has not been well studied, fucose is present in several nonstarch polysaccharides and is a component of glycoproteins and glycolipids from nearly every species. Thus, depending on dietary composition and bioavailability of the fucose in plant or animal glycans, humans and other mammals are likely to ingest variable amounts of fucose. These subjects have not yet been carefully studied. In addition, intestinal microflora synthesizes fucosylated glycans that could be catabolized to generate free fucose with subsequent uptake by the colon.

In limited clinical experience with LAD II patients, fucosylated glycan expression is severely impaired in multiple cell types in the absence of fucose supplementation. These observations demonstrate that at least in a handful of children fed normal diets, flux through the salvage pathway does not generate cytosolic GDP-fucose concentrations sufficient to overcome the relatively modest defect in transport of GDP-fucose into the Golgi that is characteristic of LAD II cells. Provision of oral fucose, however, restored fucosylation to one LAD II patient, indicating that the salvage pathway maintains the capacity to generate GDP-fucose concentrations sufficient to overcome defective GDP-fucose import in this disease if supplied with supraphysiological concentrations of fucose. In support of this conclusion, glycoconjugates are undetectable in multiple adult tissues in mice with a targeted mutation of the FX locus unless exogenous fucose is supplied in the chow or water. These results in fucosylation-deficient humans and mice recapitulate findings with cultured mammalian cell lines with defects in GMD or the FX protein.

Fucose catabolism by bacteria is well described, varies according to the species, and can be robust enough to allow some bacteria to use fucose as a carbon source. These observations imply that free fucose in the diet, or released in the digestive tract from ingested fucose-

containing foods, may be diverted to catabolic pathways before absorption by the digestive tract. The degree to which intestinal bacteria contribute to oral fucose disposition is not understood. In contrast, a catabolic pathway for utilization of free fucose as an energy source has not been identified in mammalian cells. However, in a study involving intravenous administration of [1-14C] fucose to human patients, it was reported that 39% of the injected radioactivity was excreted as $14CO_2$, suggesting that fucose could be metabolized to smaller metabolic units. Subsequent work in rats corroborated this finding, but the proportion of administered fucose that was metabolized in this manner was much smaller, with only 1.6% of the intraperitoneally injected [1-14C] fucose appearing as $14CO_2$.

Fucoidan therapy

Fucoidans are well known as L-selectin blockage agents and were found to inhibit neutrophil extravasion into the peritoneal cavity. Clotting parameters were also assessed. The most active inhibitors were fucoidans from *Laminaria saccharina* and *Fucus evanescens*, which inhibited neutrophil extravasation by more than 90% whereas fucoidan from *Fucus distichus* and *Fucus spiralis* inhibited the neutrophil transmigration by only 60%. Other parameters investigated included P selectin inhibition in a platelet aggregation model, coagulation parameters and cancer cell adhesion to platelets *in vitro*. All fucoidans, except that from *Cladosiphon okamuranus*, exhibited anticoagulant activity *in vitro* and *in vivo*.

Fucoidans from *Laminaria saccharina, Laminaria digitata, Fucus serratus, Fucus distichus,* and *Fucus vesiculosus* has been found to effectively inhibit breast carcinoma cell adhesion to platelets. Several studies have demonstrated that fucoidan is effective as an anti-tumor and immune modulating agent.

Others have investigated the relative effects of higher and lower molecular weight fractions of the same fucoidan source and found significant differences in their bioactivity. It has been shown that orally delivered higher molecular weight fraction of fucoidan was more effective than an unfractionated crude fucoidan in inhibiting liver fibrosis. The key findings were increases in CD8 expression in spleens of the highest molecular weight fucoidan fed mice. The CD4/CD8 ratio tended to decrease, and the number of cells expressing CD11b cells (NK, monocytes and macrophage cells) tended to increase as compared to the lower molecular weight fucoidan fed mice. Investigations on the differential effects of high and low molecular weight fractions of fucoidan in an arthritis model showed a marked contrast in the two

preparations. The high molecular weight fraction activated the inflammatory process, whereas the low molecular weight fraction inhibited the disease.

Nontoxic, orally delivered therapeutics to address both short term and long term inflammation are highly desirable. Fucoidan's potential lies in its pleiotropic anti-inflammatory effects. These include inhibition of selectins, inhibition of complement and enzyme inhibitory activity. Fucoidan also has significant enzyme inhibitory activity against a number of enzymes including matrix metalloproteases, hyaluronidases and elastases. This inhibitory activity limits tissue breakdown in inflammatory settings caused by injury and disease and can even inhibit metastasis.

Several recent studies indicate a role of fucoidan in addressing the symptoms of osteoarthritis. It has been shown that orally administered *Undaria pinnatifida* fucoidan successfully inhibited pain. In a small human clinical study, osteoarthritis symptoms were significantly inhibited by oral administration of fucoidan rich seaweed extract. Over three months, symptoms were reduced by 52%. This result is a marked improvement for osteoarthritis symptoms. There was no reduction in TNF alpha which was used as inflammation marker, but an accompanying study in healthy volunteers showed a decrease in Interleukin 6, a marker for chronic inflammation.

The development of fucoidan fractions requires attention to the source and the required characteristics of the fraction, in addition to consideration of the route of delivery. Oral delivery appears promising, with research indicating therapeutic potential in different areas. Increasing bioavailability is likely to be important for orally delivered fucoidan.

Larch Arabinogalactan

Arabinogalactans are long, high-molecular weight (10,000-120,000) polysaccharides found in highest concentration in species of the genus Larix, including the Western Larch (*Larix occidentalis*) and Mongolian Larch (*Larix dahurica*). Arabinogalactan is highly branched with a backbone of β-D-galactopyranose residues joined primarily by (1→3) linkages in the interior of the molecule and (1→6) linkages on the exterior side chains. AGs are FDA approved and based on food grade status and numerous studies supporting the safety of larch arabinogalactan, it is considered to be orally safe even in large doses.

Arabinogalactan is fermented by human intestinal bacteria at a slower rate than other carbohydrates due to its branched structure. This unique nontoxic dietary fiber is receiving increased attention as a clinically useful nutraceutical agent due to the potent biological activity, immune-enhancing properties, and peculiar solution properties.

Fig.25 Arabinogalactan from larch tree

The primary source of arabinogalactan is the larch tree. Larch arabinogalactan is reported to be composed of a β-D-(1,3)-galactan backbone with galactose and arabinose side chains. Chemical characterization of larch extract was found to contain 93% carbohydrate and total sugar analysis found galactose to represent 90% and arabinose 9%, with smaller amounts of mannose and glucose. Unique properties of larch AG are its complete solubility and stability over a wide range of concentrations, pHs and temperature. AG is fermented in the colon by bacteria such as *Bifidobacterium* and *Bacteroides.* Both bifidobacteria and lactobacilli are beneficial bacterial populations believed to maintain and restore normal intestinal balance. Increased concentrations of these organisms have been associated with decreased fecal concentrations of potentially pathogenic bacteria and decreased levels of carcinogenic and putrefactive compounds in digesta. Some species and strains of Lactobacilli may have immunomodulating activities, such as enhancing phagocytic activity in the peripheral blood.

In a recent study on rabbits, it has been demonstrated that AG shows mucoadhesive properties useful for retention on the eye surface. The AG formulation tested on a dry eye model exerted a protective effect against the appearance of dry spots on the corneal epithelium, and significantly increased the healing rate of corneal wounds. It has also been demonstrated that AG lowers total plasma cholesterol. There is a variety of potential cholesterol lowering mechanisms associated with the consumption of dietary fiber. These mechanisms are related to viscosity, SCFA production and bacterial proliferation. There is some research to support that propionate may be the hypocholesterolemic short-chain fatty acid. Also, Lactobacilli bacteria may lower serum cholesterol levels, although the mechanisms are unclear. The microflora may be involved in the deconjugation of bile salts and subsequent inefficient cholesterol absorption, or they may possibly assimilate cholesterol and remove it from the colon. Evidence indicates consumption of larch arabinogalactan has a significant effect on enhancing gut microflora by increasing the production of short-chain fatty acids, principally butyrate and propionate.

The absolute concentration of larch arabinogalactan absorbed following an oral dose is unclear. In animal models, following injection, larch arabinogalactan is cleared from the blood with a half-life of 3.8 minutes. Ninety minutes post-injection, concentrations are highest in the liver (52.5%) and in urine (30%), with hepatic clearance following first order kinetics and having a half-life of 3.42 days. Purified larch arabinogalactans are known to bind *in vitro* with liver asialoglycoprotein receptors. *In vivo* experimental evidence also demonstrates this strong binding property to liver asialoglycoprotein receptors. Larch arabinogalactan, reaching the liver through the portal circulation, is rapidly and specifically internalized within hepatocytes by receptor-mediated endocytosis. Because of the high percentage of larch arabinogalactan arriving the liver, and its uptake by hepatocytes, it has been suggested that arabinogalactan might be an ideal vehicle to deliver drugs to the liver.

Decreasing blood ammonia concentration with larch arabinogalactan

It has been suggested arabinogalactan might have clinical value in the treatment of porto-systemic encephalopathy because of the ability to lower the generation and subsequent absorption of ammonia. In demonstrating studies ammonia may have been reduced due to the significant increases in total anaerobes. Some anaerobic colonic bacteria prefer to utilize ammonia as a nitrogen source rather than amino acids or peptides when fermenting

carbohydrates. As an example strain of Eubacterium species is reported to have a strict requirement for ammonia. High colonic ammonia levels may have detrimental health implications. Studies have shown that ammonia levels as low as 5 mmol/L can have cytopathic effects on colonic epithelial cells. Ammonia is reported to be toxic toward epithelial cells, a circumstance which leads to their increased turnover. Patients with liver disease who are unable to detoxify ammonia have been successfully treated with antibiotics and lactulose. Lactulose is fermented in the colon by bacteria that utilize ammonia as a nitrogen source, thus decreasing colonic ammonia concentration. It has been demonstrated that pectin, lactulose and arabinogalactan yield similar amounts of SCFA when fermented by intestinal bacteria, suggesting that as dietary supplements they would make similar contributions to total energy requirements. Arabinogalactan might have some value in the treatment of porto-systemic encephalopathy, as it will tend to lower ammonia absorption without the drastic purgation or side effects which may attend the use of lactulose.

Macrophage activation by arabinogalactan

Larch arabinogalactan from *Larix occidentalis* has been shown to increase circulating peripheral blood monocytes. Tumor cells pretreated with larch arabinogalactan enhanced NK cell cytotoxicity and phagocytic capacities of macrophages and lymphocytes, and increased release of various cytokines, such as IFN-γ, TNF-α, IL-1β, and IL-6. The enhancement of cytotoxicity occurred through stimulation of various cytokines, particularly interferon γ. Experimental studies have also indicated larch arabinogalactan can stimulate and enhance other functional aspects of the immune system, and inhibit the metastasis of tumor cells to the liver. Healthy donor blood treated with a combination of larch arabinogalactan, *E.purpurea*, and *E. angustifolia* in 24-hour incubation showed significant increase in macrophage cell density, and the greatest immune cell stimulation and proliferation when compared to single agent vitamins and minerals. The same study showed the combination Echinacea and larch arabinogalactan had a greater immune-enhancing effect than the individual effects of either Echinacea or larch arabinogalactan alone. Other studies have supported the benefits of larch arabinogalactan in reducing symptoms and recovery time from acute respiratory tract infections, such as the common cold and influenza. The results from a pilot study showed that a dose of 4.5 g per day for approximately 10 weeks increased the antibody response of healthy volunteers. Another study demonstrated that acidic arabinogalactan, was effective in activating macrophages to cytotoxicity against tumor cells and micro-organisms. Furthermore,

AG induced macrophages to produce tumor necrosis factor (TNF-alpha), interleukin-1 (IL-1), and interferon-beta 2. Arabinogalactan did not activate B cells and did not induce T cells to produce interleukin-2, interferon-beta 2, or interferon-gamma, but it did induce a slight increase in T-cell proliferation. These and other findings suggests that AG may have therapeutic implications in the defense against tumor and infectious diseases.

Discussion and conclusion

Many studies have been conducted in an attempt to define the structure of glycans and the precise roles oligosaccharide chains play in the function of glycoproteins. The conformations of the sugars in oligosaccharides chains vary depending on their linkages and proximity to other molecules with which the oligosaccharide may interact. These interactions lead to changes of cellular activity. A principal feature of glycans which explains many of their biological actions is that they bind specifically to a variety of molecules such as other glycans, certain viruses, many bacteria and some parasites. It is important to define the precise nature of these interactions in order to develop drugs or other agents that will specifically inhibit attachment. Appropriate analytical techniques are now available helping to disclose the sugar code and uncover many new biological interactions that are sugar dependent. Until recently, cellular use of dietary sugars for glycan biosynthesis had not been studied because cellular sugars where assumed to be derived from glucose alone. Although the utilization of monosaccharides from dietary polysaccharides is yet poorly understood, recent science has demonstrated that dietary mannose and galactose can be directly incorporated into glycoproteins and dietary mannose has been shown to be preferentially used over glucose for glycoprotein synthesis. Certain polysaccharides have attracted growing scientific interest for their ability to exert marked effects on immune system function, gastrointestinal functions, inflammation, and cancers.The discipline of glycobiology contributes to the understanding of human health and disease through research, most of which is published in peer-reviewed scientific journals, and has contributed to the development of several drugs. On the other hand conventional and complementary medicine has a history of discovering plant extracts without any harmful or toxic side effects that has contributed to numerous health benefits for mankind. Although more research needs to be done on the beneficial properties of naturally derived plant saccharides, there is the potential for a strong link between glyconutrients and glycobiology.

References

1. Ivan A. Ross *Medical Plants of the World: chemical constituents, traditional and modern medical uses* (2003). Humana Press Inc.

2. Varki A, Cummings R, Esko J, et al. *Essentials of glycobiology* (2009). CSHL Press.

3. McAnalley B. *The science behind aloe: The healing plant* (2009).

4. Aubery M. *Glycans in cell interaction and recognition* (2001). Harwood academic publishers.

5. Taylor ME, Drickamer K. *Introduction to glycobiology* (2006). Oxford university press.

6. Murray RK, Bender DA, Botham KM, et al. *Harpers biochemistry* (2009). McGraw-Hill.

7. P.de Lonlay P, Seta N. *The clinical spectrum of phosphomannose isomerase deficiency, with an evaluation of mannose treatment for CDG-Ib*. Biochimica et Biophysica Acta 1792 (2009): 841-843.

8. Jaeken J, Hennet T, Matthijs G, et al. *CDG nomenclature: Time for a change!* Biochimica et Biophysica Acta 1792 (2009): 825-826.

9. Niehues R, Hasilik M, Alton G, et al. *Carbogydrate-deficient Glycoprotein Syndrome Type Ib.* J.clin. Invest. (1998): 1414-1420.

10. Panneerselvam K, Etchison JR, Freeze HH. *Human fibroblasts prefer mannose over glucose as a source of mannose for N-glycosylation. Evidence for the functional importance of transported mannose.* J Biol Chem. (1997): 23123-9.

11. Alton G, Hasilik M, Niehues R. *Direct utilization of mannose for mammalian glycoprotein biosynthesis.* Glycobiology. (1998): 285, 291-92.

12. Freeze HH. *Disorders in protein glycosylation and potential therapy: Tip of an iceberg?* J pediatr (1998): 593-600.

13. Gianfranco P, Sylvain J, Inka B, et al. *Over-expression of ST3Gal-I promotes mammary tumorigenesis*. Glycobiology vol. 20. (2010): 1241.

14. Manna S, McAnalley BH. *Determination of the position of the O-acetyl group in a beta-(1-->4)-mannan (acemannan) from Aloe barbardensis Miller*. Carbohydr Res. (1993): 241, 317-19.

15. Yamaguchi I, Mega N, Sanada H. *Components of the gel of Aloe vera (L.) burm. f.* Biosci Biotechnol Biochem. (1993): 1350-52.

16. Talmadge J, Chavez J, Jacobs L, et al. *Fractionation of Aloe vera L. inner gel, purification and molecular profiling of activity.* Int Immunopharmacol. (2004): 1757-73.

17. Femenia A, Garcia-Pascual P, Simal S, et al. *Effects of heat treatment and dehydration on bioactive polysaccharide acemannan and cell wall polymers from Aloe barbadensis Miller.* Carbohydr polymers. (2003): 397-405.

18. Turner CE, Williamson DA, Stroud PA, et al. *Evaluation and comparison of commercially available Aloe vera L. products using size exclusion chromatography with refractive index and multi-angle laser light scattering detection.* Int Immunopharmacol. (2004): 1727-37.

19. Danhof IE, McAnalley BH. *Stabilized aloe vera: effect on human skin cells.* Drug and Cosmetic Industry (1983): 52-54-105-106.

20. Panneerselvam K, Freeze HH. *Mannose enters mammalian cells using a specific transporter that is insensitive to glucose.* The Journal of Biological Chemistry (1996): 9417-9421.

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

22. Fitton JH. *Therapies from fucoidan; multifunctional marine polymers.* Mar Drugs (2011): 1731-60.

23. Li B, Lu F, Wei X. *Fucoidan: structure and bioactivity.* Molecules (2008): 1671-95.

24. Senni K, Pereira J, Gueniche F, et al. *Marine Polysaccharides: A Source of Bioactive Molecules for Cell Therapy and Tissue Engineering.* Mar. Drugs (2011): 1664-1681.

25. Berteau O, Mulloy B. *Sulfated fucans, fresh perspectives: structures, functions, and biological properties of sulfated fucans and an overview of enzymes active toward this class of polysaccharide.* Glycobiology (2003): 29R-40R.

26. Wang J, Jin W, Zhang W, et al. *Hypoglycemic property of acidic polysaccharide extracted from Saccharina japonica and its potential mechanism.* Carbohydr Polym. (2013): 143-7.

27. Tokita Y, Nakajima K, Mochida H, et al. *Development of a fucoidan-specific antibody and measurement of fucoidan in serum and urine by sandwich ELISA.* Biosci Biotechnol Biochem. (2010): 350-7.

28. Irhimeh MR, Fitton JH, Lowenthal RM, et al. *A quantitative method to detect fucoidan in human plasma using a novel antibody.* Methods Find Exp Clin Pharmacol. (2005): 705-10.

29. Nakazato K, Takada H, Iha , et al. *Attenuation of N-nitrosodiethylamine-induced liver fibrosis by high-molecular-weight fucoidan derived from Cladosiphon okamuranus.* J Gastroenterol Hepatol. (2010): 1692-701.

30. Mišurcová L, Škrovánková S, Samek D, et al. *Health benefits of algal polysaccharides in human nutrition.* Adv Food Nutr Res. (2012): 75-145.

31. Robinson RR, Feirtag J, Slavin JL. *Effects of dietary arabinogalactan on gastrointestinal and blood parameters in healthy human subjects.* J Am Coll Nutr. 2001: 279-85.

32. Becker DJ, Lowe JB. *Fucose: biosynthesis and biological function in mammals.* Glycobiology. (2003): 41R-53R.

33. Salyers AA, Vercellotti JR, West SHE, et al. *Fermentation of mucin and plant polysaccharides by strains of Bacteroides from the human colon.* Applied and Environmental Microbiology. (1977): 319-322.

34. Vince AJ, McNeil NI, Wager JD, et al. *The effect of lactulose, pectin, arabinogalactan and cellulose on the production of organic acids and metabolism of ammonia by intestinal bacteria in a faecal incubation system.* Br J Nutr. (1990): 17-26.

35. Kelly GS. *Larch arabinogalactan: clinical relevance of a novel immune-enhancing polysaccharide*. Altern Med Rev. (1999): 96-103.

36. Ramberg JE, Nelson ED, Sinnot RA. *Immunomodulatory dietary polysaccharides: a systematic review of the literature.* Nutr J. (2010): 9: 54.

37. Udani JK, Sing BB, Barrett ML, et al. *Proprietary arabinogalactan extract increases antibody response to the pneumonia vaccine: a randomized, double-blind, placebo-controlled, pilot study in healthy volunteers.* Nutrition Journal (2010): 9:32.

38. Burgalassi S, Nicosia N, Monti D, et al. *Larch arabinogalactan for dry eye protection and treatment of corneal lesions: investigations in rabbits.* J Ocul Pharmacol Ther. (2007): 541-50.

39. Grieshop CM, Flickinger EA, Fahey GC Jr. *Oral Administration of Arabinogalactan Affects Immune Status and Fecal Microbial Populations in Dogs*. J. Nutr. (2002): 478-482.

40. Anderson JW, Nicolosi RJ, Borzelleca JF. *Glucosamine effects in humans: a review of effects on glucose metabolism, side effects, safety considerations and efficacy.* Food Chem Toxicol. (2005):187-201.

41. Alavi A, Fraser O, Tarelli E, et al. *An open-label dosing study to evaluate the safety and effects of a dietary plant-derived polysaccharide supplement on the N-glycosylation status of serum glycoproteins in healthy subjects.* Eur J Clin Nutr. (2011): 648-56.

42. McCarthy EF. *The Toxins of William B. Coley and the Treatment of Bone and Soft-Tissue Sarcomas.* Iowa Orthop J. (2006): 154–158.

43. Wiemann B, Starnes CO. *Coley's toxins, tumor necrosis factor and cancer research: a historical perspective. Pharmacol Ther.* (1994): 529-64.

44. Schnaar RL, Freeze HH. *A "glyconutrient sham"*. Glycobiology (2008): 652-7.

45. Nelson ED, Ramberg JE, Best T, et al. *Neurologic effects of exogenous saccharides: a review of controlled human, animal, and in vitro studies.* Nutr Neurosci. (2012): 149-62.

46. Best T, Kemps E, Bryan J. *Association between dietary saccharide intake and self-reported memory performance in middle-aged adults.* Br J Nutr. (2009): 93-9.

47. Best T, Kemps E, Bryan J. *A role for dietary saccharide intake in cognitive performance.* Nutr Neurosci. (2007): 113-20.

48. Berger V, Périer S, Pachiaudi C, et al. *Dietary specific sugars for serum protein enzymatic glycosylation in man.* Metabolism. (1998): 1499-503.

49. Roseman S. *Reflections on glycobiology.* J Biol Chem. (2001): 41527-42.

50. Ghazarian H, Idoni B, Oppenheimer SB. *A glycobiology review: carbohydrates, lectins and implications in cancer therapeutics.* Acta Histochem (2011): 236-47.

51. Lefkowitz DL, Stuart R, Gnade BT, et al. *Effects of a glyconutrient on macrophage functions.* Int J Immunopharmacol.(2000): 299-308.

52. Kim LS, Waters RF, Burkholder PM. *Immunological activity of larch arabinogalactan and Echinacea: a preliminary, randomized, double-blind, placebo-controlled trial.* Altern Med Rev. (2002): 138-49.

53. Luettig B, Steinmüller C, Gifford GE, et al. *Macrophage activation by the polysaccharide arabinogalactan isolated from plant cell cultures of Echinacea purpurea.* J Natl Cancer Inst. (1989): 669-75.

54. Marzorati M, Verhelst A, Luta G, et al. *In vitro modulation of the human gastrointestinal microbial community by plant-derived polysaccharide-rich dietary supplements*. Int J Food Microbiol. (2010): 168-76.

55. Best T, Kemps E, Bryan J. *Saccharide effects on cognition and well-being in middle-aged adults: A randomized controlled trial*. Dev Neuropsych (2010): 66-80.

56. Wang C, Szabo J.S, Dykman RA. *Effects of a carbohydrate supplement upon resting brain activity*. Integr Physiol Behav Sci (2004): 126-38.

57. Balzarini J. *Targeting the glycans of glycoproteins: a novel paradigm for antiviral therapy*. Nat Rev Microbiol. (2007): 583-97.

58. Stechmann B, Bai SK, Gobbo E, et al. *Inhibition of retrograde transport protects mice from lethal ricin challenge.* Cell. (2010): 231-42.

59. Kleinnijenhuis J, Oosting M, Joosten LA, et al. *Innate immune recognition of Mycobacterium tuberculosis.* Clin Dev Immunol. (2011): 1-12.

60. Torrelles JB, Schlesinger LS. *Diversity in Mycobacterium tuberculosis mannosylated cell wall determinants impacts adaptation to the host.* Tuberculosis (Edinb). (2010):84-93.

61. Nishida C, Uauy R, Kumanyika S, et al. *The joint WHO/FAO expert consultation on diet, nutrition and the prevention of chronic diseases: process, product and policy implications.* Public Health Nutr. (2004): 245-50.

62. Di Lella S, Sundblad V, Cerliani JP, et al. *When galectins recognize glycans: from biochemistry to physiology and back again.* Biochemistry. (2011): 7842-57.

63. Azuma K, Ishihara T, Nakamoto H, et al. *Effects of oral administration of fucoidan extracted from Cladosiphon okamuranus on tumor growth and survival time in a tumor-bearing mouse model.* Mar Drugs. (2012): 2337-48.

64. King GK, Yates KM, Greenlee PG, et al. *The effect of Acemannan Immunostimulant in combination with surgery and radiation therapy on spontaneous canine and feline fibrosarcomas.* J Am Anim Hosp Assoc. (1995): 439-47.

65. Ni Y, Turner D, Yates KM, et al. *Isolation and characterization of structural components of Aloe vera L. leaf pulp.* Int Immunopharmacol. (2004): 1745-55.

66. Tai-Nin Chow J, Williamson DA, Yates KM, et al. *Chemical characterization of the immunomodulating polysaccharide of Aloe vera L.* Carbohydr Res. (2005): 1131-42.

67. Harris C, Pierce K, King G, et al. *Efficacy of acemannan in treatment of canine and feline spontaneous neoplasms.* Mol Biother. (1991): 207-13.

68. Wu XZ, Chen D. *Effects of sulfated polysaccharides on tumour biology.* West Indian Med J. (2006): 270-3.

69. Brandley BK, Schnaar RL. *Cell-surface carbohydrates in cell recognition and response*. J Leukoc Biol. (1986): 97-111.

70. Varki A. *Biological roles of oligosaccharides: all of the theories are correct*. Glycobiology (1993): 97-130.

71. Sharon N, Lis H. *History of lectins: from hemagglutinins to biological recognition molecules.* Glycobiology (2004): 53R-62R.

72. Raman R, Raguram S, Venkataraman G, et al. *Glycomics: an integrated systems approach to structure-function relationships of glycans.* Nat Methods. (2005): 817-24.

73. Freeze HH. *Human disorders in N-glycosylation and animal models.* Biochim Biophys Acta. (2002): 388-93.

74. Ohtsubo K, Marth JD. *Glycosylation in cellular mechanisms of health and disease.* Cell. (2006): 855-67.

75. Freeze HH, Aebi M. *Altered glycan structures: the molecular basis of congenital disorders of glycosylation.* Curr Opin Struct Biol. (2005): 490-8.