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Targeting cells for drug and gene delivery: Emerging applications of mannans and mannan binding lectins

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This review outlines exclusive applications of mannose, mannan conjugates, and their specific interacting proteins (macrophage mannose receptors, dendritic cell receptors) in gene delivery and drug targeting, and their scope in development of targeted delivery systems.

Keywords: MBL/MBP, DC receptors, Gene delivery, Mannan coated nano particles, Mannose Receptors, Mannosylated liposomes

Introduction

Many specific carbohydrate-based ligand-receptor mechanisms have been implicated in inflammation, cell surface communication, and immune response. Carbohydrate-based drugs are making an impact in cancer, diabetes, AIDS, influenza, bacterial infections and rheumatoid arthritis. This review outlines exclusive applications of mannose, mannan conjugates, and their specific interacting proteins (mannose binding lectins and mannose receptors) in drug targeting, and their future scope in development of targeted delivery systems.

Carbohydrates-Lectins Interactions

Carbohydrates exhibit properties of potential interest when developing drug-delivery system (DDS)[1]. Carbohydrate-based therapeutics[2,3], targeting of drugs to specific disease cells via carbohydrate–lectin interactions, and carbohydrate based anti-thrombotic agents have been developed. Many human pathogens possess surface proteins that complex with specific membrane-bound oligosaccharides on human cells (Table 1)[4-8]. Variety of carbohydrate structures that occur on diseased cells gives rise to highly complex carbohydrate–lectin interactions and signaling processes. Emerging roles of carbohydrates and glycomimetics in anticancer drug design are being recognized[9].

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Lectins as Tools in Cell Recognition

Lectins agglutinate cells and/or precipitate glycoconjugates without affecting their covalent linkages and act as mediators of cell recognition in biological systems[10,11]. Lectin activities specific for different monosaccharides or glycans (fucose, galactose, mannose, N-acetylglucosamine, N-acetylgalactosamine, N-acetylneuraminic acid and heparin) have been identified. Most of them show a cellular specificity and developmental regulation. But some of them seem to be involved in signaling events both intracellularly (nuclear lectins) or at the cell surface by autocrine and paracrine mechanisms. In animals, many endogenous lectins have been implicated in a variety of immunological functions including first-line of defense against pathogens, cell trafficking, and immune regulation. Among cell surface receptors, glycolipids, glycoproteins and proteoglycans have been shown to interact with lectins on surface of animal cells. These molecules are of great interest to immunologists mainly because of their ability to interact with lymphocytes and to induce blast cell transformation. As bioadhesins, lectins enhance absorption of drugs and vaccines at mucosal

Table 1— Carbohydrates as attachment sites for bacterial/viral/fungal pathogens on animal tissues [4-6, 31, 35]

Organism	Target tissue	Carbohydrate as ligand
Actinomyces naeslundii	Mouth	Galß3GalNAcß-
B. bassiana	Insects/ Immune system?	Gal ß1—>4 Glc a); Galß1—>3 GalNAc
C. jejuni	Intestinal	Fuca2GalßGlcNAc
E. coli Type 1	Urinary	Mana3Mana6Man Man
P	Urinary	Gala4Gal*
S	Neural	NeuAc (a2–3)Galß3GalNAc * Neu5Aca3Galß4GlcßCer, GalNAcß4(Neu5Aca3)Galß4GlcßCer Galß3GalNAcß4(NeuAca3)Galß4GlcßCer (GM1)
CFA/1	Intestinal	NeuAc (a2–8)–
F1C	Urinary	GalNAcß4Galß *
F17	Urinary	GlcNAc
K1	Endothelial	GlcNAcß4GlcNAc
K99	Intestinal	NeuAc(a2–3)Galß4Glc*
E. cloacae	GI Tract	Man9(GlcNAc)2-tyrosinamide
H. influenzae type B	Respiratory	[NeuAc(a2–3)] $_{0,1}$ * Galß4GlcNAcß3Galß4GlcNAc
H3 subtype virus	Respiratory	Neu5Ac a2-6 Gal
H1 subtype virus	Respiratory	Neu5Ac a2-6Gal ß1-4GlcNAc
H. pylori	Stomach	NeuAc(a2–3)Galß4GlcNAc
BabA	Stomach	[Fuca2]Galß3[Fuca4]GlcNAc (Lewis B)-
K. pneumoniae	Respiratory	Man
Mycoplasma	Respiratory	Neu5Aca3Galß4GlcNAcß-
N. gonorrhoea	Genital	Galß4Glc(NAc) * NeuAca3Galß4GlcNAc-
N. meningitidis	Respiratory	[NeuAc(a2–3)] $_{0,1}$ * Galß4GlcNAcß3Galß4GlcNAc
P. aeruginosa	Respiratory	L-Fuc
	Respiratory	Galß3Glc(NAc)ß3Galß4Glc *
Propionobacterium	Skin, Intestine	Galß4Glcß-Cer
*S.cerevisiae*** Lectin	?	Man; trisaccharide of mannose
S. gordonii DL1	Oral cavity	Sialic acid
S. saprophyticus	Urinary	Galß4GlcNAc-
S. typhimurium	Intestinal	Man
S.pneumoniae	Respiratory	[NeuAc(a2–3)] $_{0,1}$ * Galß4GlcNAcß3Galß4GlcNAc Galß4Glc-NAcß3Galß4Glc GlcNAcß3Galß4Glc
S. suis	Respiratory	Gala4Galß4Glc *
V. cholera	Small intestine	Galß3GalNAcß4(NeuAca3)Galß4GlcßCer (GM1)*

*Indicates predominant form in tissue as glycolipids; otherwise dominant form is glycoprotein
**Yeast - a eukaryotic model organism

surfaces[1]. Targeted drug delivery to selected sites allows reduced toxicity, enhanced efficiency and interchangeable target potential.

Classification of Animal Lectins

Lectins possess functionally diverse group of protein domains which can bind specific carbohydrate recognition domains (CRDs)/oligosaccharide structures present on cell surfaces, extracellular matrix, and secreted glycoproteins. Of 8 well-established CRD groups, 4 contain intracellular and other 4 contain extracellular lectins. Intracellular lectins [calnexin family (Calnexin and Calreticulin), M-type (related to a-mannosidases of ER and *cis*-Golgi), L-type (ERGIC-53) and P-type

(phosphomannosyl receptors)] are located in luminal compartments of secretory pathway and function in trafficking, sorting and targeting of maturing glycoproteins. Extracellular lectins [C-type (collectins, selectins, mannose receptor, and others), R-type (some proteins in macrophage mannose receptor family), Siglecs (siglec-1, -2, -3 or CD33 and -4), and S-type (galectins)] are either secreted into extracellular matrix or body fluids, or localized to plasma membrane, and mediate a range of functions including cell adhesion, cell signalling, glycoprotein clearance and pathogen recognition[6]. Collagenous lectins such as mannan-binding protein (MBPs) are multimeric proteins with CRD aligned in a manner that facilitates binding to microbial surface polysaccharides (Table 1).

Role of Lectins in Immune System

To initiate immune responses against infection, antigen presenting cells (APC) must recognize and react to microbes. Recognition is achieved by interaction of particular surface receptors on APC with corresponding surface molecules on infectious agents. Professional APCs are: i) mature dendritic cells (DC), found in lymphoid tissues and derived from immature tissue dendritic cells that interact with many distinct types of pathogens; ii) macrophages, specialized to internalize extracellular pathogens, especially after they have been coated with antibody, and to present their antigens; and iii) B cells, which have antigen-specific receptors that enable them to internalize large amounts of specific antigen, process it, and present it naïve T cell for activation. By contrast, pattern recognition receptors (PRR) recognize and interact with pathogens directly. In addition to scavenger receptors and toll-like receptors, PRR include C-type lectin-like receptors (CLR) that bind carbohydrate moieties of many pathogens[12,13]. CLR include: i) mannose receptors for mannose or its polymers[14]; ii) mannose-binding lectins (MBLs) for encapsulated group B or *C meningococci*[15]; iii) DC-SIGN and structurally related receptors (DC-SIGNR) for mannose on human immunodeficiency virus, *Leishmania*, and *Mycobacteria*[13,16]; and iv) dectin-1 and dectin-2 for ß-glucan on yeasts and fungi[17].

Mannose Receptor (Man R)

ManR of macrophages, epithelial, and endothial cells acts as a molecular scavenger, binding to and internalizing a variety of pathogenic microorganisms and harmful glycoproteins. Macrophage mannose receptor (MMR; 180-kDa), a prototype member of a family of multilectin receptors, recognize carbohydrates on cell walls of infectious organisms[12]. Receptor has an extracellular region containing multiple domains that allow recognition of a diverse range of glycoconjugate ligands. It can mediate both endocytosis and phagocytosis and can facilitate clearance of both particulate and soluble ligands. Once internalized, ligands are released from receptor following endosomal or phagosomal acidification, and receptor recycles to cell surface. Role of ManR in innate immune response is well documented, with several clinically important pathogens including *Mycobacterium tuberculosis* and *Pneumocystis carinii* subject to opsonin-independent phagocytosis by the receptor. Receptor plays a central role in coordinating innate and adaptive immune responses by enhancing uptake and processing of soluble glycoconjugates released from pathogens for presentation to T cells by major histocompatibility complex class II molecules[12,18]. Carbohydrate recognition by ManR facilitates macrophage uptake of bacteria, yeast, and parasites, thereby contributing to innate immunity towards variety of pathogens.

ManR, a type I transmembrane protein, contains an NH_2-terminal cysteine-rich domain, a domain containing fibronectin type II repeats, and 8 tandem C-type lectin CRDs (Figs 1 & 2). Domain organization of CRD-4 monomer in ManR represents extended and U-shaped conformations. N-terminal cysteine-rich domain and fibronectin type II repeat appear to increase rigidity of molecule. Rigid, extended conformation of receptor places domains with different functions at distinct positions with respect to membrane[19]. An N-terminal cysteine-rich domain mediates recognition of sulfated *N*-acetylgalactosamine, which is terminal sugar of unusual oligosaccharides present on pituitary hormones. Extracellular domains of ManR, which are linked to a transmembrane region and a small cytoplasmic domain, are also shared among other members of ManR family (phospholipase A2 receptor[20], DC receptor DEC-205[21], and a ManR-like receptor expressed in epithelium[22]). CRDs of extracellular region mediate calcium-dependent binding to sugars that are commonly found on microorganisms, but rarely seen in sufficient density in terminal positions of mammalian oligosaccharides[12,18].

Of 8 C-type CRDs, CRDs 4-8 are required for binding and endocytosis of mannose/GlcNAc/fucose-terminated ligands, but only CRD-4 has demonstrable sugar binding activity in isolation. As principal mannose-recognition

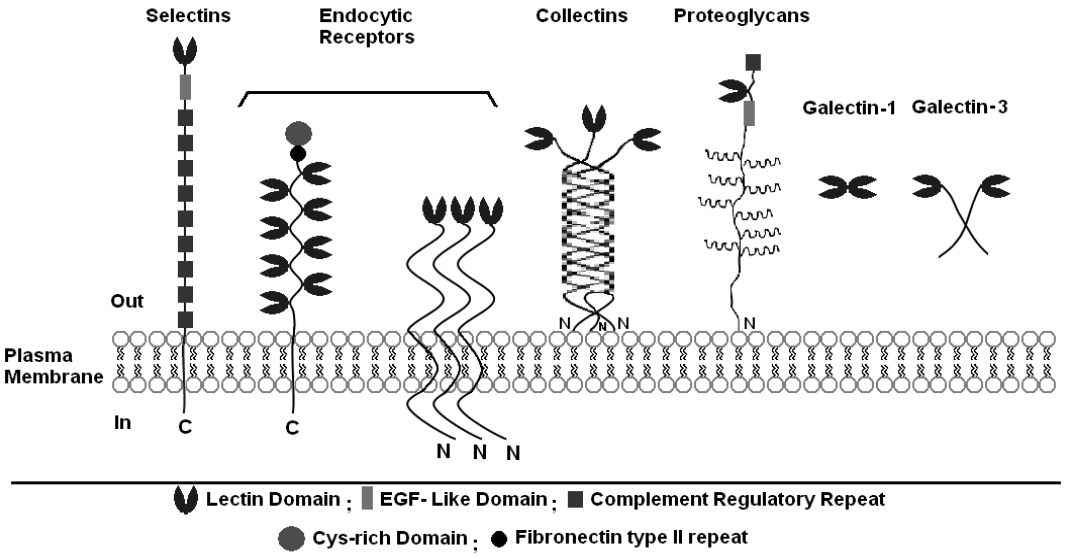

Fig. 1—Organization of CRD in different members of C-type lectin family[18]

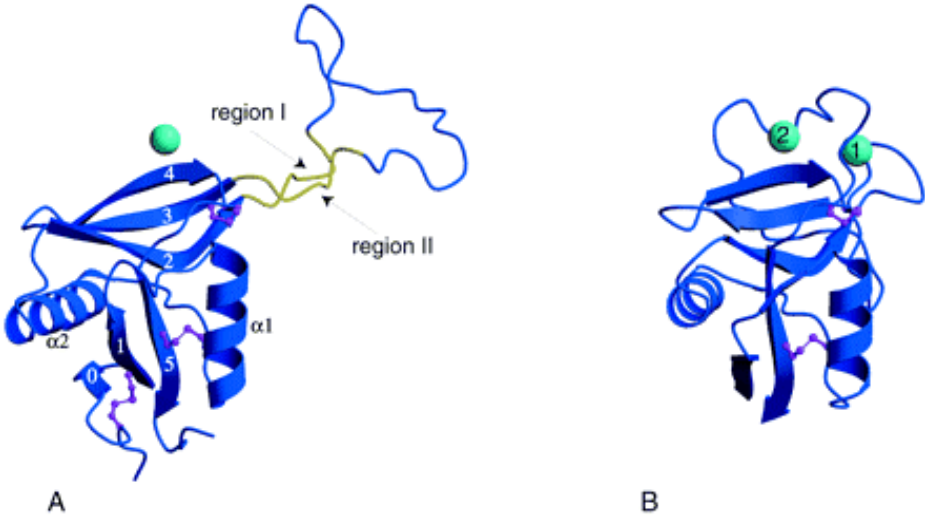

Fig. 2—Ribbon diagram of CRD-4 monomer in mannose receptor (A) and comparison to rat mannose-binding protein A (B)[23] [Disulfide bonds are shown in *pink ball-and-stick* representation, and Ca^{2+} is shown as a *blue-green sphere*. In MBP-A CRD (B), Ca^{2+} site 1 is auxiliary site, and Ca^{2+} site 2 is e principal site[24]. MBL has an oligomeric structure (400-700 kDa), built of subunits of identical peptide chains of 32 kDa each. MBL binds yeasts such as *Candida albicans*, viruses such as *HIV* and *influenza A*, many bacteria including *Salmonella* and *Streptococci*, parasites like *Leishmania*[25]]

domain of ManR (CRD4) is central ligand binding domain of receptor, multiple CRDs in whole receptor might interact with each other[23]. Although basic C-type lectin fold is preserved, a loop extends away from the core of domain to form a domain-swapped dimer in crystal (Fig. 2A). CRD-4 of mannose receptor has specificity for mannose, GlcNAc, and fucose like C-type CRDs of rat serum and liver MBPs[12,18,22-24] (Fig. 2B). Some aspects of binding of sugar and Ca^{2+} by CRD-4 are similar to those of MBP CRDs, but others are different[22] (Fig. 2A).

Mannose-Binding Lectin/Protein (MBL/MBP)

MBL/MBP plays a role in recognition of high-mannose type glycans of foreign microorganisms or plant predators.

Serum MBP, or MBL, is a Ca^{2+}-dependent lectin that belongs to family of animal lectins isolated from liver and serum of rabbits, humans and rodents. Serum MBP is specific for mannose or N-acetylglucosamine (GlcNAc), and contains both a collagen-like domain and a CRD (Fig. 2B). MBL, a multimeric lectin, recognizes a wide array of pathogens independently of specific antibody, and initiates lectin pathway of complement activation. Basic structural unit is a triple helix of MBL peptides, which aggregate into complement-fixing higher-order structures (tetramers, pentamers and hexamers). It plays a significant role during infancy before adequate specific protection is provided by adaptive immune system[12,18,24,25]. MBP in normal serum is found at low levels but is produced in increased amounts during acute-phase response. MBL acts as an opsonin for monocytes, which, unlike tissue macrophages, do not express macrophage ManR. Gene encoding MBL, *MBL2*, contains several common polymorphisms that influence transcription and assembly of molecule into multimers. Administration of recombinant or purified MBL may benefit clinical settings where MBL deficiency is associated with a high burden of infection[25].

Lectins of Dendritic Cells (DC)

Members of DC family, distributed to virtually all organs (except brain), serve as tissue resident APCs, playing critical roles in presenting environmental, microbial, and tumor-associated antigens to immune system. Several C-type lectin receptors are abundantly expressed on DC surface. Cell-surface C-type lectin receptors (DC-SIGN, L-SIGN, ManR, macrophage galactose binding lectin), and other lectins (soluble collectins and galectin-3), recognize particular glycan Ags of schistosomes and allergens, which may contribute to orchestrate Th2 associated adaptive responses. Among C-type lectins expressed by DC as PRR, DC-SIGN receptor on DC binds with high affinity to both synthetic mannose- and fucose-containing glycoconjugates. These carbohydrate structures are abundantly expressed by pathogens as demonstrated by affinity of DC-SIGN for natural surface glycans of human pathogens[13,16,26] (*M. tuberculosis, H. pylori, Leishmania mexicana, Schistosoma mansoni, and HIV-1*).

DEC-205, another mannose specific receptor, present on DC, internalizes antigens and present their fragments to naïve T lymphocytes for development of T cell dependent immunity. DEC-205, a type I membrane-integrated glycoprotein, contains 10 distinct CRD motifs in extracellular region. Anti a-DEC-205 antibodies target to DEC-205 receptor that mediates antigen presentation to T cells by DCs. Both DEC-205 and MMR mediate uptake of glycosylated antigens by DC[21,27]. Unlike DEC-205 and MMR, which contain multiple CRD motifs in NH_2-terminal ends, second group of C-type lectins consists of polypeptides that contain a single CRD in their COOH termini. Members of this group include hepatic lectin (or asialoglycoprotein receptor), macrophage galactose/ *N*-acetylgalactosamine-specific lectin (MGL), CD23, and various receptors encoded in natural killer gene complex (CD69, CD94, Ly-49, and NKG2). Thus DCs express both type I surface lectins (DEC-205 and MMR) and type II surface lectins (CD23, CD69, DCIR, dectin-1 and dectin-2 α, β, and γ isoforms)[28].

Mannose Binding Lectins from Plants

Biologically active plant lectins occur in cereals and vegetables. Mannose-specific lectins from *Amaryllidaceae* family (*Hippeastrum sp. hybrid* and *Galanthus nivalis;* GNA) inhibit HIV infection of human lymphocytic cells and suppress syncytium formation between persistently HIV-1-infected cells and uninfected $CD4^+$ T cells. In contrast to legume lectins that interact with both mannose and glucose, monocot MBLs[10,11] react exclusively with mannose and mannose-containing N-glycans. Mannose-specific lectins, structurally related to jacalin (lectins from Jerusalem artichoke, banana or rice), have been characterized. Structural diversity associated with specific recognition of high-mannose type glycans highlights importance of mannose-specific lectins as recognition molecules in drug targeting[29]. Concanavalin A (ConA), a lectin from jack bean (*Canavalia ensifomist*) seed, shows saccharide-binding specificity toward a–D-mannopyranoside or a–D-glucopyranoside ring with unmodified hydroxyl groups at 3, 4 and 6 positions. ConA activates T cells in polyclonal activation, regulates Ca^{2+} entry in human neutrophils, and specifically recognizes pentasaccharide core (ß-GlcNAc-(1→2)-a-Man-(1→3)-[ß-GlcNAc-(1→2)-a-Man-(1→6)]-Man) of N-linked oligosaccharides[30].

Lectins in Microorganisms

Bacterial lectins are typically elongated submicroscopic multi-subunit protein appendages, known as fimbriae (or pili). Until early 1980s, only bacteria specific for mannose were identified, namely type 1 fimbriated strain *E. coli*. Since then, *E. coli* strains

with diverse specificities including urinary strains carrying P fimbriae that are specific for galabiose [Gala4Gal], and neural S fimbriated strains specific for NeuAc(a2–3) Galß3GalNAc were discovered (Table 1)[4-6]. In addition, bacteria with affinities for other sugars include *Neisseria gonorrhoea*, a genital pathogen, which recognizes N-acetyllactosamine (Galß4GlcNAc, LacNAc). *Helicobacter pylori*, causative agent of peptic ulcer, expresses a number of distinct binding specificities[5,13,31]. Several of these lectins recognize NeuAc (a2–3)Galß4Glc (Sia3'Lac) and its N-acetylglucosamine analog (Sia3'LacNAc) while others are specific for Leb determinant Fuca2Galß3 [Fuca4]GlcNAc. An individual bacterium may co-express more than one lectin, certain strains of *E. coli* are both mannose and galabiose specific and those of *H. pylori* recognize simultaneously tri- and tetrasaccharides. Soluble carbohydrates (aromatic a-mannosides) recognized by bacterial surface lectins block adhesion of bacteria to animal cells *in vitro*. Significantly, lectin-inhibitory saccharides protect animals against experimental infection by lectin-carrying bacteria. Extracellular lectin (Lectin I) from *Agrobacterium radiobacter* NCIM 2443, a MBL, recognizes Man3-GlcNAc-GlcNA[31]. Plant and animal lectins with various carbohydrate specificities indicated that lectins interact primarily, but not solely, with O-side chain *of H. pylori* LPS[32]. Like bacteria, a broad range of proteins bind high-mannose carbohydrates found on the surface of envelope protein gp120 of HIV and thus interfere with viral life cycle, providing a potential new way of controlling HIV infection. These proteins interact with carbohydrate moieties in different ways[33].

On bacteria, many adhesins (one-tenth of a bacterial cell diam) are protein subunits of pili (hair), also known as fimbrae (threads), which mediate bacterial adhesion to host cell glycocalyx. Type-1 fimbriae exhibit specificity for a-mannosides and are assumed to mediate bacterial adhesion via interaction of a fimbrial lectin and a-mannosyl residues exposed on host cell surface. This carbohydrate-specific adhesive protein subunit of type 1 fimbriae is called FimH. The Fim H (29-31 kDa) subunit that possesses a carbohydrate-binding site is responsible for sugar binding activity of fimbriae. Though, FimH subunits of *E. coli* and *K. pneumoniae* are 88% homologous, two organisms differ in their fine specificity. Mannose-sensitive hemagglutinin (MSHA) of *Vibrio cholerae O1 El Tor* biotype is a member of family of type 4 pili[34]. A cell surface MBL of 40 kDa has been isolated from cell walls of a highly flocculent strain of *S. cerevisiae*. Lectin had high affinity and specificity towards branched trisaccharide of mannose, which also inhibits flocculation of yeast cells[35]. Lectin from *Beauveria bassiana* mycelium (BBL) recognizes Thomsen-Friedenreich antigen and related structures[4]. BBL preferentially reacted with some glycoproteins carrying O-linked sugar Galß1—>3 GalNAc.

Cell Targeting based on Mannan-Lectin Interactions

Synthetic glycopolymers and glycoproteins, used as carriers of covalently conjugated drugs, contain carbohydrate ligands that provide delivery specificity. However, these systems commonly rely on endogenous mechanisms, (lysosomal degradation), for release of active drug, and so unwanted release of drug at sites other than desired site of action is possible. Glycotargeting is being followed by use of oligosaccharide moiety or using lectin as a component of DDS[36-38]. Bio-recognition between lectinised DDS and glycosylated structures in intestine can be exploited for improved peroral therapy. Possibility of using lectins as a potential innovation for targeted and prolonged therapy within oral cavity is benig explored but toxicity and cost needs to be addressed before their routine use becomes a reality[36-38].

Use of Nanotechnology in Drug Targeting

Nanoparticles have been developed as an important strategy to deliver conventional drugs, recombinant proteins, vaccines and nucleotides. Nanoparticles and other colloidal DDSs modify kinetics, body distribution and drug release of an associated drug. Other effects are tissue or cell specific targeting of drugs and reduction of unwanted side effects by a controlled release. When linked with biotargeting ligands (monoclonal antibodies, peptides or small molecules), nanoparticles are used to target malignant tumors with high affinity and specificity. Long-lived quantum dots are replacing classical fluorescent dyes in staining and bioimaging. Polyethylene glycol (PEG) coated quantum dots and mannosylated PEG (Man-PEG) quantum dots, prepared for labeling macrophage exhibit extremely low cytotoxicity and are safe to macrophages[39]. Emerging implications of these platforms for advances in cancer diagnostics and therapeutics form the basis of many reviews[40-41].

Carbohydrate-directed Targeting in Cancer

Lectins affect tumor cell survival, adhesion to endothelium, or extracellular matrix, as well as tumor

vascularization and other processes that are crucial for metastatic spread and growth. This ligand-carbohydrate interaction can be made use of by development of nanoparticles containing carbohydrate moieties that are directed to certain lectins (direct lectin targeting) as well as incorporating lectins into nanoparticles that are directed to cell surface carbohydrates (reverse lectin targeting). In treatment of glioblastoma multiforme (GBM), a primary malignant tumor of brain, many nanotechnology-based devices can be applied in improvement of drug delivery to GBM[40-41]. Mannan-methotrexate conjugate showed significantly improved anti-tumor activity compared to free methotrexate in mouse model of leukemia disseminated in peritoneal cavity treated with i.p. injected chemotherapy[42].

Uptake of Polymeric Mannan-conjugates (Receptor-mediated Targeting)

Cell surface-bound receptors represent suitable entry sites for delivery of macromolecules or supramolecular structures into cells by receptor-mediated endocytosis. Carrying carbohydrate-tag, DDS can be recognized by cells/tissues and internalised by endogenous lectins at cell surface[43,44]. Mammalian mannose/fucose/galactose specific cell surface receptors are expressed on macrophages and other antigen presenting cells such as DCs in skin and M-cells in intestine. Macrophages play an important role in host immune functions such as antigen presentation. ManR on macrophages mediates internalization of a wide range of molecules or microorganisms in a pattern recognition manner. Therefore, it represents an attractive entry for specific drug, gene, or antigen delivery to macrophages and antigen-presenting DCs. Particles coated with carbohydrate ligands offer potential future. Based on this principle, DDSs containing asialofetuin, galactose, mannose, or N-acetyl-galactosamine were developed and tested for endocytois by macrophages, DCs, and liver cells. Use of carbohydrate-modified HPMA or liposomes gave improved results[45,46]. Liver or colon macrophages and mouse brain are targeted by mannosylated liposomes (Man-liposome)[47].

Asialoglycoprotein receptor (ASGP-R), also called hepatic lectin, is predominantly expressed on sinusoidal surface of mammalian hepatocytes and is responsible for clearance of glycoproteins with desialylated galactose or acetylgalactosamine residues from circulation by receptor-mediated endocytosis. Specificity of receptor for D-galactose or D-mannose is accomplished by specific hydrogen bonding of 3 and 4-hydroxyl groups with carboxylate and amide side-chains. Therefore, mutation of amino acid sequence in CRD results in a conversion of its specificity[48]. Crystal structure provides a direct confirmation for conversion of ligand-binding site of mannose-binding protein to an ASGP-R-like specificity. A number of functional mimics for CRDs of these lectins have been developed by modification of domain amino acid residues. Modified CRD that displayed 40-fold preferential binding to N-acetylgalactosamine compared with galactose proved to be a good functional mimic for ASGP-R. Mannose labeling shifted ratio to more non-parenchymal cell incorporation (majority to Kupffer cells). Therefore, alternative approaches are needed to target liposomes to hepatocytes via ASGP-R[47,49].

Mannosylated Proteins as Cell-Specific Carriers

Hepatic uptake characteristics of mannosylated bovine serum albumin (Man-BSA) were assessed as a liver-specific carrier system[50]. The ^{111}In-Man18-BSA accumulated in liver (up to 70%), in which endothelial and Kupffer cells contributed major fraction. Fewer binding sites and a greater internalization in comparison with other carriers such as galactosylated, succinylated and cationized BSAs[50] characterize uptake of ^{111}In-Man-BSA. Gliotoxin (GTX) is known to induce apoptosis of hepatic cells and to cause regression of liver fibrosis. Mannose-6-phosphate-modified human serum albumin (M6P-HSA) selectively accumulated in liver fibrogenic cells and induced apoptosis in myofibroblastic (hMFs), rat hepatic stellate cells (HSCs), and in fibrotic liver slices. *In vivo*, both GTX and conjugated GTX to M6P-HSA (GTX-M6P-HSA) attenuated the number of activated HSCs, but GTX also affected hepatocytes, suggesting that cell-selective delivery of apoptosis-inducing agent GTX is feasible in fibrotic livers[51]. Mannosylated-gelatin nanoparticles (Man-G-NPs) selectively delivered an anti-HIV drug, didanosine, intravenous administration of which significantly enhanced drug uptake by lung, liver, and lymph nodes as compared to non-coupled G-NPs or free drug[52].

Mannosylated poly(L-lysine)

A drug targeting system, which utilizes ManR-mediated endocytosis to enhance cellular uptake of oligonucleotides (ONs) in alveolar macrophages (AMs), employs a molecular complex consisting of partially substituted mannosylated poly(L-lysine) (ManPL), linked to ON. Upon recognition by macrophage ManRs, ManPL was

internalized by receptor-mediated pathway, co-transporting ON. AMs treated with ManPL: ON complex exhibited a significant increase in ON uptake over free ON-treated controls. Following cellular internalization, ON largely accumulated in endocytic vesicles[53].

Poly-(L-lysine Citramide Imide)

Commercially available quinic and shikimic acids appear as stable mannose bioisosteres, which should prove valuable tools for specific cell delivery[54]. Internalization of norfloxacin antibiotic, which is active against some intracellular bacteria, was coupled to a polymeric carrier [poly-(L-lysine citramide imide), derived from metabolites, citric acid and L-lysine]. This carrier is known to be biocompatible and slowly degradable under slight acidic conditions. It was proposed that prodrug macromolecules compete effectively with glucose oxidase and thus should be able to bring drug up to mannosyl receptor-bearing membranes of macrophages infected by intracellular bacteria[55].

Evaluation of Cyclodextrin Conjugates

Dendritic ß-cyclodextrin (ßCD) derivatives bearing multivalent mannosyl ligands were assessed for binding efficiency towards ConA and mammalian mannose/fucose specific cell surface receptor from macrophages. This type of ßCD-dendrimer construct showed high drug solubilization capability. A subtle change in structure of conjugate may have important consequences on receptor affinity[56]. McNicholas *et al*[57] synthesized amphiphilic ß-cyclodextrins bearing hexylthio, dodecylthio, and hexadecylthio chains at 6-positions and glycosylthiocarbamoyl-oligo(ethylene glycol) units at 2-positions. These amphiphilic glycosylated cyclodextrins form vesicles in water. Hexylthio assemblies exhibited selective binding to Lens culinaris lectin. A bioeliminable amphiphilic poly(ethylene oxide)-b-poly(e-caprolactone) diblock copolymer end-capped by a mannose residues showed that these colloidal systems have great potential for drug targeting and vaccine delivery systems[58].

Man-poly-ethyleneimine (ManPEI)/poly-propyleneimine (ManPPI) conjugates

DCs transfected with ManPEI/DNA complexes containing adenovirus particles are effective in activating T cells of T cell receptor transgenic mice in an antigen-specific fashion[59]. Lamivudine (3TC), loaded with poly(propyleneimine) (PPI) and ManPPI, had higher anti-HIV activity, at a dose as low as 0.019 nM/ml, as compared to free drug. Cellular uptake of 3TC loaded on ManPPI was 21 and 8.3 times higher than that of free drug and PPI respectively. Thus, ManPPI carrier holds higher potential with reduced toxicity of antiretroviral therapy[60].

Chitosan as Carrier of Drugs

Chitosan, a natural polysaccharide, is a non-viral vector and has advantages in biocompatibility, biodegradability and low toxicity with high cationic potential. Kim *et al*[61] studied galactose or mannose ligand modification of chitosan for enhancement of cell specificity and transfection efficiency via receptor-mediated endocytosis. A colon targeted tablet formulation, using chitosan and guar gum as carriers and diltiazem hydrochloride as model drug, revealed that polysaccharides as carriers and inulin and shellac as coating materials can be used effectively for colon targeting for treating local as well as systemic disorders[62].

Konjac Glucomannan and Xanthan Gum as Drug Carriers

With xanthan gum (XG), konjac glucomannan (KGM), a water-soluble non-ionic polysaccharide, forms thermoreversible gels with biodegradation properties. KGM, which is degraded in colon but not in small intestine, makes it potentially useful as an excipient for colonic drug delivery. Mixtures of some KGM with XG offer potential to develop delivery systems capable of maintaining physical integrity and drug release[63,64]. DNA release with KGM can be controlled by changing preparation conditions and structure parameters of hydrogels. Thus, KGM hydrogels have a potential use for advanced controlled release[65]. Seed endosperm of *Cassia pleurocarpa* contains a water-soluble galactomannan, which can be exploited in biomedical applications in drug delivery and tissue engineering[66]. Repeating unit of heteropolysaccharide shows a backbone of ß(1-4) linked D-mannopyranosyl units, to which D-galactopyranosyl units are linked as side chains through a(1-6) linkages.

Mannosylated Carriers in Gene Delivery

Poly[N-p-Vinylbenzyl-O-ß-Mannopyranosyl-(1-4)-D-Glucoamide] (PV-Mannose)

PV-Mannose that contains mannose and interacts with ManR-carrying cell line strongly binds to macrophage cells, probably due to a specific interaction mediated by ManRs on cell membrane. Using a PV-mannose glycopolymer, receptor-mediated gene transfer via ManR is another method for targeted gene delivery into macrophages[67]. Polymeric nanospheres (NS) with a

polystyrene core and a glucosyloxyethyl methacrylate (GEMA) oligomer corona NS proved to be a useful material for studying sugar-biomolecule recognition and offered a potential for using a multi-lectin nanoparticle array in glycoprotein mapping[68,69]. Poly[N-p-vinylbenzyl-O-D-glucopyranosyl-(1-4)-D-glucoamide] (PV-maltose) and PV-mannose, which have specific binding ability with murine hematopoietic cells, have been suggested for gene and drug delivery for hematopoietic cells and in therapeutic settings, respectively[70].

ManPEI Coupled Silica Nanoparticles

Mesoporous silica nanoparticles (MSN) coupled with mannosylated polyethylenimine (ManPEI) were examined to transfect plasmid DNA *in vitro*[71]. ManPEI was coupled to MSN to target macrophage cells with ManR. MSN/DNA complexes showed enhanced transfection efficiency through receptor-mediated endocytosis via ManR.

Mannosylated Liposomes (MLs)

Direct respiratory delivery via inhalation of MLs to alveolar macrophages is of great interest. However, clustering of mannose residues on liposomal surfaces or mannose density of Man-liposomes was important in determining binding affinity of Man-liposomes to MBP[72]. MLs intercalated-benzyl derivative of an antibiotic MT81 (Bz2MT81) could eliminate intracellular amastigotes of *Leishmania donovani* within splenic macrophages more efficiently than liposome intercalated Bz2MT81 or free Bz2MT81. Liver and kidney function tests showed that toxicity of Bz2MT81 was reduced up to normal level when mannose grafted liposomal Bz2MT81 were administered[73]. Using cell receptors on surface of mononuclear phagocyte cells, stavudine-loaded Man-liposomal formulations have been tested for targeting HIV-infected cells. Using Con A as a model system for *in-vitro* ligand-binding, Man-liposomes showed potential applications for site-specific and ligand-directed delivery systems with better pharmacological activity[74]. In order to enhance localization to lymphatics, specifically to lymph node and spleen, surface engineered MLs showed biphasic response of zidovudine (ZDV) release. Man-liposomes appeared to be promising vesicular system for enhanced targeting of ZDV to lymphatics in AIDS chemotherapy[75].

Mannosylated Cationic Liposomes

Complexes of polylysine linked to ligands such as mannose[76,77] with DNA enhance gene expression in macrophages. However, transfection efficiency of many of these vectors is handicapped due to endosomal or lysosomal degradation. Introduction of ligands for cell-surface receptors into liposomes has improved transfection efficiency in macrophages. In most cases, liposomes were coated with macromolecular ligands[78,79] (transferrin, immunoglobulins and asialoglycoproteins). Various cationic lipids that deliver genes into cells have been synthesized. DC-Chol liposomes have been used in gene therapy in clinical settings[78]. A galactosylated cholesterol derivative with dioleoylphosphatidylethanolamine (DOPE) efficiently transfers a plasmid DNA into human hepatoma cells (HepG2) via an asialoglycoprotein receptor-mediated mechanism. However, these cationic liposomes did not exhibit any cell specificity *in vivo*.

Kawakami *et al*[80], developed a low-molecular weight lipidic ligand, a mannosylated cholesterol derivative, cholesten-5-yloxy-N-[4-{(1-imino-2-β-D-thiomannosylethyl) amino} butyl]-formamide (Man-C4-Chol), for gene delivery to hepatocytes and compared with other types of liposomes prepared with various molar ratios of Man-C4-Chol and particle size in transfection assays[80]. Gene expression with Man-C4-Chol/DOPE (6:4) liposome/ DNA complexes in liver non-parenchymal cells was significantly reduced by predosing with Man-BSA. Higher gene expression in liver following intraportal injection suggested that plasmid DNA complexed with Man-liposomes exhibited high transfection activity due to recognition by ManR both *in vitro* and *in vivo*. Intravenous (i.v.) injection of DNA/cationic liposome complexes resulted in gene expression in many tissues including heart, lung, liver, kidney and spleen[81] and supported the participation of ManR in uptake of Man-liposome/DNA complexes and in liver Kuppfer and/or endothelial cells. Like galactosylated protein, directly relating to surface density of galactose residues[82], Man-liposomes follow similar strategy in liver cells. Chemical structure and physicochemical characteristics of Man-C4-Chol seemed to satisfy the conditions for transfection in macrophages by offering a cationic charge and being recognized by mannose structure on liposomal surface. Wijagkanalan *et al*[72] demonstrated efficient targeting to alveolar macrophages by intratracheally administering Man-liposomes via ManR-mediated endocytosis in rats. Study, testing Man-liposomes with various ratio of Man-C4-Chol, suggested *in vitro* uptake of Man-liposomes in a concentration-dependent manner. Through intratracheal route of

administration of Man-7.5 and Man-5.0-liposomes, internalization was enhanced and was selective to alveolar macrophages. However, serum MBP inhibits mannosylated liposome-mediated transfection to macrophages[83]. Though, Man-C4-Chol exhibited a higher transfection activity than DC-Chol liposomes in mouse peritoneal macrophages based on a receptor-mediated mechanism, role of serum proteins has to be examined and to be overcome. Since this compound itself has a positive charge, a high density of mannose residues can be deposited on liposome surface without adversely affecting binding ability of cationic liposomes to DNA. These characteristics of liposomes with a mannosylated cholesterol derivative are reflected in their superior *in vivo* gene transfection[84].

Mannosylated-Emulsions

Carbohydrate grafted emulsions are one of the most promising cell-specific targeting systems for lipophilic drugs. Man-emulsions composed of soybean oil, EggPC and Man-C4-Chol (70:25:5) were significantly delivered to liver non-parenchymal cells (NPC) via ManR-mediated mechanism after i.v. administration in mice and supported design of pDNA/ligands-grafted cationic liposome complexes for cell-specific gene delivery[85]. *In vitro* study showed increased internalization of Man-5.0- and Man-7.5-emulsions and significant inhibition of uptake in presence of mannan. Mannose density of Man-emulsions plays an important role in both cellular recognition and internalization via a ManR-mediated mechanism[85].

Man-Cationic Liposomes/CpG DNA Complex

To combat refractory peritoneal dissemination, immunotherapy using immunostimulatory CpG DNA is a promising therapeutic approach. A mannosylated cationic liposomes/immunostimulatory CpG DNA complex (Man/CpG DNA lipoplex) is an effective inhibitor for peritoneal dissemination in mice. Intraperitoneal administration of Man/CpG DNA inhibited proliferation of tumor cells more efficiently than Bare/CpG DNA lipoplex and Gal/CpG DNA lipoplex. Therefore, Man/CpG DNA lipoplex can be used for efficient immunotherapy to combat peritoneal dissemination[86].

DC-targeted Vaccines

Adjuvant Role of DCs

Efficient delivery of antigens to antigen presenting cells (DCs) is an important challenge for new generation vaccines. Multivalency is fundamental requirement for carbohydrate-protein interactions. DCs work as a natural adjuvant to elicit T cell immunity. Antigen has been targeted to DCs through DC-specific receptors (ManR, DEC205, DC-SIGN) and dying cell receptors. However, antigen captured by DCs in absence of danger signals induces tolerance. Therefore, duration and/or magnitude of danger signals play a crucial role in generating an immunogeneic response. It is, therefore, crucial to determine optimal conditions for antigen delivery to DCs in an environment suited to maximally stimulate immune system[87].

ManR Targeting Vaccines

Targeting antigens to endocytic receptors on professional APCs represents an attractive strategy to enhance vaccines efficacy. Such APC-targeted vaccines have ability to guide exogenous protein antigens into vesicles that efficiently process antigen for MHC class I and class II presentation. Efficient targeting not only requires high specificity for receptor that abundantly express on APCs surface, but also ability to be rapidly internalised and loaded into compartments that contain elements of antigen-processing machinery. ManR and related C-type lectin receptors are especially designed to sample antigens, much like pattern recognition receptors, to integrate innate with adaptive immune responses. A variety of approaches involving delivery of antigens to ManR have demonstrated effective induction of potent cellular and humoral immune responses. ManR-targeted vaccines are likely to be most efficacious *in vivo* when combined with agents that elicit complementary activation signals. A better understanding of mechanism associated with induction of immune responses as a result of targeting antigens to ManR, will be important in exploiting ManR-targeted vaccines not only for mounting immune defenses against cancer and infectious disease, but also for specific induction of tolerance in treatment of autoimmune disease[88].

Blank anionic poly(e-caprolactone)-poly(ethylene glycol)-poly(e-caprolactone) (PCEC) and anionic mannan modified PCEC (MPCEC) nanoparticles with nearly same particle size and zeta potential, used to adsorb human basic fibroblast growth factor (bFGF) onto particles surface produced improved results. Higher antibody titers of Ig isotypes in mice immunized with bFGF-MPCEC complexes than those immunized by either bFGF-PCEC or bFGF-Alum suggested that MPCEC could be targeted to DCs to improve humoral immunity[89].

Liposomes prepared with multi-branched mannosylated lipids display higher binding affinity for ManR than vesicles containing mono-mannosylated analogs. Dimannosylated ligands present at liposomes surface were as efficient as tetramannosylated ones to engage in multidentate interactions with ManR of immature DCs. Antigen-associated targeted liposomes containing diantennary mannosylated lipids could be effective vectors for vaccines when combined with additional DC activation signals[90]**.** *Bordetella bronchiseptica* antigens containing dermonecrotoxin (BBD) were loaded in mannosylated chitosan microspheres (MCMs) or chitosan microspheres (CMs). BBD-loaded MCMs (BBD-MCMs) bound to ManR on murine macrophages and resulted in enhanced immune-stimulating activities through mucosal delivery due to a specific interaction between MCMs and ManR on macrophages[91].

DEC-205: A Target for Immunotherapy

DEC-205, present on DC, is a mannose specific receptor. One of the major functions of DEC-205 is to internalize antigens and present to naïve T lymphocytes for development of T cell dependent immunity. To assess potential of antigen targeting to DC to improve immunity, Bonifaz *et al*[92] incorporated ovalbumin into a mAb to DEC-205, which is abundantly expressed on these cells in lymphoid tissues. Simultaneously, agonistic a-CD40 antibody was also injected to mature DCs. A single low dose of antibody-conjugated ovalbumin initiated immunity from naive $CD4^+$ and $CD8^+$ T cell repertoire. By antibody-mediated antigen targeting via DEC-205 receptor, efficiency of vaccination for T cell immunity increased in disease models[92]. Badiee *et al*[93] prepared anti-human DEC-205 immunoliposomes (anti-hDEC-205 iLPSM) and compared their uptake by monocyte-derived DC (MyDC) and blood DC (BDC) with conventional liposomes (cLPSM). Confocal microscopy confirmed that anti-hDEC-205 iLPSM were phagocytosed by DC and available for antigen processing. Thus, DEC-205 is one of the effective targets for delivering liposomes to human DCs.

The observation that anti-a-DEC-205 antibodies target to DEC-205 receptor that mediates antigen presentation to T cells by DCs, was exploited for immunization strategies by conjugating melanoma antigen tyrosinase-related protein (TRP)-2 to a-DEC-205 antibodies and immunization of mice with these conjugates together with DC-activating oligonucleotides (CpG). Upon grafting of melanoma cells, a-DEC-TRP immunized mice showed substantially slow growth of implanted tumors in tumor bearing hosts and animals (70%) were cured from existing tumors. Thus, targeting of DCs *in situ* by antibody-antigen conjugates may be an effective mode to induce long-lasting antitumor immunity[94].

DC-SIGN-mediated Targeting

Gieseler *et al*[95] studied liposomal compound delivery to monocyte-derived myeloid dendritic cells (MyDCs) by specifically addressing DC-SIGN (CD-209), a DC-associated lectin implicated in transmission of HIV-1 to $CD4^+$ T helper cells. DC-SIGN was superior target than other MyDC markers. Liposomal targeting to DC-SIGN and related C-type lectins may afford therapeutic intracellular drug delivery to MyDCs and other cells susceptible to HIV-1 infection. Synthetic oligomannose dendrons display multivalent oligomannoses in high density and interact with DC-SIGN with high efficacy. A second-generation Man_9 dendron was identified as a potential immunogen for HIV vaccine development and as a potential antiviral agent[96].

Mannan-coated Nanoparticles in Vaccination

Genetic immunization using naked plasmid DNA (pDNA) has been used to elicit humoral and cellular immune responses. In search of a cell-targeted delivery system, cationic nanoparticles (NPs) were coated with plasmid DNA to produce pDNA-coated NPs. An endosomolytic lipid and/or a DC-targeting ligand (mannan) were incorporated in or deposited on NPs to enhance immune responses in mice. Plasmid DNA-coated NPs, especially with both an endosomolytic lipid and DC-targeting ligand, resulted in 16-fold increase in IgG titer and 3-fold release in Th1-type cytokine over naked pDNA, indicating that engineered pDNA-coated NPs could enhance *in vitro* cell transfection and enhance *in vivo* immune responses. pDNA-coated NPs, especially mannan-coated pDNA-NP with DOPE, resulted in significant enhancement in both antigen-specific IgG titers and splenocyte proliferation over naked pDNA alone[97,98]. To overcome disadvantages associated with conventional methods used to mannosylate antigens, Sheng *et al*[99] developed a mannose-based antigen delivery system that utilizes a polyamidoamine (PAMAM) dendrimer. Mannosylated dendrimer ovalbumin (MDO) potently induced OVA-specific T cell response *in vitro* and showed exceptional adjuvanticity.

Cell Targeting in Intestine: A Strategy for Mucosal Vaccination and Drug Delivery

Various lectins and lectin containing pathogens bind specifically to oligosaccharides on intestinal cells. Antigen-sampling M cells offer a portal for absorption of colloidal and particulate delivery vehicles, including bacteria, viruses and inert microparticles. While ManR is found on lymphatic endothelial cells of small intestine, intestinal serosa revealed a regular, dense, planar network of cells with prominent dendritic morphology within external muscular layer and with increasing frequency along intestine length. Serosal-disposed layers show a significant fraction of DCs that express DEC-205, langerin, and various other molecules. This network of DCs needs to be explored for drug targeting and mucosal vaccination through ManR, DEC-205, and langerin present on intestinal cells[100,101].

Lectins as Drug Carriers (Reverse Targeting)

In use of lectins towards glycotargeting, DDS is decorated with lectins of certain carbohydrate specificity so that it can interact with glycosylated surfaces. Nonpathogenic strains of some bacteria can also be utilized through this approach. In addition to plant lectins recognizing glycans and mannans in particular, trimannoside-recognizing peptide sequences have been identified in T7 phage [PSVGLFTH (8-mer) and SVGLGLGFSTVNCF (14-mer)], which need to be examined for development of inhibitors or DDSs targeting polysaccharides[102].

ConA-conjugated-nanoparticles/liposomes as Carriers

Lectin-conjugated gliadin nanoparticles are potential candidates for targeted drug delivery and are useful in treatment of H. pylori[103]. Ulex Europaeus Agglutinin I (UEA I) and ConA lectins, bound to gliadin nanoparticles (GNP) bearing acetohydroxamic acid (AHA) were effective in inhibiting H. pylori binding. In addition, antimicrobial activity of UEA-GNP and Con A-GNP was two-fold higher compared to GNP.

Lectinised Liposomes

Lectinised liposomes bind alveolar type II epithelial cells[104] for epithelial drug delivery. Amphiphiles, which carry many mannose residues as side chains, are incorporated in liposomes and recognized by ConA. Interaction between sugar residues on liposome and lectin was largely affected by degree of polymerization and surface density of amphiphile in liposomes. Positive entropy change for binding of ConA to mannose residues on liposome surface indicated that recognition in liposome system is largely promoted by release of water molecules from both sugar residues on liposome surface and binding site of Con A[105]. As compared to plain liposomes, binding to A549 cells increased upon surface modification with WGA, ConA or soybean agglutinin. In search of non-viral vectors for gene therapy of cystic fibrosis, lectins were screened for binding and uptake into living human airway epithelium[106]. Whereas ConA was internalised within 1 h, lectins from *Erythrina cristagalli* and *Glycine max*, peanut lectin, and Jacalin were taken up into epithelium within 4 h.

Insulin Delivery Systems

An implantable ConA based, glucose-responsive insulin delivery system, which can be used for long-term diabetes treatment, has been described[107]. *In vitro* release experiments with ConA conjugate and glycosyl-poly(ethylene glycol) (G-PEG)-insulin complex enclosed in membrane device indicated a pulsative, reversible release pattern for G-PEG-insulin in response to glucose challenges, demonstrating feasibility of release system for chronic *in vivo* studies with diabetic-pancreatectomized dogs. Lectin-modified solid lipid nanoparticles (SLNs), containing insulin after oral administration of peptide and protein drugs, indicated that SLNs and WGA-modified SLNs promote oral absorption of insulin.

ConA-polystyrene-HIV-1 Nanospheres in Immunization

Polystyrene derivatives contain mannose moieties that interact with ManR-carrying cell lines (DCs, macrophages) or mannan binding proteins. ConA, coupled to polystyrene nanoparticles via a poly (ethyleneoxide) linker, protects protein conformation and activity. ConA-coated particles bind selectively to a series of different glycoproteins, and ConA-immobilized polystyrene nanospheres (ConA-NS) efficiently capture HIV-1. Intranasal immunization with inactivated HIV-1-capturing nanospheres (HIV-NS) produces vaginal anti-HIV-1 IgA antibody in mice. Intranasal immunization of macaques with ConA-NS or inactivated simian/HIV-KU-2-capturing nanospheres (SHIV-NS) and then intravaginally challenged with SHIV KU-2 exhibited partial protection[108,109].

PHA-L4 Isolectin Binds Malignant Tumors

Phaseolus vulgaris agglutinin-L isolectin (L -PHA), which interacts with ß1-6 branching N-acetylglucosamine (ß1-6 GlcNAc), has been used for

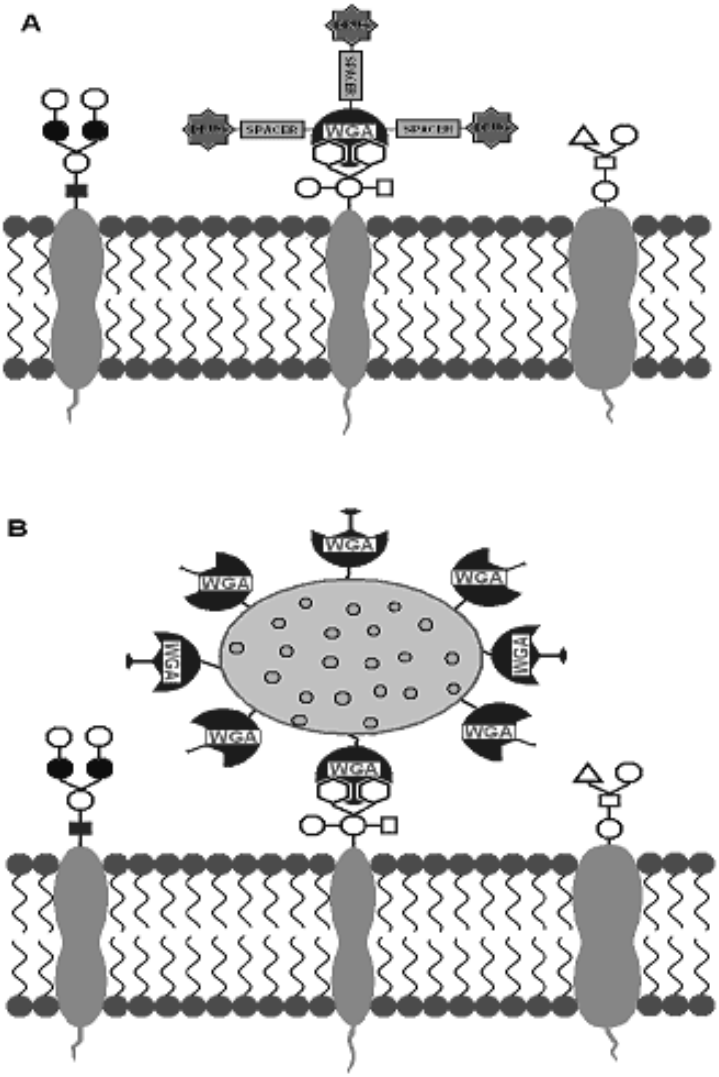

Fig. 3—Lectin-grafted formulations: a) Lectin-grafted prodrug; b) Lectin-grafted carrier system[112] (Symbols ⬡ ○ ● ■ □ and △ denote different extracellular carbohydrate moieties; ⬡ denotes galactose, which recognizes WGA, a galactose specific lectin)

in situ cancer diagnosis. Bionanocapsules (BNCs), hollow particles (~ 80 nm), composed of hepatitis B surface antigen (HBsAg) and a lipid bilayer have been developed as human liver-specific nanocapsules for *in vivo* DDS. PHA-BNCs systemically targeted mouse xenograft, which could accumulate in ß1-6 GlcNAc-expressing malignant tumors. PHA-BNCs were able to deliver DNA to malignant cancer cells. This study opens usefulness of L -PHA lectin as a targeting agent in DDS, and of PHA-BNCs as a nano-device for tumor-specific bioimaging[110].

Lectin-grafted Formulations

Carbohydrate-mediated biorecognition of lectins, as mucoadhesion, cytoadhesion, and/or cytoinvasion, might be advantageous for drug delivery to small intestine[111]. Two approaches to drug carrier formulations are basically pursued (Fig. 3): a) preparation of prodrugs consisting of lectin as glycotargeting moiety, drug as active ingredient, and spacer as a link; and b) development of lectin-grafted carrier systems. A reservoir such as microparticles, nanoparticles or liposomes contains drug and lectins are immobilised at outer surface of reservoir. Lectin should help to guide drug container to site of absorption getting closer to the sight of imagination. Both cell types, enterocytes and M-cells, are involved in transcytosis of particulate matter. Thus, this pathway for drug delivery can be extended by use of lectins[112].

a) Lectin-grafted Prodrug

In prodrug design, drug (doxorubicin) was coupled to WGA by a cis-aconityl spacer. Targeting effect of

colon-cancer directed prodrug derives from both high WGA-binding capacity of colon-cancer cells and release of cytostatic agent not until reaching acidic lysosomal milieu of target cell. To get evidence if lectin-mediated cytoadhesion and cytoinvasion can facilitate absorption of proteins, fluorescent labelled BSA was coupled to WGA via stable amide bonds (Fig. 3A). WGA was found to mediate cellular uptake of even high molecular weight proteins. But cut-off for uptake of proteins was still higher since IgG was also transported into cells by active transport mechanisms after conjugation to WGA, showing that membrane barrier can be surmounted with the help of lectins. Diverse strategies like incorporation of enzyme inhibitors or shielding by matrix systems can solve the problem[112]

b) Lectin-grafted Carrier Systems

Contrary to prodrugs, it is possible to achieve therapeutic levels of drugs by administration of microparticles, nanoparticles or liposomes (Fig. 3B). Matrix of drug carrier systems, which is preferably biocompatible and biodegradable, renders controlled release of drugs possible. Incorporation in appropriate carrier systems not only protects drugs against enzymic and acidic degradation in intestine but also affords an increase in payload. Decoration of drug carrier system with lectins will enrich drug on glycosylated surfaces of gastrointestine (GI). Tomato lectin, useful for drug delivery as well as for oral vaccination, exhibits adjuvant activity by priming systemic and mucosal immune responses[112]. In such an approach, coupling of lectins to NPs led to higher transcytosis than that of free lectins. Moreover, rate of transcytosis increased with lectin density on surface of NPs, which open a trafficking route different to that of free lectin. Characterisation of interaction between colloidal carriers and cells is most important for *in vitro* evaluation of lectinised nanospheres. Cytoassociation rate strongly depends on orientation of device and agitation of suspension[112].

Bioadhesion via lectins may be applied not only for GI tract but also for other biological barriers like nasal mucosa, lung, buccal cavity, eye and blood-brain barrier. Entering vesicular pathway by receptor mediated endocytosis part of conjugated drug is accumulated within lysosomes. Part of the drug is supposed to be transported across epithelium. As exemplified by lectin-grafted prodrug and carrier systems, this strategy is expected to improve absorption and probably bioavailability of poorly absorbable drugs, peptides and proteins as well as therapeutic DNA[112]. Conjugation of WGA onto PLGA nanoparticles effectively improved intestinal absorption of TP5 due to specific bioadhesion on GI cell membrane[113].

Plant lectins, specific for complex glycosyl side chains such as *P. vulgaris* agglutinin from red kidney beans and lectin from *Robinia pseudoacacia*, strongly and reversibly bound to villous and crypt epithelia as well as M-cells followed by high rates of endocytosis and transcytosis[57]. In comparison to *P. vulgaris* agglutinin and lectin from *R.. pseudoacacia*, lectin from snowdrops and other mannose-specific lectins exhibited only slight binding to jejunal epithelial cells and moderate binding to M-cells[114]. It appears that multivalency enhancement/ cluster effect plays a significant role in binding events. Binding of glycodendrimers to fluorescent-labelled ConA was small, in agreement with its widely spaced binding sites, whereas it was large for GNA, with its 12 more closely spaced binding sites. Dendrimer-fitted chip represents a valuable screening tool for multivalency effects[115].

c) Bipartite Drug-delivery System

Robinson *et al*[116] described a bipartite DDS that exploits (i) endogenous carbohydrate-lectin binding to localize glycosylated enzyme conjugates to specific, predetermined cell types followed by (ii) administration of a prodrug activated by that predelivered enzyme at desired site. Combined *in vivo* and *in vitro* techniques demonstrated successful activation of a-L-rhamnopyranoside prodrug. Competition experiments revealed enhanced, specific and a strongly carbohydrate-dependent, 60-fold increase in selectivity towards target cell hepatocytes that generated >30-fold increase in protein delivered. Therapeutic effectiveness of lectin-directed enzyme-activated prodrug therapy was shown by construction of doxorubicin prodrug, Rha-DOX, and its application to reduce tumor burden in a hepato-carcinoma model[116].

Toxicity of Plant Lectins – Boon or Bane?

DDSs that are based on interaction between carbohydrates and exogenous lectins are directed to whole organs[49], and can be harmful to normal tissues, unless thoroughly evaluated. Lectin from red kidney beans causes diarrhea, malabsorption, and growth reduction; but it also stimulates overgrowth of mannose-sensitive *E. coli*. Kidney bean lectin-induced *E. coli*

overgrowth in small intestine is blocked by *Galanthus nivalis* (Snowdrop) (GNA), a mannose-specific lectin[114]. On the other hand, lectins such as tomato lectin and WGA are considered safe in man. Lectin from *Salanum tuberosum* (potato) and *helix pomatia* (edible snail) showed no evidence of acute irritancy when administered intradermally in rabbits[117]. However, when cytotoxicity of lectin to cells derived from oral mucosa was considered, cytotoxicity was evident for most of the lectins, particularly at higher concentrations and over longer period[118].

With few exceptions, toxicity of lectins has been observed when used in milligram quantities, whereas glycotargeting requires lectin amount in micrograms[118] and can be exploited in cancer therapy. ConA is highly toxic to animals and ConA-induced hepatitis has been used as a model of T cell-mediated liver injury, in which IFN-γ plays an essential role by inducing apoptosis of liver cells. Though, hepatitis occurring after ConA administration in mice is thought to be a T lymphocyte-mediated disease, neutrophils are key initiators of lymphocyte recruitment and liver injury caused by ConA[119]. Since ConA stimulates IFN-γ production from liver natural killer T (NKT), stimulation of IFN-γ from liver NKT cells can display an antitumor effect in liver without liver injury. Therefore, a nonhepatotoxic low dose of ConA might serve as an immunomodulator that can preferentially activate innate immune NKT cells to induce an antitumor effect against metastatic liver tumor. Mistletoe extracts, rich in mistletoe lectins, are under clinical trials for cancer therapy and offer better opportunities for drug carriers and for therapeutic applications[120]. Lectins from *C. ensiformis* bind most intensely to metastatic tumors of lymph nodes relative to non-metastasising cells. Lectins from *T. vulgaris*, *C. ensiformis, and P. vulgaris* are inhibitory to *in vitro* proliferation of melanoma cells. Therefore, cytotoxic lectins can be used as drug carriers with beneficial effects in cancer[21].

Anti-viral Properties of Mannose-Binding Plant Lectins

Plant lectins [*Galanthus nivalis* (GNA) and *Hippeastrum hybrid* (Amaryllis) (HHA)] selectively inhibit a wide variety of HIV-1 and HIV-2 strains and clinical isolates in different cell types, and also efficiently inhibit infection of T lymphocytes by a variety of mutant virus strains. GNA and HHA are non-cytotoxic, antimetabolically active, or mitogenic to human primary T lymphocytes at concentrations that exceed their antivirally active concentrations by 2-3 orders of magnitude. GNA and HHA are heat stable at 50°C, can be easily formulated in gel preparations for microbicidal use, and non-toxic to mice on i.v. administeration[122].

Mannose-specific lectins from *Cymbidium hybrid* (CA), *Epipactis helleborine* (EHA) and *Listera ovata* (LOA) are highly inhibitory to HIV-1, HIV-2 and simian immunodeficiency virus (SIV) in MT-4, and show anti-human cytomegalovirus (CMV), respiratory syncytial virus (RSV) and influenza A virus activity in HEL, HeLa and MDCK cells, respectively. CA and EHA are potent inhibitors of syncytium formation between persistently HIV-1- and HIV-2-infected HUT-78 cells and $CD4^+$ Molt/4 cells[122]. Initially, it was thought that the mannose-specific plant lectins (*Galanthus, Hippeastrum, Narcissus, Epipactis helleborine,* and LOA), and N-acetylglucosamine-specific lectin from *Urtica dioica* would primarily be targeted at virus-cell fusion process. However, later studies suggested that mannose specific lectins interfere with an event in HIV replicative cycle that is subsequent to fusion process[122].

Conclusions

Carbohydrates are involved in a vast array of biological processes. This led to develop therapies such as targeting cells for drugs and genes, and development of vaccines through endocytic cells. To fully exploit these opportunities, it is essential to develop efficient methods for synthesis of carbohydrates. With development of newer mannosylated glycopolymers, it will be simpler to develop newer strategies to further glycotargeting and drug therapies.

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