Carcinoma-associated fucosylated antigens are markers of the epithelial state and can contribute to cell adhesion through CLEC17A (Prolectin)

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Abstract

One of the critical steps in cancer progression is the epithelial to mesenchymal transition (EMT), through which cancer cells lose their polarity, detach from their original tissue and acquire invasive properties. This process is reversible and cells need to go through a mesenchymal to epithelial transition (MET) to colonize ectopic tissues. Many glycosylation alterations have been identified in cancer cells, which, in particular, affect fucosylated and sialylated motifs found at the termini of N-linked, O-linked or lipid-linked glycan chains.

FACS analysis on mammary cell lines with a panel of antibodies and bacterial lectins revealed that expression of some fucosylated antigens (e.g.: Lewis Y) decreases during EMT. Moreover, we showed that these changes could be monitored by staining cells with the fucose-specific bacterial lectin BC2L-C-Nt. Interestingly, the ability to distinguish epithelial and mesenchymal cells did not seem to be a general property of fucose binding lectins. Furthermore, gene expression analysis demonstrated that the FUT1 and FUT3 fucosyltransferase genes, although associated with poor prognosis when overexpressed, were down-regulated in mesenchymal cells.

We then looked for endogenous lectins of the metastatic micro-environment, susceptible to interact with fucosylated antigens upon their re-expression following MET. CLEC17A, also called Prolectin, is a human fucose/mannose specific C-type lectin expressed on B cells of the lymph node germinal centers, that shares ligand specificities with BC2L-C-Nt. Accordingly, we showed that Prolectin also bound preferentially to epithelial rather than to mesenchymal cells, although the difference was not as marked as with BC2L-C-Nt, due to the mannose recognition of Prolectin. Using microfluidic experiments, we showed that Prolectin behaves as a cell adhesion molecule. Adhesion of cells to Prolectin under shear flow conditions was found to be mainly
dependent on the expression of fucosylated rather than of mannosylated ligands. Consistently, cells in the epithelial state adhered to a Prolectin coating much more efficiently than cells in the mesenchymal state. Furthermore, expression of Prolectin ligands was increased in tumors in comparison to healthy tissue and analysis of paired primary tumors/lymph node metastases showed selected increase in metastasis. Based on these results, we suggest a model in which invasive cancer cells infiltrating into the lymph node would increase their fucosylated ligands expression as they return to a more epithelial phenotype through MET. The epithelial state-associated fucosylated structures would thus enable carcinoma cells to use Prolectin as a colonization factor in lymph nodes, thereby contributing to the metastasis process.
Exploring glycosignatures of nontypeable \textit{Haemophilus influenzae} and recognition by human surfactant protein D

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Keywords: NTHi, lipooligosaccharide, bacteria microarrays, QCM, SP-D.

Abstract

Bacterial surfaces are coated with distinct carbohydrate structures that are targeted by host receptors for triggering defence responses. Therefore, typification of bacterial glycosignatures may be of particular relevance when characterising clinical isolates or mutant libraries, especially in order to ascertain virulence correlations. Recently, we have established a proof-of-concept for the use of designer microarrays in the exploration of glycosylation motifs of the bacterial surface, using \textit{Klebsiella pneumoniae} as model\textsuperscript{1}. This human pathogen presents a capsular polysaccharide and a lipopolysaccharide (comprising lipid A, a core oligosaccharide, and an O-polysaccharide chain), which are important mediators of bacterial virulence and can elicit strong immune responses. Other Gram-negative bacteria are nonencapsulated and/or do not present O-antigen-containing lipopolysaccharides. This is the case for nontypeable \textit{Haemophilus influenzae} (NTHi), an opportunistic nonencapsulated pathogen frequently isolated from adult and pediatric patients and responsible for causing otitis media, sinusitis, conjunctivitis, pneumonia, and even invasive infections. NTHi synthetises lipooligosaccharide (LOS) molecules containing only lipid A and a branched oligosaccharide core. In this work, we have examined the recognition of NTHi by different lectins. A library of mutant strains of the clinical isolate 375 lacking specific glycosyltransferases involved in LOS synthesis was generated and evaluated, together with other clinical isolates from different origin. LOSs of interest were isolated, quantified, and immobilised in the microarrays alongside the corresponding whole bacteria strains. As first step, a panel of plant lectins with well-defined oligosaccharide-binding specificities was used as pattern-reading tool. Competition assays with lectin-specific haptons served to confirm carbohydrate-dependent recognition. In addition, a real-time kinetics study was performed for selected bacteria-lectin pairs by Quartz Crystal Microgravimetry (QCM), using novel bacteria nano-chips. Both microarray and QCM techniques revealed strain- and lectin-dependent binding. Having thereby confirmed the availability of carbohydrate structures on the NTHi surface for lectin recognition, binding in the bacteria/LOS microarray set-up of human pulmonary surfactant protein D (SP-D), which is known to enhance pathogen clearance by regulating both adaptive and innate immune functions, was examined. A strain-selective recognition of the whole bacteria and of the isolated LOSs was observed, the LOS' inner core appearing as likely target. To the best of our knowledge, the results here presented provide the first experimental evidence for direct binding of SP-D to NTHi, of potential relevance for the development of novel therapeutic strategies against infections caused by this pathogen.

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Identification of a D-galactose specific lectin from blue mussel (*Mytilus edulis*) belonging to an unclassified family of lectins

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Keywords: GalNAc/Gal-specific lectin, *Mytilus edulis*, antibacterial activity

Abstract

Marine invertebrates lack adaptive defense system and so, their immune response is mainly based on cell components such as the hemocytes and a large number of proteins that are part of a humoral component. Lectins play an important role in the defense of these organisms as they have the ability to recognize surface antigens that are expressed in bacteria such as LPS (lipo-polysaccharides) and carry out agglutination mechanisms and hemolysis. Very little is known about the defense mechanisms from these organisms which are important due to commercial interests. *Mytilus edulis* is a bivalve organism native from Baja California, Mexico coasts and is commercially important, some lectins were reported in Extrapalial fluid like MeML, this lectin was suspected to play a role in the capture of food particles.

In this work a *Mytilus edulis* lectin (MEL) was purified by affinity chromatography using α-lactose-agarose resin. Molecular weight of the lectin determined by SDS-PAGE and MALDI-TOF mass spectrometry was estimated to be 17,000 Da. MEL was found to agglutinate all types of human erythrocytes together with rabbit and was not dependent on divalent cations. In hemagglutination inhibition assays, D-galactose, and N-acetyl-D-galactosamine were the most potent inhibitors among the monosaccharides tested. Out of the oligosaccharides containing non-reducing terminal D-galactose, melibiose, and raffinose were found to be strong inhibitors. On the other hand the amino-acid sequence analysis and cDNA sequence showed that MEL belongs to a new lectin family. The lectin exhibits antibacterial activity and might be involved in the recognition and antibacterial activity in the mussel.
Galectin-8 as a Modulator of Cancer Metastasis

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Prostate cancer is the second leading cause of cancer death in men worldwide. The lethal form of this disease occurs when metastatic lesions progress, most commonly to the bone. Here we show that galectin-8, known to be secreted, from prostate cancer cells, induces the expression of pro-inflammatory and pro-metastatic agents such as the chemokines SDF-1, MCP-1 and other NFkB target genes, from osteoblasts. The mechanism by which galectin-8 induces expression of SDF-1, MCP-1 and other inflammation agents in osteoblasts involves the activation of JNK and NFkB signaling pathways. Metastasis of prostate cancer cells to bone is facilitated and guided by SDF-1 and MCP-1. Indeed, we could show that migration of PC3 cells (derived from metastatic prostate cancer patient) toward osteoblasts treated with galectin-8 was significantly faster than towards naïve osteoblasts. Our findings implicate galectin-8 as a novel regulator of chemokine and cytokine gene expression in osteoblasts. Our results further suggest that galectin-8 could facilitate the metastasis process of cancer cells to the bone, by promoting chemo attraction between prostate cancer cells and osteoblasts through the SDF-1/CXCR4 and the MCP-1/CCR2 axis.
Galectin-3 Ablation Increases High-Fat Diet Induced Obesity, Liver Steatosis and Metaflammation In Adipose Tissue and Pancreatic Islets

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Keywords: Galectin-3, obesity, steatosis, metaflammation

Introduction: Increased visceral adiposity enhances the risk of insulin resistance, liver steatosis and type 2 diabetes. The mechanisms involved in obesity-associated chronic inflammation in metabolic tissues (metaflammation) that leads to insulin resistance, dysregulated glucose metabolism and increased hepatic accumulation of lipids are incompletely defined. Galectin-3 (Gal-3), a β-galactoside-binding lectin, modulates immune/inflammatory responses and specifically binds to metabolic danger molecules such as advanced glycation end products (AGE). The importance of Gal-3 in obesity-associated metabolic alterations, metaflammation and liver pathology is not fully elucidated.

Materials and methods: In order to dissect the role of Gal-3 in obesity, diabetes and fatty liver, Gal-3-deficient (LGALS3−/−) and wild-type (WT) C57Bl/6 male mice were placed on obesogenic high-fat diet (HFD, 60% kcal fat) or standard chow diet (10% kcal fat) for 3 and 6 months and metabolic, gene expression, histological and immunophenotypical analyses in metabolic tissues were performed.

Results: HFD-fed LGALS3−/− mice had higher body weight and body weight gain, amount of visceral adipose tissue (VAT), hyperglycemia, hyperinsulinemia, insulin resistance, hypercholesterolemia, hypertriglyceridemia and enhanced hepatic steatosis compared with diet-matched WT mice. Compared to WT mice, enlarged VAT in obese LGALS3−/− mice contained larger adipocytes. We demonstrate enhanced inflammation in VAT and pancreatic islets in LGALS3−/− mice fed HFD compared with diet-matched WT controls. VAT of LGALS3−/− mice fed HFD for 3 months contained more numerous Type-1 T and NKT cells, pro-inflammatory F4/80+CD11c+CD11b+ macrophages expressing NLRP3 inflammasome and IL-1β and less numerous Tregs and M2 macrophages. In contrast to WT mice, the number of CD11c+ dendritic cells and proinflammatory F4/80+high macrophages, CXCR3+ and CD8+ T cells increased in VAT of Gal-3 deficient mice after 6 months of high-fat feeding. Severe insulitis and pronounced infiltration of macrophages expressing IL-1β were observed in islets of LGALS3−/− mice after 3 months of HFD. This was accompanied with increased accumulation of AGE and receptor for AGE (RAGE) expression in islets, and elevated phosphorylated nuclear factor-κB (NF-κB) p65 and mature caspase-1 protein expression in LGALS3−/− islets and VAT. Furthermore, LGALS3−/− mice fed HFD for 6 months developed more pronounced liver steatosis which was accompanied with significantly increased pro-steatotic PPAR-γ, Cd36, Abca-1 and FAS mRNA expression in livers compared to diet-matched WT controls.

Conclusion: We provide evidence that Gal-3 ablation results in enhanced HFD-induced adiposity and inflammation in adipose tissue and islets, insulin resistance, hyperglycemia and marked liver steatosis. Thus, Gal-3 represents an important regulator of obesity-associated immunometabolic alterations.
Regulation of oxidative burst in hemocytes by a serum lectin of crayfish

C. quadricarinatus

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Keywords: CqL, Lectin receptor, oxidative burst, immune system

Abstract

In the last years, it has reported increase in pathogens that affect all crustaceans. In these organisms, it has been determinate the presence of innate immune mechanisms that defend them against infectious agents. Lectins are proteins that present high affinity recognition for carbohydrates. These molecules show a broad repertory of biological functions, they have been considered as humoral receptors for pathogen-associated molecular patterns. Nowadays, crustacean lectins present different molecular structures, and participate in all of the immune mechanisms reported so far. In the present study, we purified and characterized a serum lectin (CqL) from the hemolymph of Cherax quadricarinatus by affinity chromatography and determined its participation in the regulation of hemocytes' oxidative burst. CqL is a 290-kDa in native form, constituted by 108, 80, and 29-kDa subunits. It is mainly composed of glycine, alanine, and a minor proportion of methionine and histidine. It showed no carbohydrates in its structure. CqL is composed of several isoforms, as determined by 2D-electrophoresis, and shows no homology with any crustacean protein as determined by LC/MS mass spectrometry. CqL agglutinated mainly rat and rabbit erythrocytes and showed a broad specificity for monosaccharides such as galactose, glucose, and sialic acid, as well as for glycoproteins, such as porcine stomach and bovine submaxillary mucin and fetuin. CqL was able to bind to 8% of crayfish granular hemocytes and increased 4.2-fold the production of hemocytes' reactive oxygen species (ROS) in vitro assays when compared with non-stimulated hemocytes; moreover, production of ROS showed a decrease in presence of CqL specific carbohydrates. It suggests that the receptor in hemocytes cell membrane presents a glycosylation recognized by CqL. Superoxide dismutase and diphenyleneiodonium chloride were effective inhibitors of ROS production. The CqL receptor is a 120-kDa glycoprotein identified in the hemocytes lysate. These results allow us to suggest that CqL participates actively in the production of oxygen radicals through NADPH-dependent mechanism; and elimination of pathogens becomes more efficient in presence of this lectin. PAPIIT IN214315, IN204315 (UNAM, México), CONACyT´s scholarship (376926).
Galectin-1-producing regulatory T-cells are essential immunoescape driven by 3-MCA-induced fibrosarcoma tumor cells
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Abstract
Galectin-1 is identified capable of delineating tumor progression mainly by suppressing T cell responses. However, the importance of tumor progression versus galectin-1-producing immune cells is undefined. Here we show preliminary evidence for galectin-1 role in the expansion and immunosuppressive activity of CD4+CD25+FoxP3+ T regulatory (Treg) cells driven by 3-MCA-induced fibrosarcoma tumor cells. Wild-type mice developed tumors in galectin-1-enriched milieu, while tumors were eradicated in galectin-1-deficient (Lgals1−/−) mice. Adoptive transfer of galectin-1-producing splenocytes into Lgals1−/− mice following by i.f.p injection of tumor cells induced tumor progression and adoptive transfer of galectin-1-deficient splenocytes into irradiated wild-type mice inhibited the tumor growth. In a search for immune population-producing galectin-1 responsible for the tumor development in Lgals1−/− mice, we found TregGal1+ cells augmenting tumor growth but not galectin-1-deficient Treg (TregGal1−) cells. Injection of recombinant galectin-1 (rGal-1) into the peritoneum of Lgals1−/− mice revealed high incidence of tumor growth as revealed in wild-type mice. As well, spleens and local tumors doubled the number of CD4+CD25+FoxP3+ cells in wild-type and rGal-1-injected Lgals1−/− mice 8 and 14 days after tumor injection relative to Lgals1−/− mice. Comparable number of DX5+, CD4+, and CD8+ cells was counted in both mouse strains and in the rGal-1-treated Lgals1−/− mice. Moreover, high concentration of TGFβ was measured in blood of wild-type and rGal-1-injected Lgals1−/− mice 8 days after tumor injection relative to Lgals1−/− mice, while secretion of other cytokines, IL-2, IL-10, IFNγ was not changed. Our preliminary findings demonstrated an essential role for Treg-endogenous galectin-1 in tumor immunoescape.
Deciphering host-pathogen interactions using mammalian lectin microarrays

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Keywords: Pathogen, commensal, microarray, PAMPS, pathogen recognition receptors.

Abstract

Lectin and glycan microarrays are increasingly utilised in glycoscience to study important biological interactions involving carbohydrates. The low sample and probe consumption combined with high data yield potential makes these platforms highly desirable. Although mammalian lectins are of great importance in development, inflammation and disease processes, their use as probes on a microarray platform has been a slow development due to their natural instability, scarcity and narrow functional parameters. To address this gap in glycobiological research, we immobilised a panel of selectins, siglec and pattern recognition receptors (C-type innate immune lectins) onto functionalised glass slides. A number of technical considerations designed to maintain the function of the mammalian lectins on the microarray were tested and optimised including print buffers, incubation buffers and storage conditions. Carbohydrate binding was assessed using relevant glycoproteins and neoglycoconjugates and binding inhibition was performed with mono- and oligosaccharides.

The interactions of clinical and laboratory strains of a panel of gastrointestinal tract-associated pathogenic and commensal bacterial strains, in addition to a number of clinically relevant Staphylococcus aureus strains, were profiled on the novel microarray to examine potential pathogen-associated molecular patterns (PAMPS) and associate these with host infection responses. The interactions of exosomes secreted by the pathogenic fungus Paracoccidioides brasiliensis with potential host receptors were also examined. Overall, several interactions were correlated with literature and novel pattern recognition receptor interactions were also revealed. The mammalian lectin microarray platform will accelerate research into host-microbial interactions and help shed light on immune responses.
Generation of tailored engineered mammalian lectins, for on demand use and application

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Keywords: mammalian, lectin, tailored, on-demand, application

Abstract

Pattern recognition receptors (PRRs), lectins of the innate immune system, recognise a wide variety of pathogen-associated molecular patterns (PAMPs) and can trigger immune response. Unlike plant and bacterial lectins, Mammalian lectins have not been widely utilised to date, due to their limited availability, expense and associated issues of stability and presentation/format (labelled, in suspension or surface bound) for use. Therefore, a need for the generation of these mammalian lectins, with reduced cost, increased availability and overcoming issues such as stability and presentation for application need to be addressed. To address these needs, we have set about generating on-demand mammalian lectins, produced at point of use.

Utilising molecular engineering approaches, we have synthesized tailored gene encoding constructs based on reported mammalian lectins. This expression of fusion-tagged mammalian lectin permits a wide range of existing secondary reagents i.e. labelled antibodies, streptavidin-biotin complexes, functionalized surfaces and beads to be utilized for detection, visualization and quantification. Expression and functional activity, of the lectins tested to date, have demonstrated the encoded lectins to be correctly presented, being produced rapidly at the point of use and easily purified. Currently, we are investigating both in suspension and surface presented expression and assay formats.
CD33-related Siglecs in Primates Reveal Evolutionary Arms Race

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Keywords: Sialic acids, N-acetylneuraminic acid, N-glycolylneuraminic acid, Non-human primates, Innate Immunity.

Abstract

All living cells are covered with a dense coat of sugar chains (glycans), and every organism has a unique and characteristic pattern in this cloak. In mammals, the tips of these sugar chains carry acidic sugars (Sialic acids). Siglecs are sialic acid-binding Ig-like lectins that recognize sialoglycans via amino-terminal V-set domains. CD33-related Siglecs (CD33rSiglecs) on innate immune cells recognize endogenous sialoglycans as “self-associated molecular patterns” (SAMPs), thereby dampening unwanted immune responses against one’s own cells via cytosolic ITIM motifs that recruit tyrosine phosphatases. However, certain pathogenic bacteria take advantage of this phenomenon and mask themselves with these very same mammalian sugars, in order to evade the host immune system through molecular mimicry. Meanwhile other pathogens like influenza viruses are using mammalian sialic acids as binding targets for attack. In response to all these pressures, the mammalian sialic acids and receptors continually evolve, to evade the pathogens that exploit sialic acids as invasion targets. This whole cycle would start all over again when the pathogens then adjust further. We hypothesized that these opposing selection forces have accelerated CD33rSiglec evolution. We used various experimental approaches to demonstrate the rapid evolution of these CD33rSiglecs at the functional level. We address this by comparative analysis of major CD33rSiglecs (Siglec-3, -5, and -9) orthologs in humans, chimpanzees and baboons. Recombinant soluble molecules displaying ligand-binding domains show marked quantitative and qualitative interspecies differences in interactions with strains of the sialylated pathogen group B Streptococcus, and with sialoglycans presented as gangliosides or in the form of sialoglycan microarrays, including variations such as N-glycolyl and O-acetyl groups. Primate Siglecs also show quantitative and qualitative intra- and inter-species variations in expression patterns on leukocytes, both in circulation and in tissues. Taken together our data explain why the CD33rSiglec-encoding gene cluster is undergoing rapid evolution via multiple mechanisms, driven by the need to maintain self-recognition by innate immune cells, while escaping two distinct mechanisms of pathogen subversion.