The 15th Symposium
of Japan Consortium for Glycobiology and Glycotechnology

Expanding of Glycoscience Research:
From basic and applied biology to health and medical applications

October 26-27, 2017
at Centennial Hall of Faculty of Medicine, Kyushu University (Fukuoka)

Program on October 26 (Thur), 2016

Opening Address
Daisuke Kohda (Kyushu University)

Welcome Address
Naoyuki Taniguchi (JCGG President)

On behalf of the Japan Consortium for Glycoscience and Glycobiology (JCGG) I wish to welcome all of you who are joining the 15th JCGG symposium. This year Dr. Daisuke Koda, Distinguished Professor at the Medical Institute of Biological Regulation, Kyushu University, has kindly accepted the meeting chair and organized this meeting entitled "Expanding of Glycoscience research: From basic and applied biology to health and medical science." This is the first JCGG symposium to be held in the Kyushu area.

Dr. Koda is an eminent scientist and a leader for the Grant-in-Aid for Scientific Research on Innovative Areas entitled "Novel measurement techniques for visualizing 'live' protein molecules at work".

Glycoscience is now aiming to continue to develop from basic science to applied science and combine with other fields of research and it is essential for us to accept that glycoscience will progress from fundamental science to applied science. Dr. Koda has actually focused on this issue. In this symposium Dr. Branch Moody, Brigham and Women's Hospital, will present a talk on lipidomics regarding T cell and glycolipid and the main themes in this symposium are the following.

Interactions between glycans and microorganisms, especially infectious disease and immune systems. Structural Biology on glycan related proteins. Glycan, food and industrialization. Nanomedicine and Polysaccharide materials. Dr. Koda has planned excellent programs as above in collaboration with many professors and scientists who are working in the Kyushu area such as Drs. Kaoru Takegawa, Makoto Ito, Yoshikatsu Sumita, Yasuo Suda and Kazuo Sakurai.

Recently Glycoscience in the US is now planning to assemble glycoscientist

groups in Harvard University and CCRC at the University of Georgia. In Japan it is noteworthy that Gifu University has launched a Center for the Highly Advanced Integration of Nano and Life Science (Director, Dr. Hideharu Ishida), and many distinguished scientists including glycoscientists will participate in the center as principle investigators. Although recently in Japan most of the universities and institutes have the problem of limited fund and budgets, we admire this effort and think that it will be promising and exciting for our glycocommunity. In this occasion I would like to offer a short explanation of the JCGG organization. The JCGG was originally started in 2003 after extensive discussions in working group of Japanese Society for Carbohydrate Research with the late Dr. Yoshitaka Nagai, Drs. Tomoya Ogawa, Shoichi Kusumoto, Sumihiro Hase, Hironobu Hashimoto, Toshisuke Kawasaki, Akemi Suzuki, and Hisashi Nariamtsu and others and finally the GCGG launched.

Our JCGG is characterized as a high activity organization with rapid decision making followed by taking action based on the discussion of board of directors or planning committee members.

However, being different from CFG (Consortium for Functional Glycomics) in the USA established by Dr. James Paulson (Scripps Res Inst) supported by NIH, our JCCG was not be able to be funded by the government. Therefore, we personally donated income derived from published books etc. and established the JCGG using these funds. The secretariat was located in the systems glycobiology group at RIKEN and we asked Mr. Keiichi Yoshida to serve as the secretary general, since he was also the secretary general for the Mizutani Glycoscience Foundation. Owing to his tremendous effort we are able to continue this consortium activity. Once a year we hold the JCGG symposium and asked the project leader funded by the government to serve as the meeting chair, and also asked several institutes or universities such as AIST, RIKEN, Osaka University, Nagoya University and Tokyo Medical Dental University to host the meeting. For the last couple of years we have published many books in both English and in Japanese. Last year we had established the Tamio Yamakawa award and selected Dr. Mark Aebi as the first awardee. This year the planning committee members of JCGG (chair, Dr. Tamao Endo) held extensive discussions concerning a new plan and asked many glycoscientists in Japan for their valuable opinions. As a result, glycoscientists in Japan are now planning to publish the Road map for Glycoscience in Japan. The road map represents contributions from many glycocommunity people in Japan (see the next page and the plenary lecture by me). This proposal contains a more integrated future concept as compared to those previously proposed that were more focused on glycoscience in future in Japan.

In the coming December, I will step down from my position as JCGG president and Dr. Tamao Endo has kindly agreed to take it over. Finally I would like to acknowledge the many glycoscientists from all over the world who generously

supported our activity, and to also thank the members of the planning committee of JCGG, the board members of JCGG, the Mizutani Glycoscience Foundation and all companies who supported our JCGG activity during the past 14 years. I also deeply thank Mr. Keiichi Yoshida and my colleagues in our group at RIKEN. Without their support JCGG would not be able to carry out many of its routine activities. I hope that JCGG will be able to continue this activity in the future.

October 25, 2017

Session 1 Interactions through Glycan Chains between Microbes and Higher Organisms

Chairs : Kaoru Takekawa and Takane Katayama

Biological roles of pyruvic acid-containing oligosaccharides in microorganisms

Kaoru Takekawa (Kyushu University)

Pyruvylation onto the terminus of oligosaccharide, widely seen from prokaryotic to eukaryotic microorganisms, confers negative charges on the cell surface and seems to be functionally similar to sialylation. However, detailed molecular mechanisms underlying pyruvylation have not been clarified well. In the fission yeast, we showed that pyruvylation negatively regulates nonsexual flocculation by capping galactose residues in N-linked galactomannan. Therefore, the fission yeast cells may possess novel recognition system for pyruvylated galactose. Pyruvate is added to the oligosaccharides of glycoproteins by the pyruvyltransferase Pvg1p, which is localized to the Golgi membrane. We determined the crystal structure of fission yeast pyruvyltransferase Pvg1p. The folding pattern of Pvg1p resembled that of the type-B glycosyltransferase, including sialyltransferases. By combining molecular modeling with mutational analysis of active site residues, we obtained a Pvg1p mutant ($Pvg1p^{H168C}$) that efficiently transferred pyruvyl moiety onto a human-type complex glycopeptide. The resultant pyruvylated human-type complex glycopeptide recognized similar lectins on lectin arrays as the α2,6-sialyl glycopeptides.

References

1) Yoritsune A, Matsuzawa T, Ohashi T, Takegawa K: FEBS Lett 587, 913, 2013

2) Matsuzawa T, Morita T, Tanaka N, Tohda H, Takegawa K: Mol Microbiol 82, 1531, 2011

3) Higuchi Y, Yoshinaga S, Yoritsune K, Tateno H, Hirabayashi J, Nakakita S, Kanekiyo M, Kakuta Y, Takegawa K: Sci Rep 6, 26349, 2016

Symbiosis and co-evolution between humans and bifidobacteria, driven by human milk oligosaccharides

Takane Katayama (Kyoto University)

Lactation is a common feeding strategy of eutherian mammals, but its functions go beyond feeding the neonates. Ever since Tissier isolated bifidobacteria from the stool of breast-fed babies, human milk has been assumed to contain compounds that selectively stimulate the growth of bifidobacteria in infant intestines. Predominance of bifidobacteria in the infant gut ecosystem continues during breast-feeding; however, the population drastically decreases after weaning. We have revealed that infant gut-associated bifidobacteria are equipped with a series of genetic and enzymatic sets dedicated to assimilation of oligosaccharides contained in milk (human milk oligosaccharides: HMOs). Among different gut microbes, occurrence of HMOs-degrading enzymes is essentially limited to infant gut-associated bifidobacteria. These results suggest that HMOs serve as selected nutrients for bifidobacteria in the gut. HMOs have apparently no-nutritional value for infants even though they are included as the third most abundant component in milk; nonetheless, mothers produce HMOs in mammary gland at the expense of great energy. In this talk, I shortly summarize our previous studies on functional and structural analysis of bifidobacterial enzymes involved in HMOs assimilation, and then show some recent results of microbiota analysis on infant stools. Finally, based on those findings, I suggest the co-evolution between bifidobacteria and humans, driven by HMOs.

References

1) Katayama T: Biosci Biotechnol Biochem 80, 621, 2016

2) Sakurama H, Kiyohara M, Wada J, Honda Y, Yamaguchi M, Fukiya S, Yokota A, Ashida H, Kumagai H, Kitaoka M, Yamamoto K, Katayama T: J Biol Chem 288, 25194, 2013

3) Yamada C, Gotoh A, Sakanaka M, Hattie M, Stubbs KA, Katayama-Ikegami A, Hirose J, Kurihara S, Arakawa T, Kitaoka M, Okuda S, Katayama T, Fushinobu S: Cell Chem Biol 24, 515, 2017

Structure-function relationships of a new family of β-mannosyl-transferases from Candida pathogenic yeasts

Yann Guérardel (Université de Lille)

The yeast *Candida albicans* belongs to the human commensal digestive and vaginal flora. Nevertheless, immunocompromised patients may develop systemic infections by *C. albicans* that are characterized by high morbidity and mortality. As is in other yeasts, the *C. albicans* cell wall is a complex structure containing a network of β-1,3 and β-1,6 glucans, surrounded by a mannose-rich glycoconjugate

outer layer mainly composed of PPM (phosphopeptidomannan; sometimes referred as 'mannan') and mannoproteins. The pioneering work of Shigeo Suzuki's research group from Tohoku Pharmaceutical University established that *C. albicans* expresses β-1,2-oligomannosides (β-Mans) at the surface of its cell wall, in contrast to most non-pathogenic yeast species such as *Saccharomyces cerevisiae* [1]. These β-Man are found in the most pathogenic Candida species (primarily *C. albicans*, but also in non-albicans species such as *C. glabrata* and *C. tropicalis*) and are considered as virulence factors. Indeed, they were shown to display stronger antigenicity than α-linked oligomannosides during the humoral antibody response of mammals and promote fungal adherence to epithelial cells [2]. Moreover, the role of β-Mans as adhesins for *C. albicans* has been established in a number of *in vitro* and *in vivo* model systems.

During this presentation, I will recapitulate our on-going efforts to characterize both structure and functions of enzymes responsible for the biosynthesis of β-1,2-oligomannosides in *C. albicans*. They started in 2008 with the identification of a family of nine genes, named CaBMT1-9, that encode for putative β-1,2-mannosyltransferases (Bmts) [3]. The aim of our studies is to characterize CaBmts activities in order to better understand the biosynthetic pathways of β-Mans and the catalytic mechanism of each enzyme, with the longer term objective to guide the design of putative inhibitors. In particular, the availability of well-defined enzymes will permit us to assay the inhibitory activities of a library of synthetic carbohydrates toward individual steps of β-mannosylated oligosaccharides biosynthesis. Here I will focus on the analysis of CaBmtl, CaBmt3 and CaBmt4 that were shown to sequentially prime, elongate and polymerize β-1,2-oligomannosides on α-1,2-oligomannosides. Indeed, using naturally derived and synthetic oligomannosides, we have identified the precise acceptor substrates and characterized the enzymatic parameters of individual enzymes. Then, combining small angle X-ray diffraction (SAXS), in silica molecular modelization and site-directed mutagenesis of recombinant proteins, we propose a structural and catalytic model of CaBmts.

References

1) Kobayashi H, Shibata N, Nakada M, Chaki S, Mizugami K, Ohkubo Y, Suzuki S: Structural study of cell wall phosphomannan of Candida albicans NIH B-792 (serotype B) strain, with special reference to ^{1}H and ^{13}C NMR analyses of acid-labile oligomannosyl residues. Arch Biochem Biophys 278, 195-204, 1990

2) Miyakawa Y, Kuribayashi T, Kagaya K, Suzuki M, Nakase T, Fukazawa Y: Role of specific determinants in mannan of Candida albicans sero-type A in adherence to human buccal epithelial cells. Infect Immun 60, 2493-9, 1992

3) Mille C, Bobrowicz P, Trinel PA, Li H, Maes E, Guerardel Y, Fradin C, Martinez-Esparza M, Davidson RC, Janbon G: Identification of a new family of genes involved in β-1,2-mannosylation of glycans in Pichia pastoris and Candida albicans. J Biol

Chem 283, 9724-36, 2008

Session 2　Complex Glycans and Infection
Chairs : Sho Yamasaki and Daisuke Kohda

Fungal strategies for escaping from host immune system: Novel functions of glycolipid hydrolases
Makoto Ito (Kyushu University)

Endoglycoceramidase (EGCase) is an enzyme capable of cleaving the glycosidic linkage between oligosaccharides and ceramides of various glycosphingolipids[1]. We found two homologues of EGCase in *Cryptococcus neoformans* and named EGCrPl and EGCrP2, which are the first identified glucocerebrosidase[2] and sterylglucosdase [3], respectively, in fungi. EGCrPl is responsible for removing immature glucosylceramides (GlcCer) by selective hydrolysis of immature one during the maturation step of GlcCer. This step is deeply related to the capsule formation in *C. neoformans*[2]. On the other hand, disruption of EGCrP2 gene accumulated ergosterylglucoside (EG) in *C. neoforma* [3] and reduced the pathogenicity to mice and survival in mice. Thus, we examined whether EG can activate the host innate immune system. The author will report in this symposium that EG activates a C-type lectin coupled to γ chain of Fc receptor and stimulates the IL8 production in human monocyte-derived dendritic cells. Collectively, we identified two EGCase homologues which are involved in the capsule formation and degradation of immunostimulatory glycolipids in pathogenic fungi. These results may indicate the novel functions of fungal glycolipid-degrading enzymes which function for escaping fungi from host innate immune system.

References

1) Ito M, Yamagata T: J Biol Chem 261, 14278-82, 1986
2) Ishibashi Y et al.: J Biol Chem 287, 368-81, 2012
3) Watanabe T et al.: J Biol Chem 290, 1005-19, 2015

Complement and inflammasome activation caused by GPI-anchor dysregulation
Taroh Kinoshita (Osaka University)

More than 150 different proteins are anchored to the outer leaflet of plasma membrane via glycosylphosphatidylinositol (GPI). GPI is synthesized in the endoplasmic reticulum and attached by transamidation to the C-terminus of precursor proteins bearing a C-terminal GPI-attachment signal sequence. When GPI is not attached, the precursor proteins are degraded or secreted, resulting in GPI-

anchored protein deficient cells. GPI deficiency causes paroxysmal nocturnal hemoglobinuria (PNH). PNH is an acquired disorder of hematopoietic stem cells. Somatic mutation in X-linked PIGA gene caused GPI-deficiency. Two complement regulatory proteins, CDSS and CD59, are GPI-anchored and PIGA- defective red cells are highly sensitive to complement, leading to hemolysis when complement is activated. Recently, atypical PNH, in which GPI-deficiency was caused by a combination of germline and somatic loss of PIGT gene, was found. PIGT-defective PNH was associated with inflammatory symptoms such as recurrent urticaria, joint pain and non-infectious meningitis, suggesting inflammasome activation. PIGA acts at the initial step in GPI biosynthesis whereas PIGT acts in attachment of preassembled GPI to proteins. It seems therefore that PIGA-defective cells do not generate any GPI intermediate but that PIGT-defective cells accumulate free GPI that is not used as a protein anchor, suggesting a causal relation to inflammation.

References

!) Kinoshita T, Fujita M: Biosynthesis of GPI-anchored proteins: special emphasis on GPI lipid remodeling. J Lipid Res 57, 6-24, 2016

2) Krawitz PM, Hochsmann B, Murakami Y, Teubner B, Kriiger U, Klopocki E, Neitzel H, Hollein A, Parkhomchuk D, Hecht J, Robinson PM, Mundlos S, Kinoshita T, Schrezenmeier H: A case of paroxysmal nocturnal hemoglobinuria (PNH) caused by a germline mutation and a somatic mutation in PIGT. Blood 122, 1312-5, 2013

Glycoconjugates from cell surface: Analysis of their immune balance modulation with chemically synthesized compounds

Yukari Fujimoto (Keio University)

Cell surface glycolipids often induce immunomodulatory activities by the recognition of innate immune receptors or antigen-presenting proteins. In order to understand and to modulate the NKT-cell-mediated cytokine induction via CD1d-ligand recognition, we have synthesized natural compounds from microbes, and also designed compounds to control the binding affinity of the ligands to enhance and to modulate the selectivity. One series of the target natural compounds are inositol phospholipids as the partial structures of GPI anchor proteins. As one of unique microbial GPI-anchor type inositol phospholipids, we synthesized protozoan glycoconjugates, and observed their cytokine inductions including INF-y and IL-4[1]. On the other hand, we have also designed and synthesized a series of lipid conjugates as the CD1d ligands that have special affinity in the binding site of the CD1d, for modulation of the NKT-cell-mediated cytokine induction. Namely, we designed the ligands that can interact with a few polar residues deep inside of a large hydrophobic binding pocket of CD1d, with introducing a polar functional group such as an amide, as the "anchor" in the long fatty acyl chain of α-GalCer. The

biological activities and the molecular dynamics calculations will be also discussed for further understanding of the binding mode of glycolipids in the receptor proteins[2].

References.

1) (a) Aiba T, Suehara S, Choy SL, Maekawa Y, Lotter H, Murai T, Inuki S, Fukase K, Fujimoto Y: Chem Eur J 23, 8304, 2017. (b) Choy SL, Bernin H, Aiba T, Bifeld E, Lender SC, Miihlenpfordt M, Noll J, Eick J, Marggraff C, Niss H, Roldan NG, Tanaka S, Kitamura M, Fukase K, Clos J, Tannich E, Fujimoto Y, Lotter H: Sci Rep 7, 9472, 2017
2) Inuki S, Aiba T, Hirata N, Ichihara O, Yoshidome D, Kita S, Maenaka K, Fukase K, Fujimoto Y: ACS Chem Biol 11, 3132, 2016

Immune regulation of bacterial glycolipids via C-type lection receptors

Sho Yamasaki (Osaka University)

C-type lectin receptors (CLRs) comprise a large family of receptors that bind to carbohydrates in a calcium-dependent manner. Recent studies have shown that some CLRs are coupled with activating motifs and act as pattern recognition receptors (PRRs) against pathogens. We found that Macrophage-inducible C-type lectin (Mincle, Clec4e) is an FcRγ-coupled activating receptor that recognizes mycobacterial glycolipid, trehalose dimycolate, to promote innate and acquired immunity. Related CLRs, such as MCL (Clec4d), Dectin-2 (Clec4d) and DCAR (Clec4bl), also recognize characteristic mycobacterial glycolipids and mediates their adjuvanticity. In addition to pathogen-derived glycolipids, we recently found that an intracellular metabolite, β-glucosylceramide, released from damaged cells activates myeloid cells via Mincle. These results suggest that CLRs may act as sensors for damaged-self (DAMPs) and non-self pathogens (PAMPs) to maintain immune homeostasis. In this symposium, the physiological advantage and potential risk of the immune responses triggered by CLRs will be discussed.

References.

1) Ishikawa E, Ishikawa T, Morita YS, Toyonaga K, Yamada H, Takeuchi O, Kinoshita T, Akira S, Yoshikai Y, Yamasaki S: Direct recognition of the mycobacterial glycolipid, trehalose dimycolate, by C-type lectin Mincle. J Exp Med 206, 2879-88, 2009
2) Yonekawa A, Saijo S, Hoshino Y, Miyake Y, Ishikawa E, Suzukawa M, Inoue H, Tanaka M, Yoneyama M, Oh-Hora M, Akashi K, Yamasaki S: Dectin-2 is a direct receptor for mannose-capped lipoarabinomannan of mycobacteria. Immunity 41, 402-13, 2014
3) Toyonaga T, Torigoe S, Motomura Y, Kamichi T, Hayashi JM, Morita YS, Noguchi N, Chuma Y, Kiyohara H, Matsuo K, Tanaka H, Nakagawa Y, Sakuma T, Ohmuraya M, Yamamoto T, Umemura M, Mastuzaki G, Yoshikai Y, Yano I, Miyamoto T, Yamasaki

S: C-type lectin receptor DCAR recognizes mycobacterial phosphatidyl-inositol mannosides to promote a Th1 response during infection. Immunity 45, 1245-57, 2016

Industry-Academia Joint Session

An originally developed reagent for highly efficient preparation of exosome and its proteome analysis

Ayako Kurimoto (Miraka Co.)

Special Lecture

The surprising nature of human T cell response to glycolipids

D. Branch Moody (Brigham and Women's Hospital)

T cell recognition of glycolipids.

T cells are a mainstay of host defense because they kill extracellular pathogens and those hidden within host cells. The most extensively studied system for T cell activation involves MHC I and MHC II proteins, which differ in structure among each human [1]. MHC proteins bind and present peptide antigens to T cells, leading to the widespread belief that peptide antigens were the only natural type of antigens for human T cells (Figure 1). Accordingly, nearly all approaches to vaccination and T cell based diagnosis of disease relied on peptide biochemistry.

However, increasing evidence demonstrates that αβ T cells recognize MHC-like proteins known as CD1, which are abundantly expressed on antigen presenting cells such as dendritic cells or B cells [2]. In the human system, CD1a, CD1b, CD1c and CD1d present lipids to T cell receptors using a mechanism whereby the alkane chains are anchored within CD1, leading to the presentation of carbohydrate, phosphate or sulfate moieties to T cells (Figure 1). This discovery expands the spectrum of natural antigens for T cells to include cellular glycolipids, opening up broad new avenues into study of how lipids can activate human immune response or even serve as vaccines. This overview talk will provide new insights into the molecular mechanism by which lipids are recognized by T cells, describe the types of human and bacterial lipids presented to T cells and provide some insight into the *in vivo* and medical applications of lipids and glycolipids to immune responses.

Classical models of glycolipid recognition by T cells: the headgroup recognition model.

Until recent years, CD1-reactive T cells were known through a few clones studied in detail. For example we used a human T cell clone known as LDNS to isolate CD1b-binding mycobacterial antigen for T cells known as glucose monomycolate[3]. Structure-function studies supported by the Mizutani Foundation for Glycoscience

showed that substituting glucose with mannose or other hexose sugars completely blocked the T cell response, whereas changes in the lipid component of the antigen, did not block T cell response. This pattern of recognition was consistent with a mechanism proposed in 1997 and illustrated in in cartoon form in Figure 1[4].

Crystal structures of CD1b-lipid-T cell receptor.

However, the first crystal structure of CD1b bound to lipid and a T cell receptor (TCR) was solved in 2016 [5]. This structure reveals in detail how the TCR forms a hydrogen bonding network with the glucose moiety, which is recognized in a "tweezer" mechanism. Two follow up studies now show how CD1b presents phospholipids with small (glycerol) or larger (inositol, choline) head groups to T cell receptors (Shahine et al, Science Immunology, in press; Shahine and Van Rhijn et al, manuscript in preparation). These three CD1b-glycolipid-TCR structures will be discussed in detail in Fukuoka to highlight that TCRs, which were previously thought to solely recognize peptide antigens, bind very specifically to glycolipids and phospholipids.

In addition, the plenary talk will describe the discovery two additional lipid antigens presented by human CD1c and CD1a proteins, known as mannosyl-β1-mycoketides[7),8)] and dideoxymycobactins[9)] (Figure 2). Collectively these studies have supported molecular models for mycobacterial lipid presentation: CD1a presentation of dideoxy-mycobactin, CD1b presentation of glucose monomycolate and CD1c presentation of phosphomycoketide. These reagents have been broadly implemented by other laboratories and served as the key reagents to develop immunological detection reagents known as 'tetramers.'

Tetramers, first described in the MHC system by Altman and Davis, represent a rapid way to identify antigen specific T cells from among complex mixtures of T cells. For CD1, individual proteins are complexed into fluorescent tetramers, which are then loaded with the antigen of interest so that any cell expressing a T cell receptor that recognizes a given CD1-lipid pair will be stained. This system can be used to discover lipid-specific T cells or track antigen specific T cells in disease. Using this method, the Moody laboratory developed working tetramers of human CD1a, CD1b and CD1c proteins as summarized in Figure 2. The plenary talk in Fukuoka will discuss recently published evidence that makes clear that non-classical, lipid-specific T cells exist as polyclonal populations in human blood. In fact, initial attempts to count cells suggests that the combined numbers of T cells recognizing non-polymorphic antigen presenting molecules is similar to or might outnumber MHC-restricted T cells [6].

A broader view of cellular lipid capture by CD1 proteins.

A central unresolved question is whether the CD1 system supports broad immune surveillance of nearly all cellular lipids, phospholipids and glycolipids, or instead

focuses a few specialized lipids. Human CD1 proteins have clefts of differing volume (1250–2250 Å^3) and architecture (Figure 3) [2]. Yet, the extent to which the four human CD1 antigen presenting molecule types capture the same or different lipids is unknown. Any CD1 isoform-specific or lipid class-specific motif, equivalent to motifs that routinely guide peptide epitope mapping, are unknown. Comprehensively solving human peptide sequence motifs took decades because MHC polymorphism generates many hundreds of structurally distinct MHC grooves. Further because nearly every human expresses differing subset of MHC proteins, broadly applying binding motifs to even a single pathogen-derived or autoantigenic protein across human populations is complex or impossible. In contrast, CD1 genes are nearly non-polymorphic and no functional polymorphisms near CD1 clefts are known. Thus, the human CD1 system is expected to have four general lipid binding mechanisms, one for each isoform, which apply to nearly all humans.

Establishing a lipidomics platform.

Current evidence regarding CD1-lipid complex formation derives mostly from crystallography or binding experiments in which lipids are chosen by the experimentalist. An unbiased approach to determine the spectrum of lipids that are captured by each of the four human CD1 protein types, when exposed to the natural complement of self cellular lipids, has not been answered. Extending prior shotgun mass spectrometry methods, we developed a mammalian lipidomics platform that met strategic targets for low false positive ligand detection, separation of highly divergent compounds in a single run, semi-quantitative detection of diverse lipids, enumeration of compounds present and reliable alignment of lipid detection across multiple proteins (Figure 4). The plenary lecture will discuss lipidomic technology development.

Broad survey of cellular lipids.

This lipidomics system provides evidence for hundreds of self lipids binding to human CD1 proteins, supporting a mechanism is which the cellular lipidome undergoes broad immunosurveillance (Huang et al, manuscript in preparation). The systematic listing of named and unnamed ligands for the four CD1 proteins comprises the Human CD1-Lipid Resource (Figure 5). Using this new platform and database, analysis of head groups and anchors present in hundreds of ligands resolves controversies about whether the system captures few or many lipids. Further, the extent to which each CD1 type captures the same or differing lipids provides insight into a unique capture mechanism for CD1b proteins. Overall, these lipidomic studies provide a new perspective that the range of natural self antigens for T cells is broader than previously thought and includes many classes of self cellular lipids.

References

1) Parham P, Lomen CE, Lawlor DA, Ways JP, Holmes N, Coppin HL, Salter RD, Wan AM, Ennis PD: Nature of polymorphism in HLA-A, -B, and-C molecules. PNAS 85, 4005-9, 1988, PubMed PMID: 1540.

2) Van Rhijn I, Godfrey DI, Rossjohn J, Moody DB: Lipid and small-molecule display by CD1 and MRl. Nat Rev Immunol 15(10), 643-54, 2015, PubMed PMID: 26388332.

3) Moody DB, Reinhold BB, Guy MR, Beckman EM, Frederique DE, Furlong ST, Ye S, Reinhold VN, Sieling PA, Modlin RL, Besra GS, Porcelli SA: Structural requirements for glycolipid antigen recognition by CD1b-restricted T cells. Science 278(5336), 283-6, 1997, PubMed PMID: 9323206.

4) Moody DB, Besra GS, Wilson IA, Porcelli SA. The molecular basis of CDl-mediated presentation of lipid antigens. Immunol Rev 172, 285-96, 1999, PubMed PMID: 4839.

5) Gras S, Van Rhijn I, Shahine A, Cheng TY, Bhati M, Tan LL, Halim H, Tuttle KD, Gapin L, Le Nours J, Moody DB, Rossjohn J: T cell receptor recognition of CD1b presenting a mycobacterial glycolipid. Nat Commun 7, 13257, 2016 PubMed PMID: 27807341; PMCID: PMC5095289.

6) Godfrey DI, Uldrich AP, McCluskey J, Rossjohn J, Moody DB: The burgeoning family of unconventional T cells. Nat Immunol 16(11), 1114-23, 2015. PubMed PMID: 26482978.

7) Roy S, Ly D, Castro CD, Li NS, Hawk AJ, Altman JD, Meredith SC, Piccirilli JA, Moody DB, Adams EJ: Molecular analysis of lipid-reactive Vdelta1 gammadelta T cells identified by CD1c tetramers. J Immunol 196(4), 1933-42, 2016. PubMed PMID: 26755823; PMCID: PMC4744554.

8) Moody DB, Ulrichs T, Muhlecker W, Young DC, Gurcha SS, Grant E, Rosat JP, Brenner MB, Costello CE, Besra GS, Porcelli SA: CD1e-mediated T-cell recognition of isoprenoid glycolipids in Mycobacterium tuberculosis infection. Nature 404(6780), 884-8, 2000, PubMed PMID: 5132.

9) Moody DB, Young DC, Cheng TY, Rosat JP, Roura-Mir C, O'Connor PB, Zajonc DM, Walz A, Miller MJ, Levery SB, Wilson IA, Costello CE, Brenner MB: T cell activation by lipopeptide antigens. Science 303(5657), 527-31, 2004, PubMed PMID: 14739458.

Program on October 27 (Fri), 2016

Session 3　Glycan-related Proteins and Structure Biology
Chairs : Yoshimitsu Kakuta and Daisuke Kohda

Integrative structural biology approach to understand the structural and dynamic basis of Asn-glycosylation
Daisuke Kohda (Kyushu University)

Asparagine-linked glycosylation (N-glycosylation) is the most ubiquitous protein modification. The defining event in N-glycosylation is clearly the formation of the covalent bond between the oligosaccharide and asparagine, which is catalyzed by an integrative membrane enzyme, oligosaccharyltransferase (OST). The archaeal OST is a single subunit enzyme consisting only of the Ag1B protein, and thus suitable for structural studies. The acceptor asparagine resides in the N-glycosylation sequon (Asn-X-Ser/Thr, X≠Pro). The glycan donor is the lipid-linked oligosaccharide (LLO). An oligosaccharide chain is assembled on a lipid-phospho carrier. We pursue an integrative strategy for understanding the molecular mechanism of the N-oligosaccharyl transfer reaction. The approach includes the structural analyses of the Ag1B structures in complexes with the glycan donor and acceptor. Unfortunately, we have little knowledge of biochemistry about archaeal N-glycosylation. Thus, we perform the chemical structure determination of N-glycans and LLOs from several archaeal species by MS and NMR. Our integrative approach using crystallography and NMR, with the combination of disulfide-bond tethering technique revealed the structure and dynamics of the Ag1B protein, which is essential to achieve the efficient and accurate N-glycosylation reaction.

References.
1) Matsumoto S et al.: Proc Natl Acad Sci USA 110, 17868 - 73, 2013
2) Matsumoto S et al.: Biochemistry 56, 602-11, 2017
3) Taguchi Y et al.: J Biol Chem 291, 11042-54, 2016

Structural diversity of marine invertebrate lections
Tomomitsu Hatakeyama (Nagasaki University)

Carbohydrate-binding proteins (lectins) are known to play important roles in molecular and cellular recognition processes through specific binding to various carbohydrate chains. In recent years, marine invertebrate lectins have been found to be involved in immune systems by recognizing carbohydrate chains on foreign cells. We have purified several lectins from marine invertebrates, and found their diverse structural and functional features. Among them, three lectins, CEL-III, SPL, and SUL-I are described here to demonstrate their intriguing structural and functional

characteristics.

CEL-III purified from the sea cucumber *Cucumaria echinata* shows strong hemolytic activity by forming ion-permeable pores in target cell membrane. This lectin is composed of two N-terminal ricin-type carbohydrate-recognition domains (CRDs) and a C-terminal oligomerization domain. The C-terminal domain forms a transmembrane pore consisting of a β-barrel, induced by binding to carbohydrate chains on target cell surface, leading to hemolysis or cell death. Structural features and carbohydrate recognition mechanisms of the Ca^{2+}-independent C-type lectin SPL from the bivalve *Saxidomus purpuratus* and the rhamnose-binding lectin SUL-I from the venomous sea urchin *Toxopneustes pileolus* are also described.

Structures and mechanisms of glycosyltransferases
Yoshimitsu Kakuta (Kyushu University)

Glycosaminoglycan is a linear sugar chain in which two kinds of sugars are alternately bonded, and it exists in a wide range of species from vertebrates to bacteria. In humans, it is mainly present in the extracellular matrix, and involvement in functions such as skeletal structure formation and cell recognition and signaling has been reported. On the other hand, microorganisms are present in the capsule of certain bacteria, and it is thought that they avoid the immune response from the host bacteria and increase the pathogenicity.

Glycosaminoglycans synthesized by microorganisms have a structure similar to that of human sugar chains and enzymes responsible for their biosynthesis are known as stable and highly reactive glycosaminoglycan synthases. These enzymes can efficiently synthesize respective sugar chains by exactly alternating two sugars and successively undergo a sugar transfer reaction. However, its reaction mechanism is still unknown.

We have determined three crystal structures: Chondroitin polymerase of *Escherichia coli K4*, hyaluronic acid sugar chain synthetase of *Pasteurella multocida*, heparosan oligosaccharide synthase of *Escherichia coli KS*. These structures introduce the detailed understanding of the molecular mechanism of glycosaminoglycan sugar chain biosynthesis.

Session 4　Sugars, Food and Their Industrial Advances
Chair : Yasuo Suda

Cultivation of Okinawamozuku and production of fucoidan
Masahiko Iha (Southproducts Co.)

Okinawamozuku (*Cladosiphon okamuranus* Tokida) is a sea alga for food. It is a sea alga peculiar to Nansei Islands.

As for Okinawamozuku, cultivation technology was established in the 1970's. Nowadays 20,000 tons a year Okinawamozuku are cultivated, and it accounts for 90% of nationwide production.

Okinawamozuku has Fucoidan, a kind of polysaccharides. The structure of Fucoidan is reported by Nagaoka et al., in which basing on α-1,3 bond of fucose unit, sulfate group is bonded at 60% of C-4 in the fucose unit and glucuronic acid is bonded at α-1,2 bond of every 6 fucose units.

We focused on the Fucoidan contained in Okinawamozuku and came up to the idea that commercial production of Okinawamozuku would make it possible to develop new high odded value applications.

We have developed a production process to achieve high purity Fucoidan by extracting Fucoidan selectively and removing impurities with ultrafiltration. The Fucoidan obtained through this method is a high purity product containing Fucoidan not less than 80% and from which low molecular fucose and impurities are removed. Biological activities and the clinical effects of Fucoidan have been studied and now Fucoidan is applied to various functional foods, drink and cosmetic products.

References.

1) 当真武: 沖縄の海藻と海草. 出版舎 Mugen (2012)
2) Nagaoka M et al.: Glycoconj J 16, 19-26, 1999
3) Tokita Y et al.: Biosci Biotecnol Biochem 74 (2), 350-7, 2010

Production of sugar of high function from starch and its application to food industry

Kazuhiro Yoshinaga (Sanasu Co.) and Junichi Abe (Kagoshima University)

Glucose is an essential sugar for almost all organism as an energy source and as a constituent of the cells, and can be easily produced by the hydrolysis of starch. Contrary to the importance of glucose, the starch industry has been struggling to get its expansion and is looking for an attractive new-sugar and application. 1,5-Anhyro-D-fructose is produced by the elimination action of α-glucan lyase (EC 4.2.2.13) from the red seaweed. We developed the production system for this sugar from sweet potato starch and the enzyme from red sea-algae as the starting materials and launched the sugar as a food material to the market. This sugar has high ability of anti-oxidation and preventing colorization of food staff, therefore, this is very useful for the manufacturing of noodles and fruits paste in the food industries. Recently, we found this sugar has the growth-inhibitory effect of microorganisms. Strikingly, the sugar is very effective to the proliferation of the bacterial spore. The spore is troublesome for the food industry, because it is resistant to the sterilization by heating and to disinfectants. In this symposium, we will mention about some of the characteristics of this promising sugar and its

application to the food industry.

References
1) Yoshinaga Y, Fujisue M, Abe J, Hanashiro I, Takeda Y, Hizukuri S: Characterization of exo-(1,4)-alpha glucan lyase from red alga gracilaria chorda. Activation, inactivation and kinetic properties of the enzyme. Biochim Biophys Acta 1472, 447-454, 1999
2) 吉永一浩、石場秀人、吉元寧、片野盟彦：1,5-アンヒドロ— D フルクトースの機能研究と工業生産技術開発、日本応用糖質科学会誌，1 号，70-75, 2011
3) Izumi S, Yoshinaga K, Abe J: Growth restraint action of 1,5-anhydro-D-fructose on gram-positive bacteria, J Appl Glycosci 60, 87-93, 2013

Unbreakable ties between saccharide and shochu

Yumiko Yoshizaki (Kagoshima University)

Shochu is a Japanese traditional spirit. *Imo-shochu*, which is made mainly from sweet potato, has a sweet and rich flavor. Five monoterpene alcohols (MTA) and β-damascenone have been identified as the characteristic compounds in *imo-shochu*. These characteristic compounds are derived from sweet potato. The MTA, except for citronelool, exist as glycosides in sweet potato. It has been shown that the precursors of nerol and geraniol are β-glucoside forms and the precursors of linalool and α-terpineol are β-primeveroside forms. Although these precursors are widely distributed throughout the sweet potato, the concentration of the MTA precursor increases from the center toward the skin of the sweet potato. *Shochu* made from only the skin has a delicious, fruity, and sweet flavor. The precursor of β-damascenone has been isolated and characterized from sweet potato. It was shown that sweet potato contains the precursor of the β-acuminoside form. This precursor is not converted to β-damascenone during fermentation, but is converted during distillation at low pH and high temperature. The different flavors of *imo-shochu* are created by the unique combinations of saccharides and the *shochu* making process.

References
1) 高峯和則、吉崎由美子、山本優、吉竹一哉、橋本文雄、玉置尚徳、鮫島吉廣；日本醸造協会誌 107, 782-7, 2012.
2) Yoshizaki Y, Takamine K, Shimada S, Uchihori K, Okutsu K, Tamaki H, Ito K, Sameshima Y: J Inst Brew 117, 217-3, 2011

Luncheon Seminar (sponsored by Shimadzu Co.)

SALSA : A novel derivatization for sialyl linkage isomer discrimination by mass spectrometry
Takashi Nishikaze (Shimadzu Co.)

Ability and possibility of the microchip electrophoresis apparatus “MultiNA” in the analysis of carbohydrate chain
Mitsuhiro Kinoshita (Kinki University)

Session 5　Glycan Nanomedicines
Chair : Yoshiko Miura

Synthesis and application of sugar chain-immobilized fluorescent nanoparticles
Masahiro Wakao (Kagoshima University)

Fluorescent nanomaterials have been utilized in the fields of fundamental research and technical applications, such as solar cells, optical devises, and biochemical probes. In particular, quantum dots (QDs), semiconductor nanoparticles, exhibit strong fluorescence under UV-VIS irradiation with higher photostability than fluorescent organic molecules do. QDs are also functionalized by ligand exchange reaction with various biomolecules onto the surface. Because of these properties, they have been extensively exploited as analytical tools not only for biosensing but also for bioimaging. Focusing on good properties of QDs, we have developed sugar-chain immobilized fluorescent nanoparticles (SFNP).

In this paper, we introduce the synthesis of SFNP containing CdTe/CdS or ZnS-AgInS 2 (ZAIS)/ZnS core/shell structure as QDs and their application . Immobilization of sugar chain moiety was performed by ligand exchange reaction using ligand conjugates. The binding interaction between SFNP and carbohydrate-binding protein was visually detected by observing specific fluorescent aggregates. In addition, SFNP could be used for the detection of anti-carbohydrate antibody in sera from GBS patients. Fluorescent imaging and flow cytometry analysis were also examined, and the results clarified that the binding property of cell for SFNP is different depending on the cell type. Thus, our SFNP can be useful for diagnosis and cell typing

References.

I)　Shinchi H, Wakao M, Nakagawa S, Mochizuki E, Kuwabata S, Suda Y: Chem Asian J 7, 2678- 82, 2012

2) Shinchi H, Wakao M, Nagata N, Sakamoto M, Mochizuki E, Uematsu T, Kuwabata

S, Suda Y: Bioconj Chem 25, 286-95, 2014.
3) Shinchi H, Yuki N, Ishida H, Hirata K, Wakao M, Suda Y: PLoS One 10, e0137966, 2015

Polysaccharide-graft copolymers as biofunctional materials

Atsushi Maruyama (Tokyo Institute of Technology)

A variety of cationic graft copolymers having polysaccharide grafts and a polyamine backbone were prepared by a reductive amination reaction between reductive ends of polysaccharides and amino groups of backbone polymers. The graft copolymer having high grafting degree, form soluble inter polyelectrolyte complex with DNA. These comb-type copolymers exhibited nucleic acid chaperone-like activity to facilitate correct folding and assembling of nucleic acids. The activity of the copolymers was also useful to control ionic peptide folding and functions. Bio-specific function of the polysaccharide was employed to control the graft copolymer activities.

References

l) Maruyama A, Shimada N: Cationic graft copolymers for DNA engineering, in "Polymers for Biomedicine", Ed. C. Scholz, pp.297- 312, 2017, Wiley
2) Takei Y et al.: Targeted gene delivery to sinusoidal endothelial cells: DNA nanoassociate bearing hyaluronan-glycocalyx, FASEB J 18, 699-701, 2004
3) Shimada N et al.: lnter-polyelectrolyte nano-assembly induces folding and activation of functional peptides. J Controlled Rel 218, 45-52, 2015

Synthetic glycopolymers shine at predesigned location: from cancer cell targeting to cell surface engineering

Gaojian Chen (Soochow University)

Sugar chains play critical roles in biological processes such as cell signaling and molecular recognition, and synthetic polymers with pendent carbohydrate moieties widely called glycopolymers have attracted much attention in intensive research and biological engineering due to advancements of polymerization techniques and their ability of mimicking biological functions in recognition processes. Herein a few examples of designing and synthesizing glycopolymer-based materials which are targeted at different locations for variable applications are provided. The first example is to synthesize functional glycopolymer-based materials that can target cancer cells. By picking the right sugar, with other helpful elements such as pH-sensitive molecule, we rebuild them to form synthetic complex glycopolymers for recognizing cancer cells. The second example is the synthesis of GAG-Mimicking glycoolymers for cell surface engineering. Glycosominoglycans (GAGs) are linear

heteropolysaccharides virtually existing in every somatic cell and they have been extensively applied in various fields such as anti-coagulation, drug delivery and regulation of stem cell fate due to their particular specific bioactivities. In addition, pathways such as FGF2-ERK1/2 for differentiation of stem cells originate in the cell membrane, cell-surface engineering with GAG-mimicking glycopolymers is expected to play an important role in regulating the cell fate. In our study, the sulfonated unit (S) and glyco unit (G) were splitted from the sulfated saccharide building block of GAGs and polymerized. Furthermore, the synthetic GAG-mimicking glycopolymers were armored with different cell-surface-anchoring moieties such as phospholipid and cholesterol groups. The time difference of their stay on cell surface and the effects in promoting neural differentiation have been compared. The third example is the fabrication of fluorescent carbon quantum dots with nano-defined position and pattern via Sugar-Electron-beam-Writing (SEW). Precise positioning and patterning of quantum dots (QDs) is a prerequisite for realizing their actual applications in nanophotonics, surface plasmonics, biological imaging, quantum computing and beyond. We provide a novel one-step method for producing Carbon QDs (CQDs) with nano-defined position via electron- beam lithography that uses a carefully chosen resist, a synthetic glycopolymer PMAG. Marrying an electron beam with PMAG enables the in situ production of CQDs with nanoscale precision and with any patterns. Through the examples above we demonstrate the variable applications of predesigned glycopolymers with targeted and even nano-defined position in cancer therapy, stem cell regulation and beyond.

References

1) Kiessling LL, Grim J C: Glycopolymer probes of signal transduction. Chem Soc Rev 42, 4476, 2013

2) Miura Y, Hoshino Y, Seto H: Glycopolymer nanobiotechnology. Chem Rev 116 (4), 1673-92, 2016

3) Narain R: Engineered carbohydrate-based materials for biomedical applications: Polymers, Surfaces, Dendrimers, Nanoparticles, and Hydrogels. New Jersey, Wiley, 2011

Glycopolymer nanomedicine by protecting-group-free synthesis

Tomonari Tanaka (Kyoto Institute of Technology)

Glycopolymers are synthetic polymers with pendant saccharides developed as glycocluster molecules incorporating multivalent forms of saccharides. Although many different glycopolymers have been synthesized, most reported synthetic methods require multiple laborious steps such as the protection and deprotection of hydroxy groups on the saccharide, making these synthetic approaches difficult to apply to oligosaccharides such as sialyloligosaccharides. We recently developed

an approach for synthesizing glycopolymers from free saccharides without the use of any protecting group. The protecting- group-free method is applicable to not only mono- and disaccharides, but also to oligosaccharides. The first step for synthesizing glycomonomers is a direct anomeric activation using 2-chloro-1,3-dimethylimidazolinium chloride (DMC) and sodium azide in water. The second step is coupling with N-propargyl acrylamide by copper-catalyzed azide-alkyne cycloaddition reaction. The resulting glycomonomers, saccharide-carrying acrylamide derivatives, were subjected to reversible addition-fragmentation chain transfer (RAFT) polymerization. The glycopolymers strongly bound with the corresponding lectin with Ka values in the order of 10^7 M^{-1} analyzed by using the quartz crystal microbalance. Furthermore, the glycopolymer bearing a complex-type sialyl N-linked oligosaccharide was found to strongly bind with both human and avian influenza A viruses analyzed by hemagglutination inhibition assay. The strong binding was attributed to the glycocluster effect of glycopolymers.

References
1) Tanaka T: Trends Glycosci Glycotechnol 28, E101, 2016
2) Tanaka T, Ishitani H, Miura Y, Oishi K, Takahash T, Suzuki T, Shoda S, Kimura Y, ACS Macro Lett 3, 1074, 2014
3) Tanaka T, Nagai H, Noguchi M, Kobayashi A, Shoda S: Chem Commun 3378, 2009

Session 6　Frontiers of Polysaccharide Material Science
Chair : Kazunari Akiyoshi

Supramolecular materials formed by cyclodextrins
Akira Harada (Osaka University)

In the past some decades much attention has been attracted to molecular recognition, host-guest chemistry, and supramolecular complexes. Although there have been numerous attempts on self-organization of molecules, there are a few on self-assembly on macroscopic scale. If molecular recognition works in macroscopic scale, we will be able to realize a variety of architectures and functions as living things do. Here we found that macroscopic soft materials can be differentiated each other through molecular recognition. This is the first example of visualization of specific molecular recognition events on macroscopic-scale.

A host gel containing-cyclodextrin (CD) was found to bind a guest gel containing adamantane (Ad) strongly through molecular recognition. In addition, αCD gel was found to bind the guest gel containing *n*-butyl group specifically and βCD gel binds that of *t*-butyl groups selectively to give self-assembly on macroscopic scale only by mixing and shaking in water.

We found that a βCD polymer and a ferrocene-polymer formed a gel. Even when

the gel was cut in half, if the gel pieces were brought in contact, the gel pieces were bound and self-healed to the original gel. This gel is redox-responsive. Polymer gels containing β-CD and adamantane showed self-healing properties with 100% efficiency[1].

References
1) Harada Ed., "Supramolecular Polymer Chemistry", Wiley-VCH, 2012
2) Nakahata M, Mori S, Takashima Y, Yamaguchi H, Harada A: Chem. 2016,1
3) lwaso K, Takashima Y, Harada A: Nat Chem 8, 625-632, 2016

Chitin nanofibers from crab shell and its biological properties

Shinsuke Ifuku (Tottori University)

Chitin nanofibers are prepared from the exoskeletons of crabs and prawns by a simple mechanical treatment after the removal of proteins and minerals. The obtained nanofibers have fine nanofiber networks with a uniform width of approximately 10-20 nm and a high aspect ratio. A grinder, a Star Burst atomization system, and a high speed blender are all used in the mechanical treatment to convert chitin to nanofibers. Chitin and chitosan nanofibers are considered to have great potential for various biomedical applications, because they have several useful properties such as high specific surface area and high porosity. Chitin nanofiber has variety of biomedical properties. These properties are applicable for tissue engineering, wound dressing, cosmetic and skin health, stem cell technology, anti-cancer treatments and drug delivery, anti-inflammatory treatments, and obesity treatment.

References
1) Ifuku S, Saimoto H: Nanoscale 4, 3308, 2012.
2) Azuma K, Ifuku S, Osaki T, Okamoto Y, Minami S: J Biomed Nanotechnol 10, 2891, 2014

A novel polysaccharide carrier for immunocyte targeting delivery of therapeutic oligonucleotides

Kazuo Sakurai and Shinichi Mochizuki (The University of Kitakyushu)

Oligonucleotides (ODNs) are easily decomposed in biological fluids, which makes it difficult to administrate antisense DNA or CpG motif in naked state. Schizophyllan (SPG) is a natural β-(1,3)-D-glucan existing as a triple helix in water and as a single chain in alkaline solutions, respectively. When homo-polynucleotides such as poly(dA) are added to SPG alkaline solution and subsequently pH is adjusted to be neutral, the single chain of SPG forms a stoichiometric complex with the

polynucleotide. The complex can protect the bound DNA against nuclease-mediated hydrolysis. Furthermore, a receptor called Dectin-1 recognizes SPG/ODN complexes, and the complex is eventually ingested by immunocytes that express Dectin-1. The present talk will show our recent *in vivo* and *vitro* studies to prove this idea and provide a new strategy to specifically transport functional ODNs including antisense-DNA, CpG-DNA, and siRNA to APCs to cure the diseases due to disorientation of immune system.

References

I) Sakurai K, Shinkai S: Molecular recognition of adenine, cytosine, and uracil in a single-stranded RNA by a natural polysaccharide: schizophyllan. J Am Chem Soc 122 (18), 4520-1, 2000.

2) Sakurai K, Uezu K, Numata M, Hasegawa T, Li C. Kaneko K, Shinkai S: Beta-1,3-glucan polysaccharides as novel one-dimensional hosts for DNA/RNA, conjugated polymers and nanoparticles. Chem Commun (Camb) 35, 4383-98, 2005

3) Sakurai K, Mizu M, Shinkai S: Polysaccharide-polynucleotide complexes. 2. Complementary polynucleotide mimic behavior of the natural polysaccharide schizophyllan in the macromolecular complex with single-stranded RNA and DNA. Biomacromolecules 2 (3), 641-50, 2001

4) Brown GD, Gordon S: Immune recognition. A new receptor for beta-glucans. Nature 413, 36-7, 2001

5) Mochizuki S, Morishita H, Adachi Y, Yamaguchi Y, Sakurai K: Binding assay between murine Dectin-1 and β-glucan/DNA complex with quartz-crystal microbalance. Carbohydr Res 391, 1-8, 2014

6) Koyama S et al.: Plasmacytoid dendritic cells delineate immunogenicity of influenza vaccine subtypes. Sci Transl Med 2, 25ra24, 2010

7) Kobiyama et al.: Nonagonistic Dectin-1 ligand transforms CpG into a multitask nanoparticulate TLR9 agonist. PNAS 111, 3086-91, 2014

8) Zhang Q, Ichimaru N, Higuchi S, Cai S, Hou J, Fujino M, Nonomura N, Kobayashi M, Ando H, Uno A: Permanent acceptance of mouse cardiac allografts with CD40 siRNA to induce regulatory myeloid cells by use of a novel polysaccharide siRNA delivery system. Gene Ther 22, 217-26, 2015

Closing Remarks