糖鎖と免疫

深瀬浩一

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大学院理学研究科附属フォアフロント研究センター

放射線科学基盤機構

先端モダリティ･DDS研究センター（CAMaD）

天然物有機化学研究室における生物活性分子の機能研究
Functional studies of Bioactive Molecules in the Laboratory of Natural Product Chemistry

深瀬浩一、下山敦史准教授、真鍋良幸准教授、髙松正之助教
樺山一哉教授（放射線科学基盤機構
2024年2月1日～）

Prof. Antonio Molinaro
(University of Naples, Federico II)

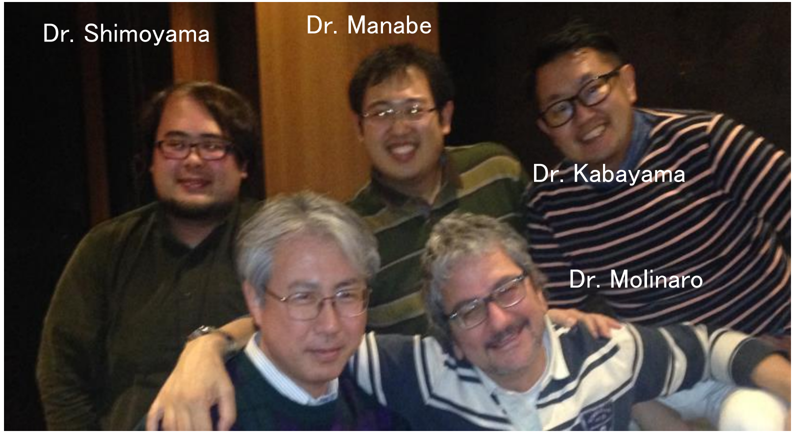
藤本ゆかり先生(2003~2014.3に在籍)
田中克典先生 (2005-2011に在籍)

Main research targets: glycans, glycoconjugates
Main research topics: chemical synthesis, biofunctional mechanism, bio-imaging

糖鎖の効率合成
鍵化合物の合成と供与（共同研究）
生物活性発現機構の解明
新規医薬や医療法への展開

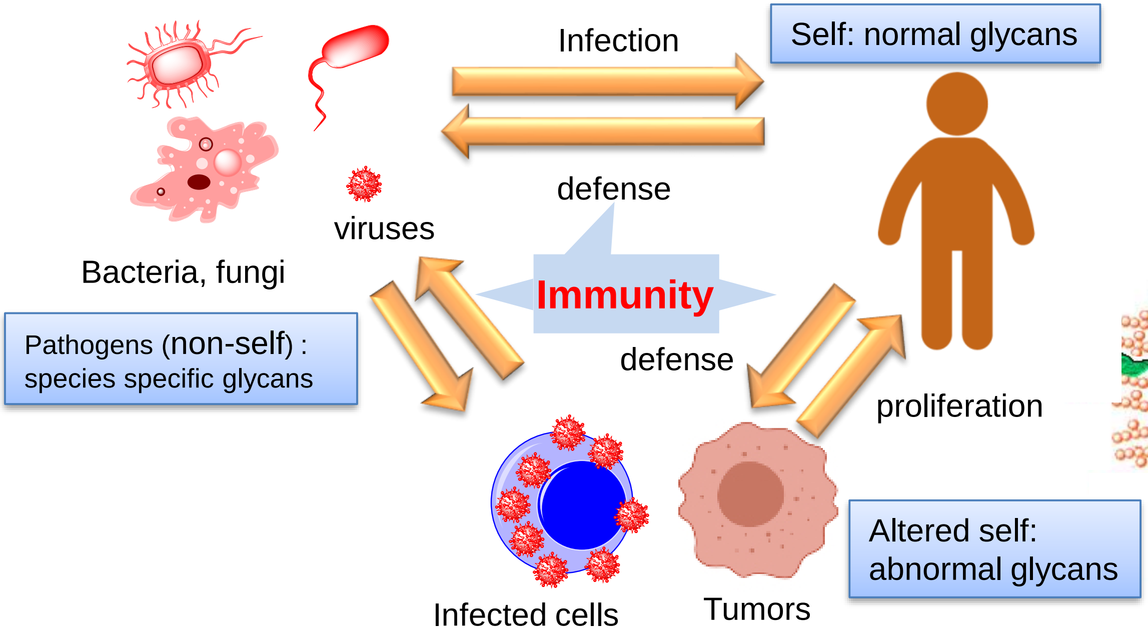
世界最先端であることが重要
発明と発見のバランス
新しい概念の提唱

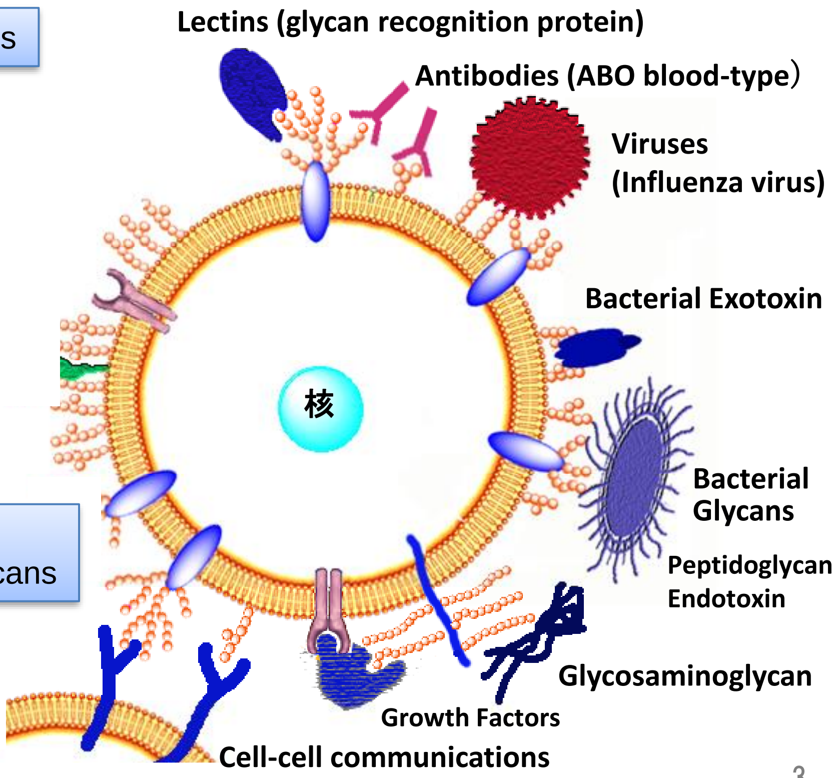
対等な共同研究



糖鎖は様々な認識イベントに関与しており、免疫応答における重要なシグナルを与える

細胞表層は糖鎖に覆われている。種々の認識に関与、自己、非自己、変性自己の認識





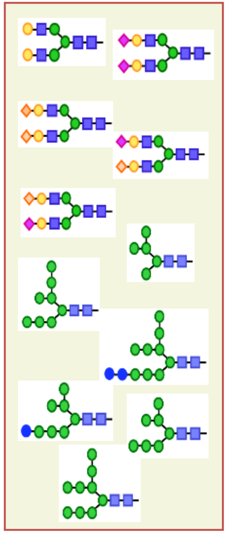
我々のミッション：**免疫における糖鎖の機能解明、ワクチンやアジュバントの開発、アルファ線核医学治療**（がんワクチンを用いた併用療法への展開）

講演の内容

- *N*-グリカンの機能解析：コアフコシル化されたIgGとデクチン-1との相互作用解析とIgGの抗炎症効果の考察
- 粘膜ワクチンのためのリピドAアジュバントの開発
- 自己アジュバント化ワクチンの開発

糖鎖の階層的グリココードの解明

Glycan libraries



多糖・オリゴ糖

部分構造の合成

要素還元主義的
アプローチ

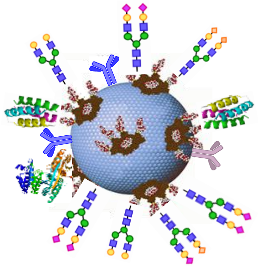
低分子
中分子

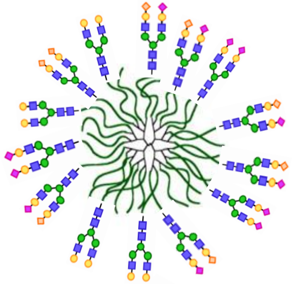
再構成

複合化

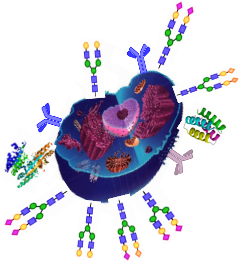
中分子
高分子

liposome

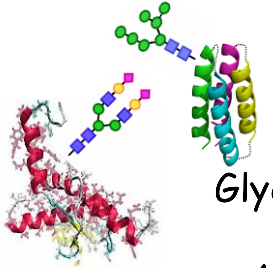




dendrimer



glycan-engineered cells



Glycoproteins
&
Analogues

1次グリココード：タンパク質の認識する構造

グリココードの同定：
糖鎖とレクチン、受容体、抗体、糖鎖の相互作用

Glycocode: K. Kasai, J. Hirabayashi,
J. Biochem. **1996**, *119*, 1.

高次グリココード：糖鎖やタンパク質の複合化・集合化により高次機能が創発

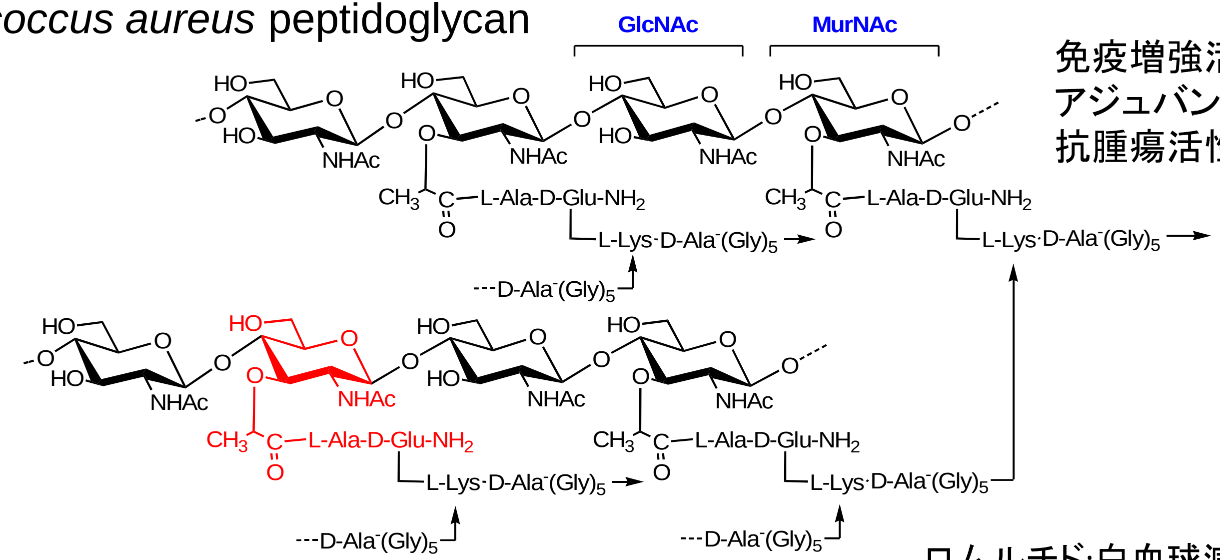
多価相互作用
異価相互作用
相乗的相互作用

システムの統合的理解

システム理論、全体論

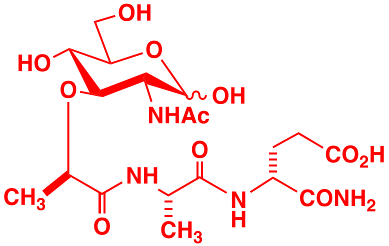
細菌細胞壁ペプチドグリカンの免疫増強作用

Staphylococcus aureus peptidoglycan



免疫増強活性
アジュバント活性（抗体生成の促進）
抗腫瘍活性

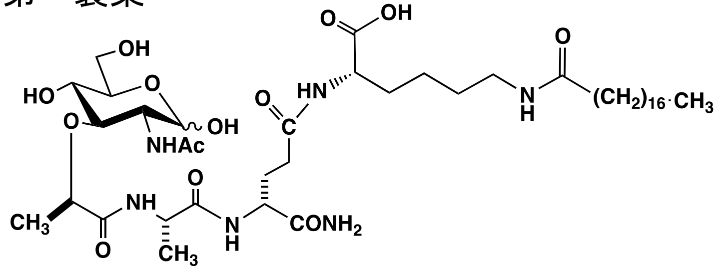
N-Acetyl-muramyl-L-alanyl-D-isoglutamine
(Muramyl dipeptide, MDP)



免疫活性化の最小構造

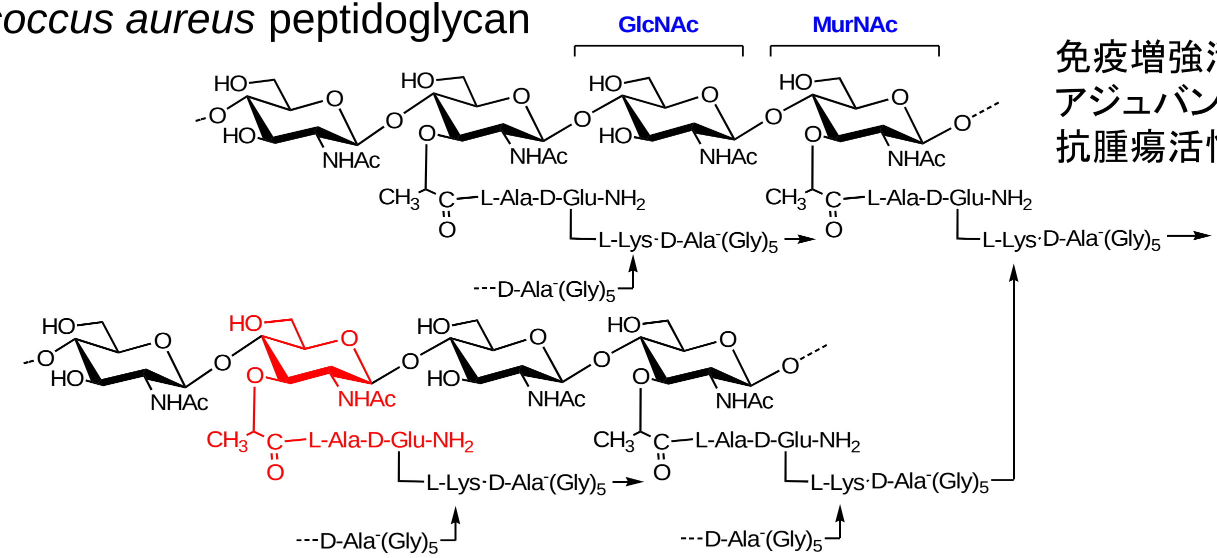
Kusumoto, Shiba, Kotani (Osaka Univ.) 1975
Lederer (Univ. of Paris-Sud) 1974

ロムルチド:白血球減少症治療薬, 免疫調節薬
第一製薬



細菌細胞壁ペプチドグリカンの免疫増強作用

Staphylococcus aureus peptidoglycan



免疫増強活性
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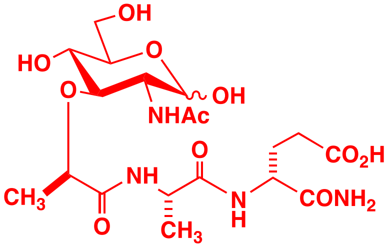


免疫活性化の最小構造

MDPの活性はPGN（ペプチドグリカン）と同一ではない
MDPはToll様受容体2 (TLR2)を活性化しない

合成と構造活性相関研究

ペプチドグリカンの一次グリココードの解析

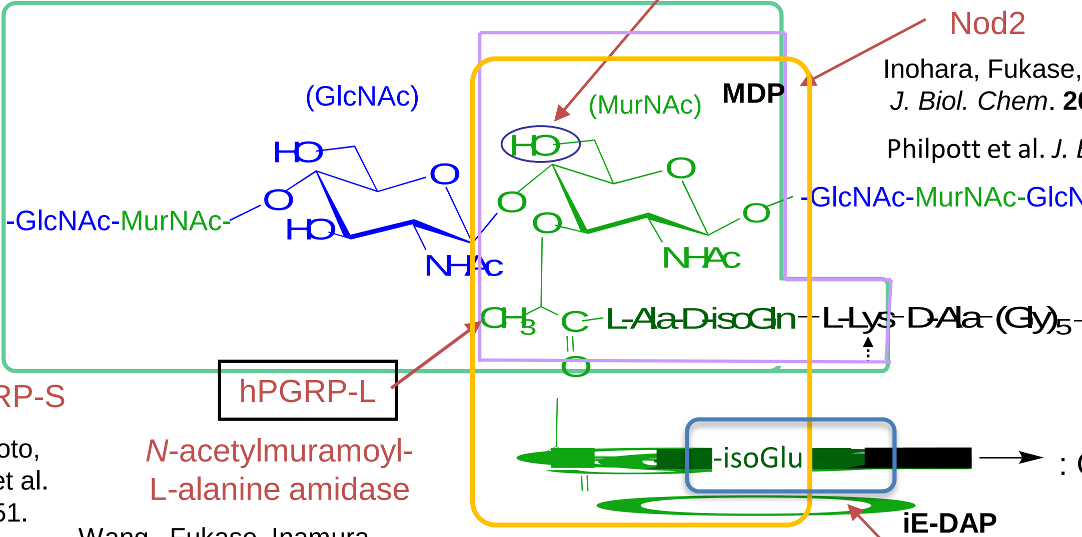
6-acyl MDP→TLR2/TLR4 recognition

Uehori, Fukase, Kusumoto, Seya, et al. *J. Immunol.*, **2005**, *174*, 7096.

Nod2

Inohara, Fukase, Inamura, Hashimoto, Kusumoto, Nuñez et al. *J. Biol. Chem*. **2003**, *278*, 5509.

Philpott et al. *J. Biol. Chem*. **2003**, *278,* 8869.



: Gram-positive bacteria

: Gram-negative bacteria

iE-DAP

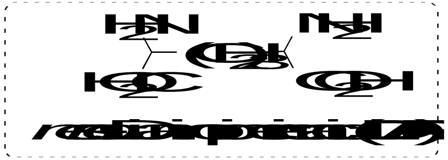
hPGRP-S

Cho, Fukase, Kusumoto, Fujimoto, Ezekowitz et al. *Blood*, **2005**, *106*, 2551.

hPGRP-L

N-acetylmuramoyl-L-alanine amidase

Wang , Fukase, Inamura, Kusumoto, Dziarski et al. *J.Biol.Chem.*, **2003**, *278*, 49044.

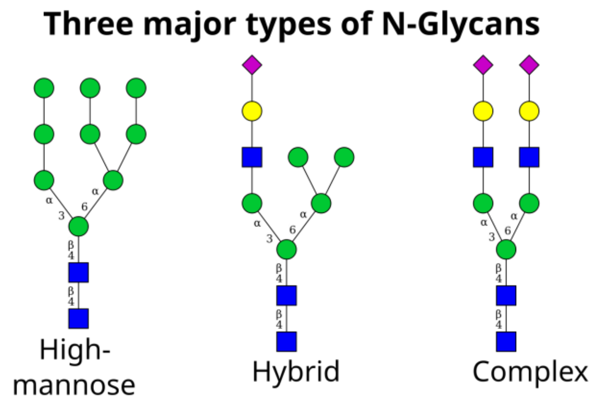


Nod1

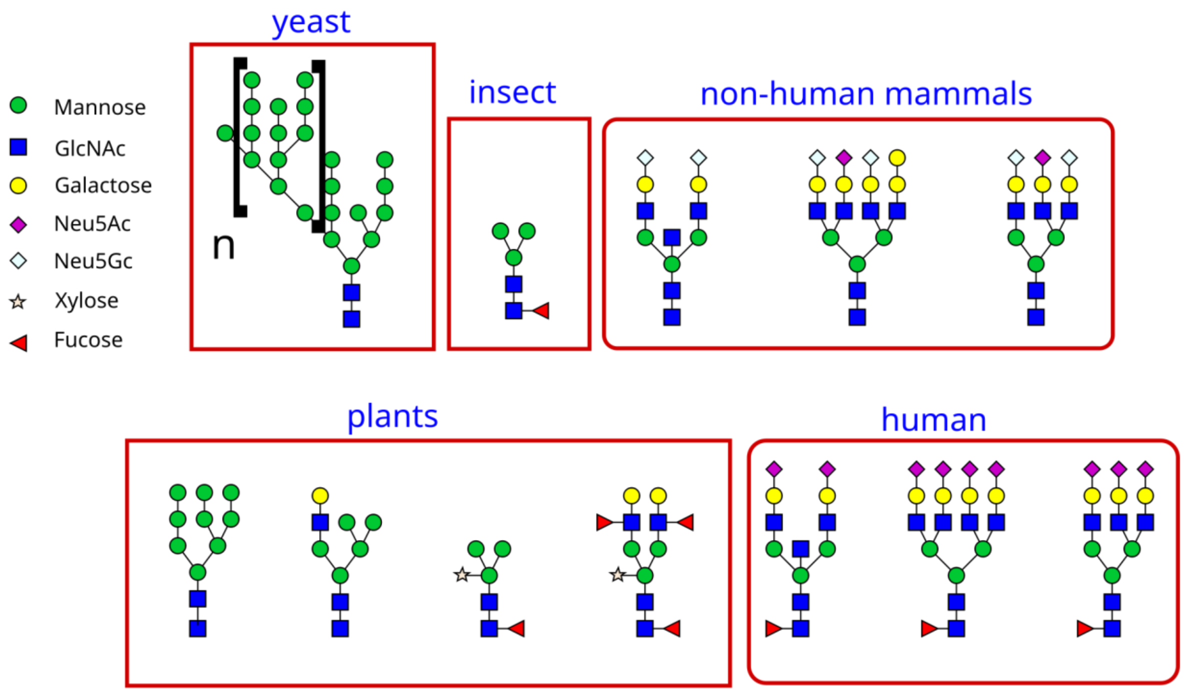
Inohara, Fukase, Kawasaki, Hashimoto, Kusumoto, Nuñez et al. *Nature Immunol.* **2003**, *4*, 702.

Philpott et al. *Science* **2003**, *300,* 1584.

アスパラギン結合型糖タンパク質糖鎖（N-グリカン）



高マンノース型　混合型　複合型



From wikipedia

高い不均一性と多様性：その意義は？

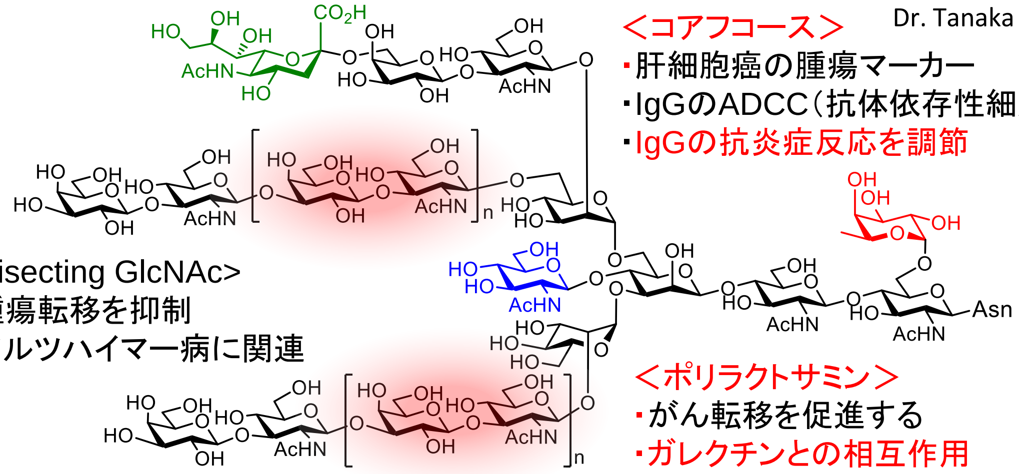
N-グリカンのグリコフォーム：構造の多様性に基づく多様な生物学的機能

N-Glycans have high diversity and are involved in a variety of important physiological events.

Dr. Tanaka

Dr. Manabe

＜シアル酸＞
・糖タンパク質の安定性を調整
・免疫応答を調節
・シグレックやセレクチン等との相互作用



＜コアフコース＞
・肝細胞癌の腫瘍マーカー
・IgGのADCC（抗体依存性細胞傷害活性）を調節
・IgGの抗炎症反応を調節

Dr. Marchetti

Dr. Silipo Dr. Molinaro

<Bisecting GlcNAc>
・腫瘍転移を抑制
・アルツハイマー病に関連

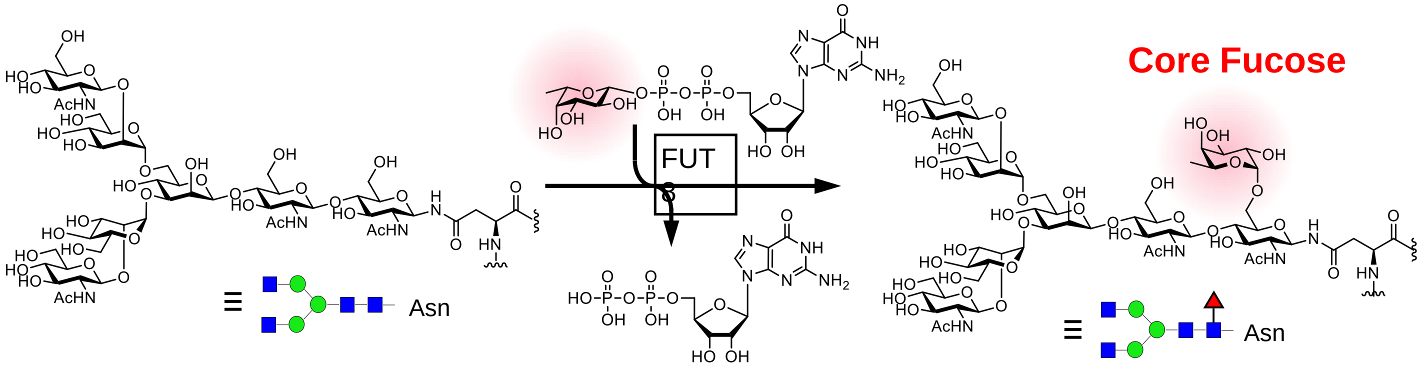
＜ポリラクトサミン＞
・がん転移を促進する
・ガレクチンとの相互作用

化学合成 ⇒ 機能研究のための均質なN-グリカン

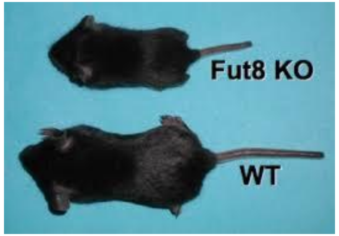
複合化を基盤とする高次グリココードの解明

Core fucose containing *N*-Glycan

< α-(1,6)-Fucosyltransferase (FUT8) >



- 3-Day mortality rate of FUT8 knockout mice is 70%.[1)]
- TGF-β response is controlled by fucosylation of TGFR. [1)]
- Antibody-Dependent-Cellular-Cytotoxicity (ADCC) is controlled by fucosylation of IgG.[2)]
- Cancer metastasis is controlled by fucosylation of cancer cells.



Molecules that recognized core fucose had not been unknown in mammals.

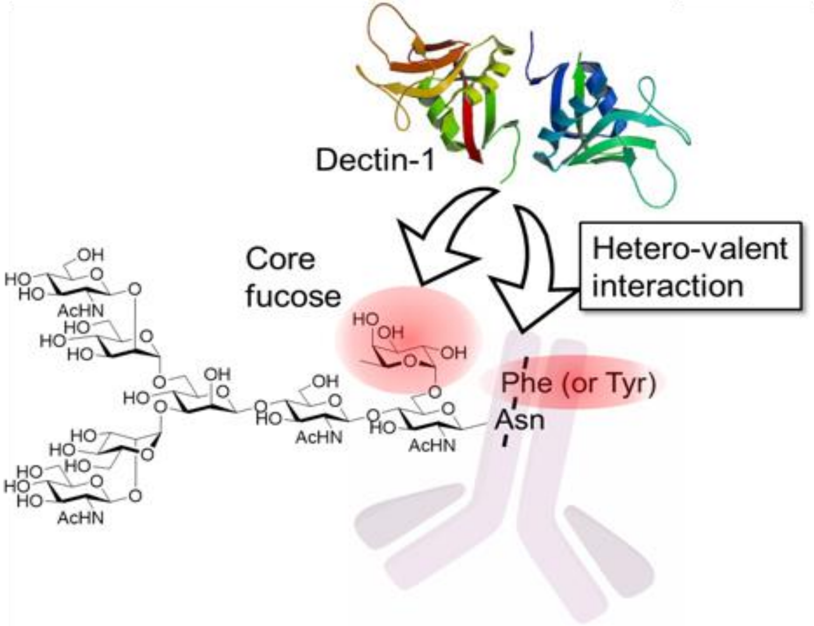
1) Wang X, Inoue S, Gu J, Taniguchi N et al., *Proc Natl Acad Sci USA*. **2005**, 102: 15791-15796.
2) Shields RL et al., *J Biol Chem*, **2002**, 277:26733–26740.

高次グリココード: dectin-1 と IgGの相互作用

Dectin-1 はコアフコシルIgGを認識する

Dectin-1: β-グルカンを認識する自然免疫レクチン
樹状細胞・マクロファージに発現

哺乳類で最初に発見されたコアフコースを認識するレクチン

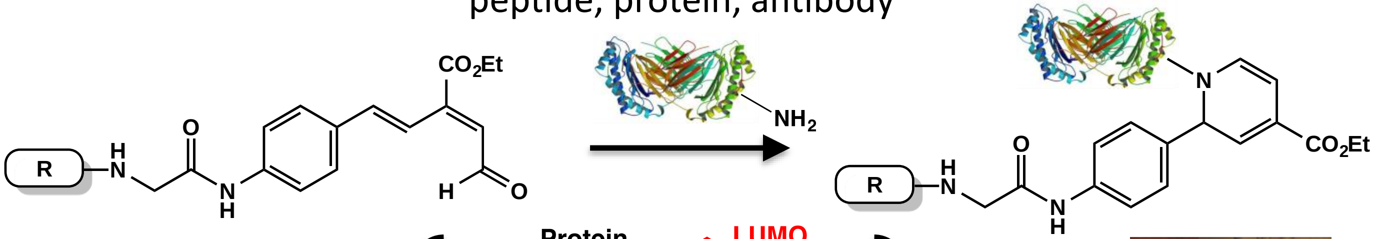


Manabe, Marchetti, Takakura, Nagasaki, Nihei, Takebe, Tanaka, Kabayama, Chiodo, Hanashima, Kamada, Miyoshi, Dulal, Yamaguchi, Adachi, Ohno, Tanaka, Silipo, Fukase, Molinaro. *Angew. Chem. Int. Ed. Engl*. **2019**, *58*, 18697.

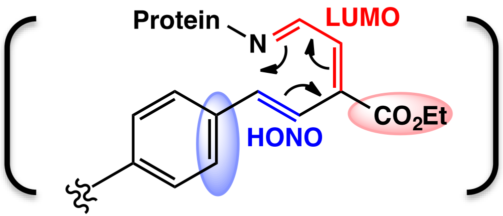
What is the physiological role of Dectin-1 with core fuocsylated IgG interaction?

New labeling and bio-conjugation by rapid 6π-azaelectrocyclization

peptide, protein, antibody



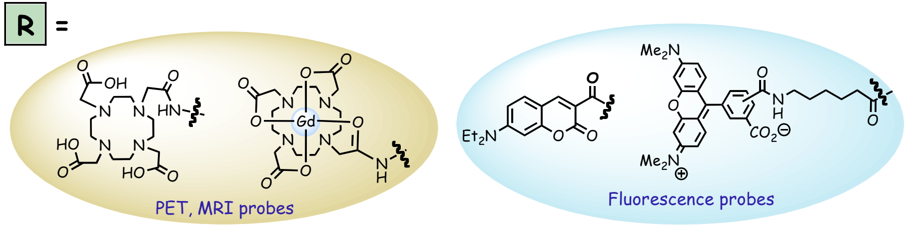
R = positron emitter
fluorescent group
glycan chain



5min,10^{-8}M, rapid and simple labeling

Dr. Katsunori Tanaka

Tanaka, K ; Mori, H.; Yamamoto, M.; Katsumura, S. *J. Org. Chem.* **2001**, *66*, 3009-3110.
Tanaka, K ; Katsumura, S. *J. Am. Chem. Soc.* **2002**, *124*, 9660-9661.

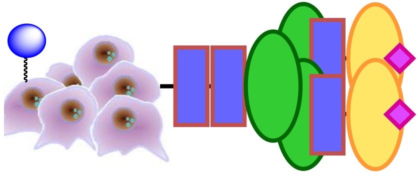


Tanaka, Fukase et. al. *Angew. Chem. Int. Ed.* **2008**, *47*, 102.

http://www.cosmobio.co.jp/export_e/products/detail/products_ksd_20091111.asp?entry_id=3888

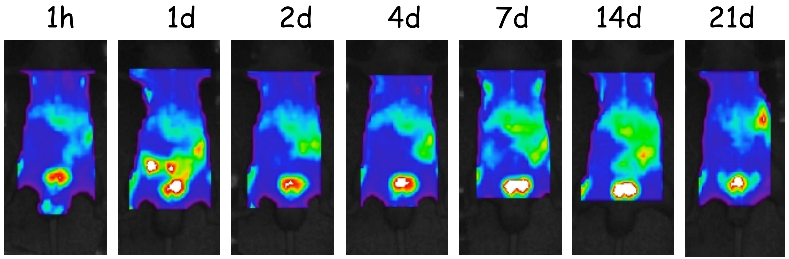
Core fucose on *N*-glycan reduces tumor metastasis capability.

Hylite730

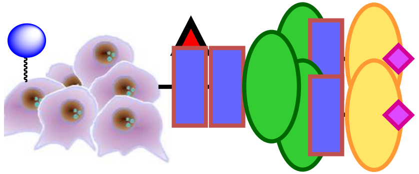


HCT-116 (mock)

Human colon carcinoma

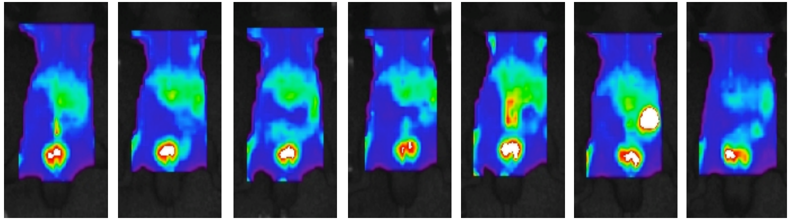


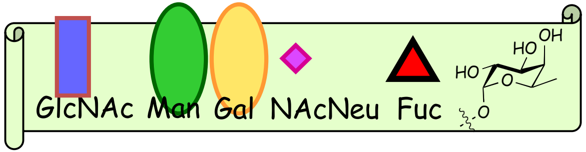
Escape from TRAIL-induced apoptosis induced by NK-cell



HCT-116 (GMDS expression)

GMDS: GDP-mannose-4,6-dehydratase





Labeled tumor cells (10^6 cellsin HBSS) were injected into the abdominal cavity.
Whole body was scanned from the back side by eXplore Optix, GE Healthcare, Bioscience (excitation at 730 nm, emission 750 nm).
Data were normalized.

BALB/cAJcl-nu/nu

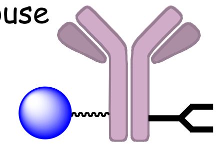
Collaboration with Prof. Eiji Miyoshi

Tanaka, K., Moriwaki, K., Yokoi, S., Koyama, K., Miyoshi, E., Fukase, K., Bioorg. Med. Chem. 2013, 21(5), 1074-1077.

Core fucose regulates IgG dynamics in nude mice

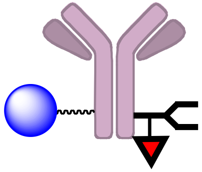
N-glycan Struc. ▲ Fucose

Fut8 KO Mouse
IgG



1μg in PBS (100μL)

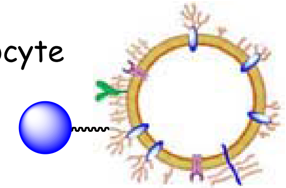
Wild Mouse
IgG



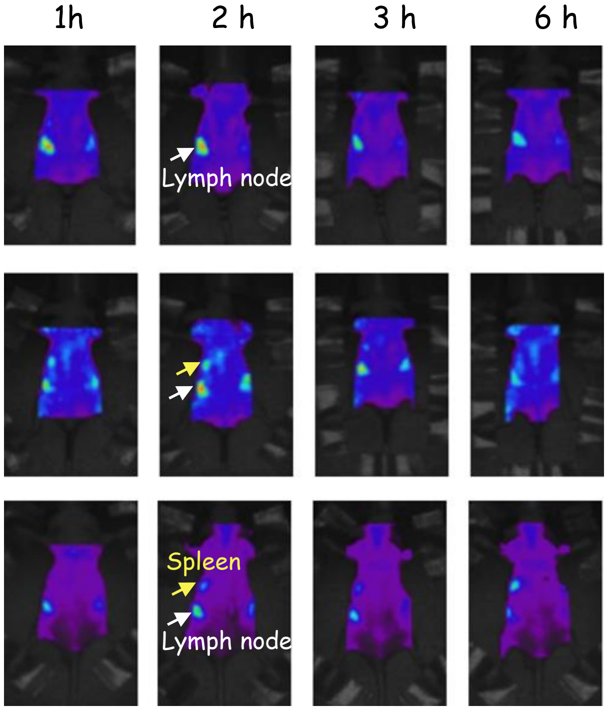
脾臓にも集積
樹状細胞？

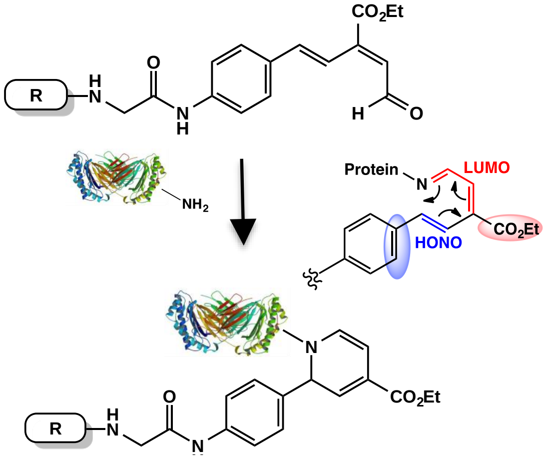
1μg in PBS (100μL)

Lymphocyte

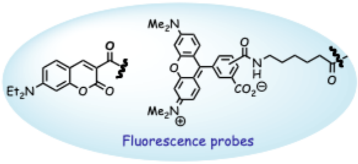


10^4 in PBS (100μL)





R =

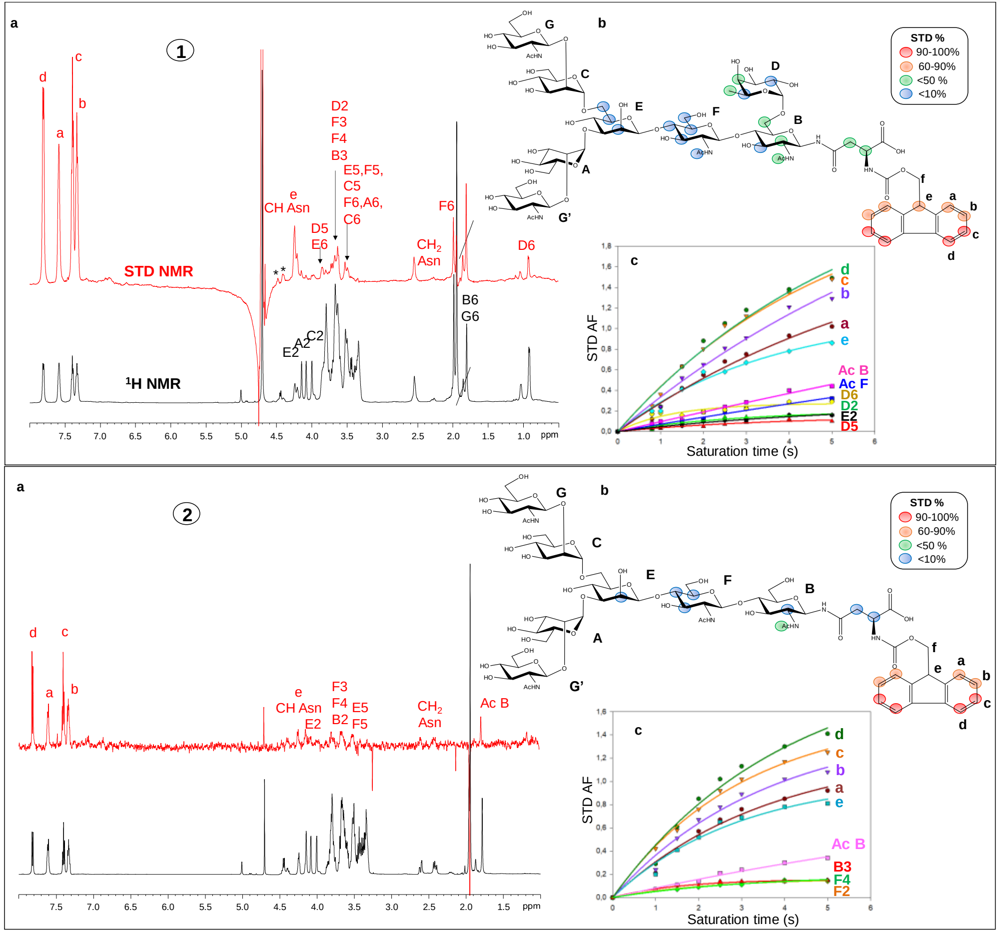


Tanaka, Fukase et. al. *Angew. Chem. Int. Ed*. **2008**, *47*, 102.

IgG: MW of 150KDa
Fucose: MW of 150

Labeled IgGs and lymphocytes were administrated intravenously and whole body was scanned from the back side by eXplore Optix, GE Healthcare, Bioscience (excitation at 646 nm, emission 663 nm). Data were normalized.

Dectin-1 とFmoc化コアフコシル*N*-グリカンのSTD-NMR



Dectin-1: β-グルカンを認識する
自然免疫レクチン
樹状細胞・マクロファージに発現

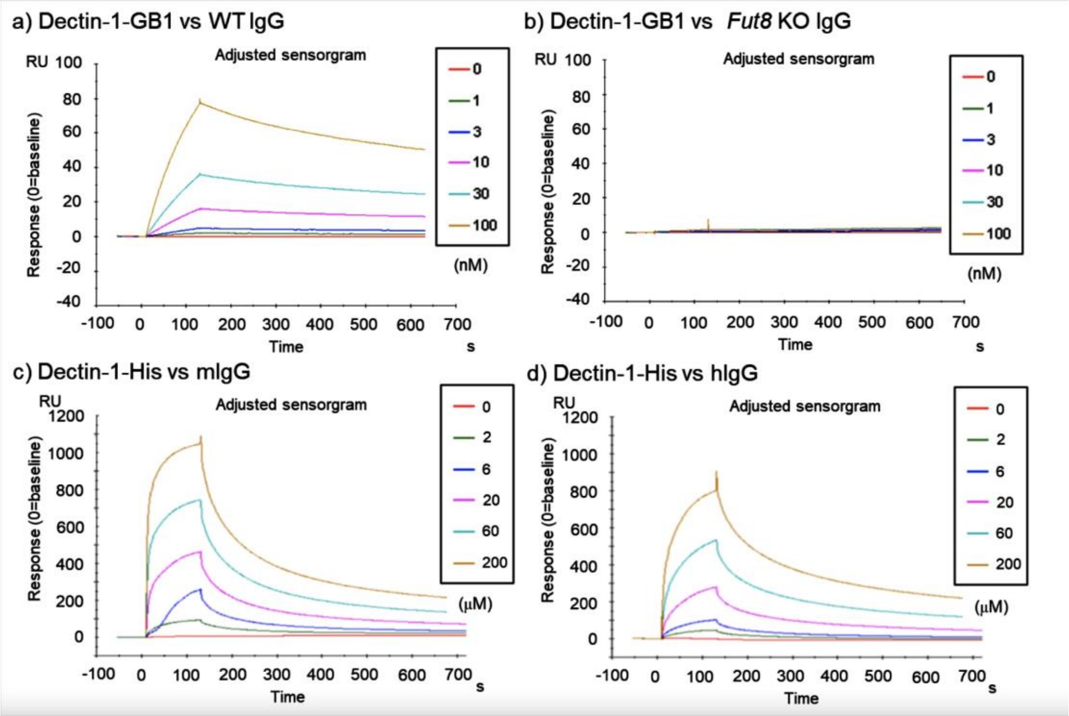
Dectin-1 とコアフコース、
Fmoc基との相互作用を確認

Fmoc化されていない糖鎖
は相互作用しない

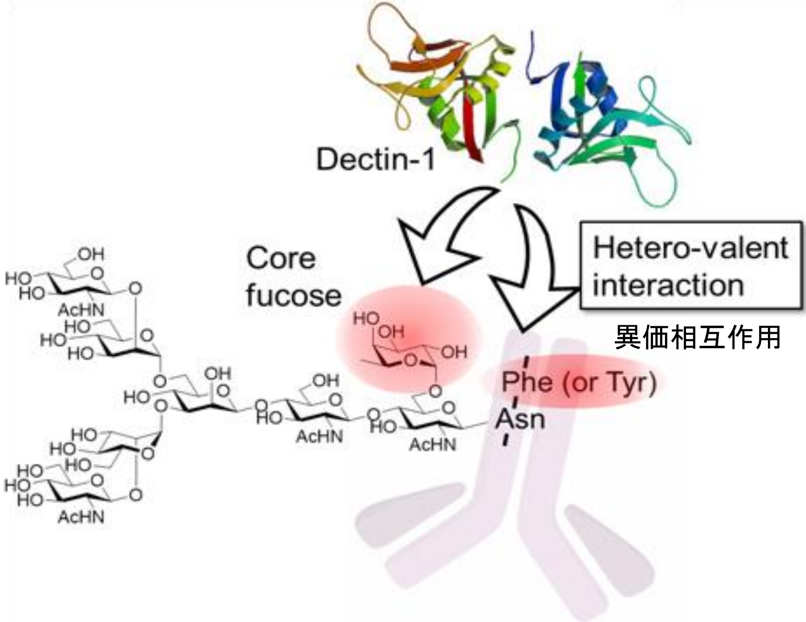
β-グルカンは相互作用に影響
しない

高次グリココード: dectin-1 と IgGの相互作用

SPR解析



GB1もIgGと相互作用する



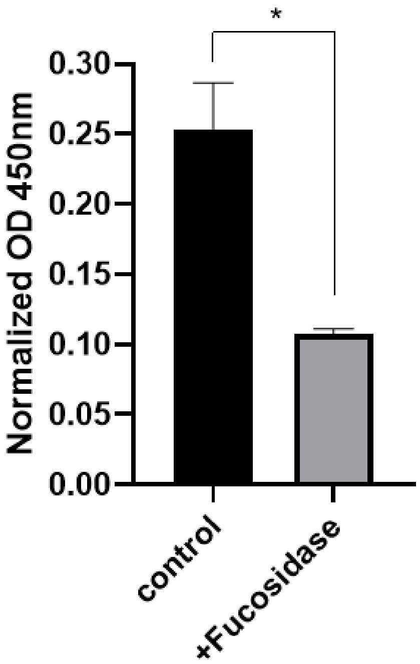
IgGのコアフコースは、抗体依存性細胞傷害作用(ADCC)を減弱

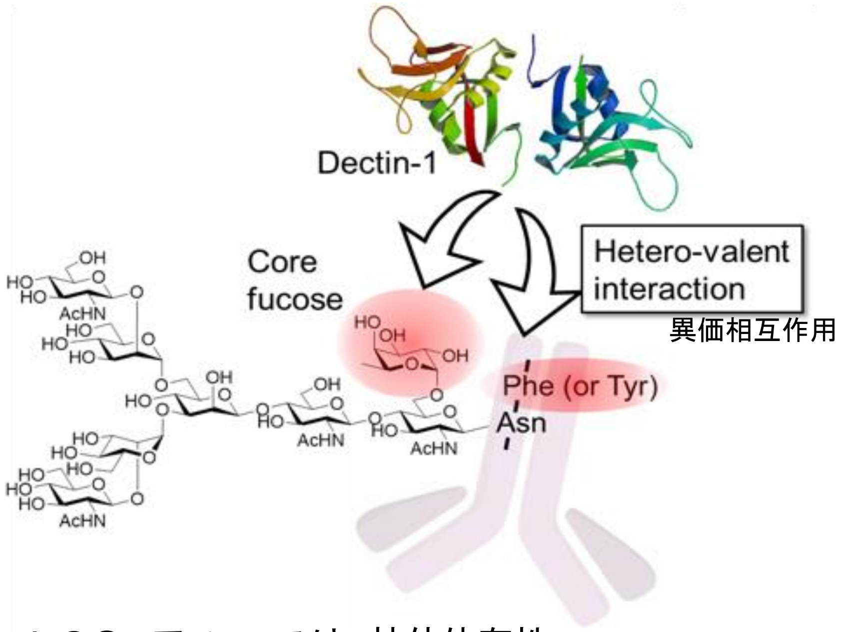
NK細胞上のFc受容体との相互作用を減弱
（NK細胞によるADCC低下）

コアフコースは免疫系の恒常性維持に寄与

高次グリココード: dectin-1 と IgGの相互作用

Dectin-1 binding to mIgG detected by ELISA.





IgGのコアフコースは、抗体依存性
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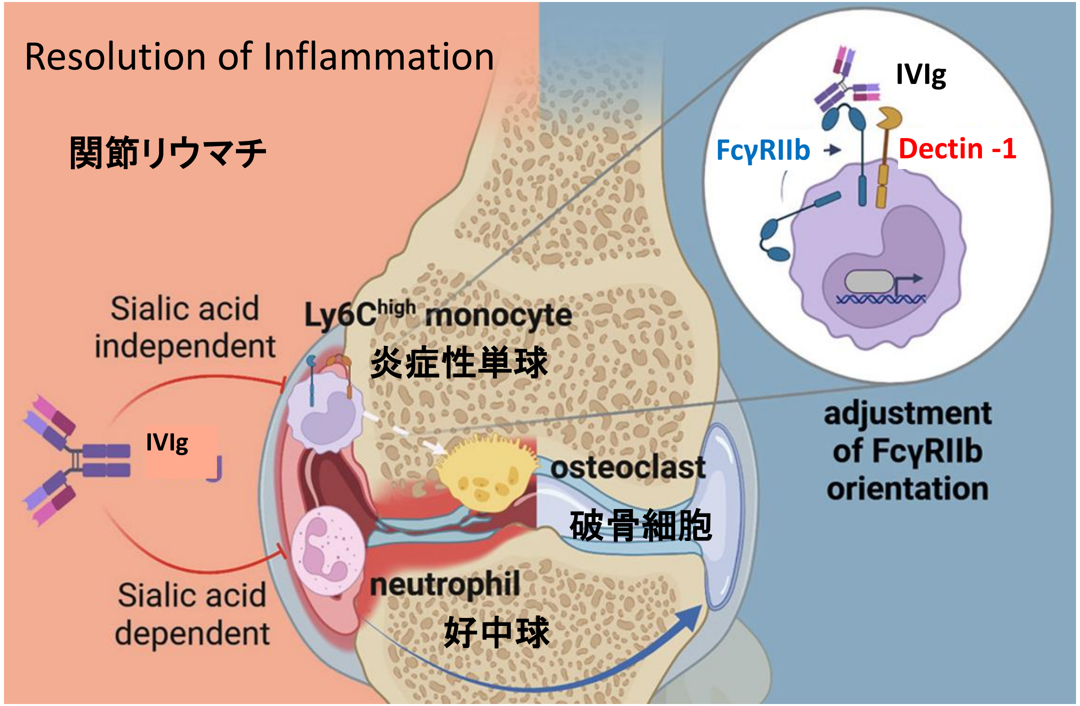
NK細胞上のFc受容体との相互作用を減弱
（NK細胞によるADCC低下）

コアフコースは免疫系の恒常性維持に寄与

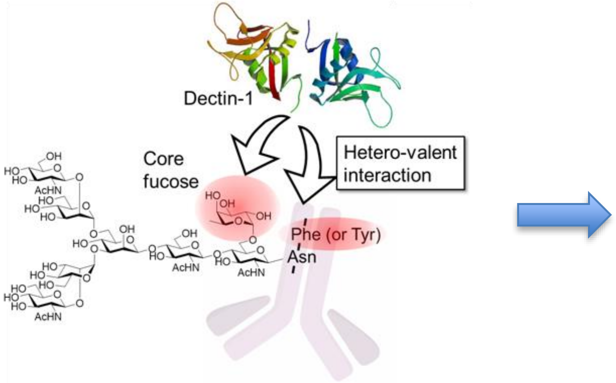
IgG-dependent inhibition of inflammatory bone remodeling requires pattern recognition receptor Dectin-1

Seeling M. et al., Immunity, 2023, doi: 10.1016/j.immuni.2023.02.019.

IVIg is serum human IgG used for immunoglobulin therapy to suppress various chronic inflammatory and autoimmune diseases.　**IVIg** は抗炎症作用を有する



Both Dectin-1 and FcγRIIb are required for IVIg anti-inflammatory response.

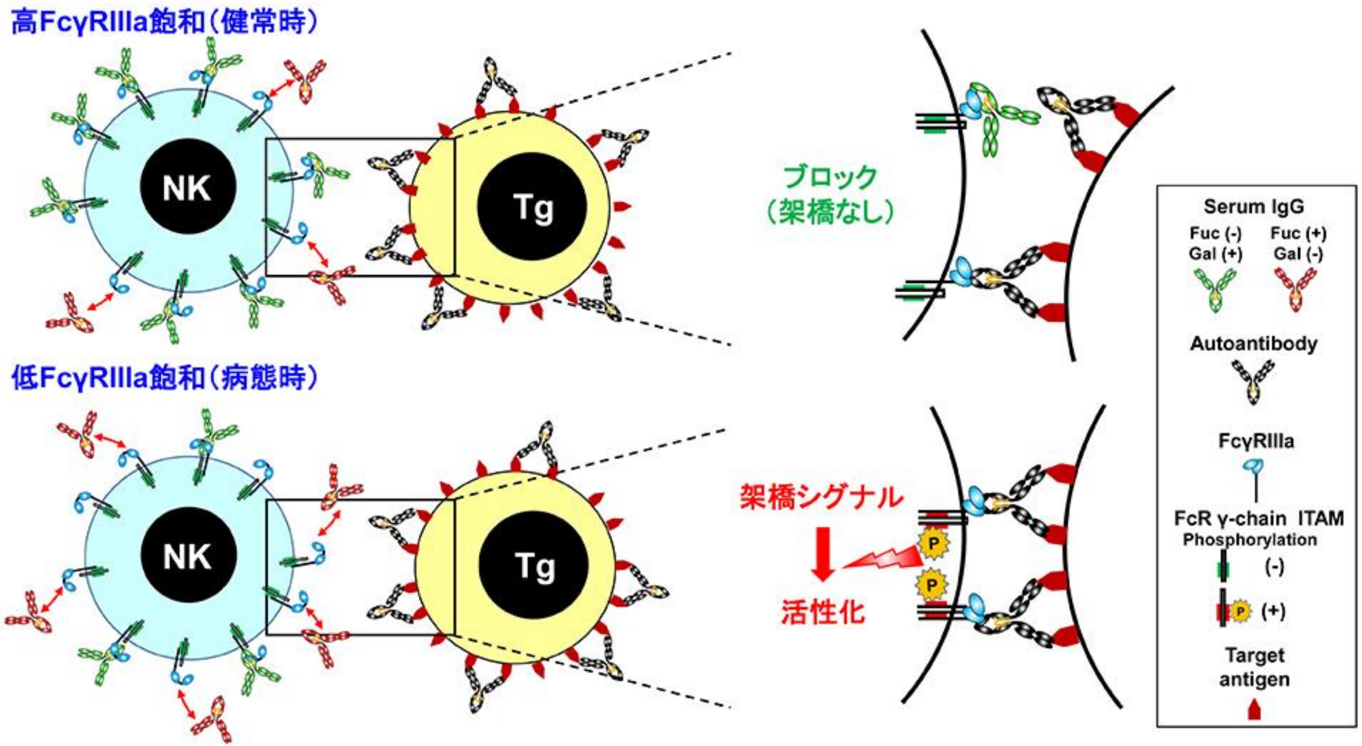


Core fucosylated IgG: secondary glycocode toward dectin-1
Core fucosylated IgG + dectin-1: tertiary glycocode toward FcγRIIb

非フコシル化IgG製剤の抗炎症作用

非フコシル化IgGとFcγRIIIaとの高い親和性：
ナチュラルキラー（NK）細胞が標的細胞（Tg）にアプローチできない

三村 雄輔先生
国立病院機構
山口宇部医療センター
臨床研究部



https://www.glycoforum.gr.jp/article/25A11J.html

N-グリカンのグリコフォーム：構造の多様性に基づく多様な生物学的機能

N-Glycans have high diversity and are involved in a variety of important physiological events.

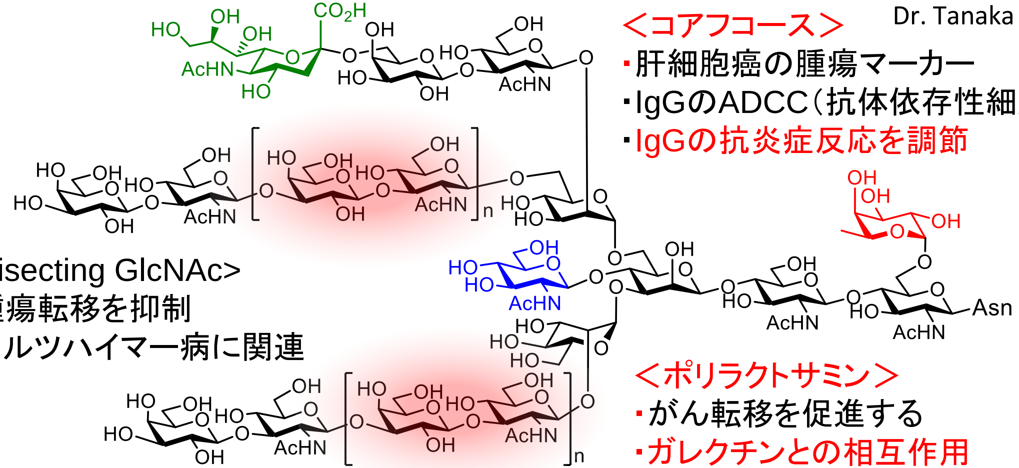
Dr. Tanaka

Dr. Manabe

＜シアル酸＞
・糖タンパク質の安定性を調整
・免疫応答を調節
・シグレックやセレクチン等との相互作用

Dr. Marchetti

Dr. Silipo　Dr. Molinaro



＜コアフコース＞
・肝細胞癌の腫瘍マーカー
・IgGのADCC（抗体依存性細胞傷害活性）を調節
・IgGの抗炎症反応を調節

<Bisecting GlcNAc>
・腫瘍転移を抑制
・アルツハイマー病に関連

＜ポリラクトサミン＞
・がん転移を促進する
・ガレクチンとの相互作用

化学合成 ⇒ 機能研究のための均質なN-グリカン

複合化を基盤とする高次グリココードの解明

バクテリア由来複合糖質の免疫増強活性研究

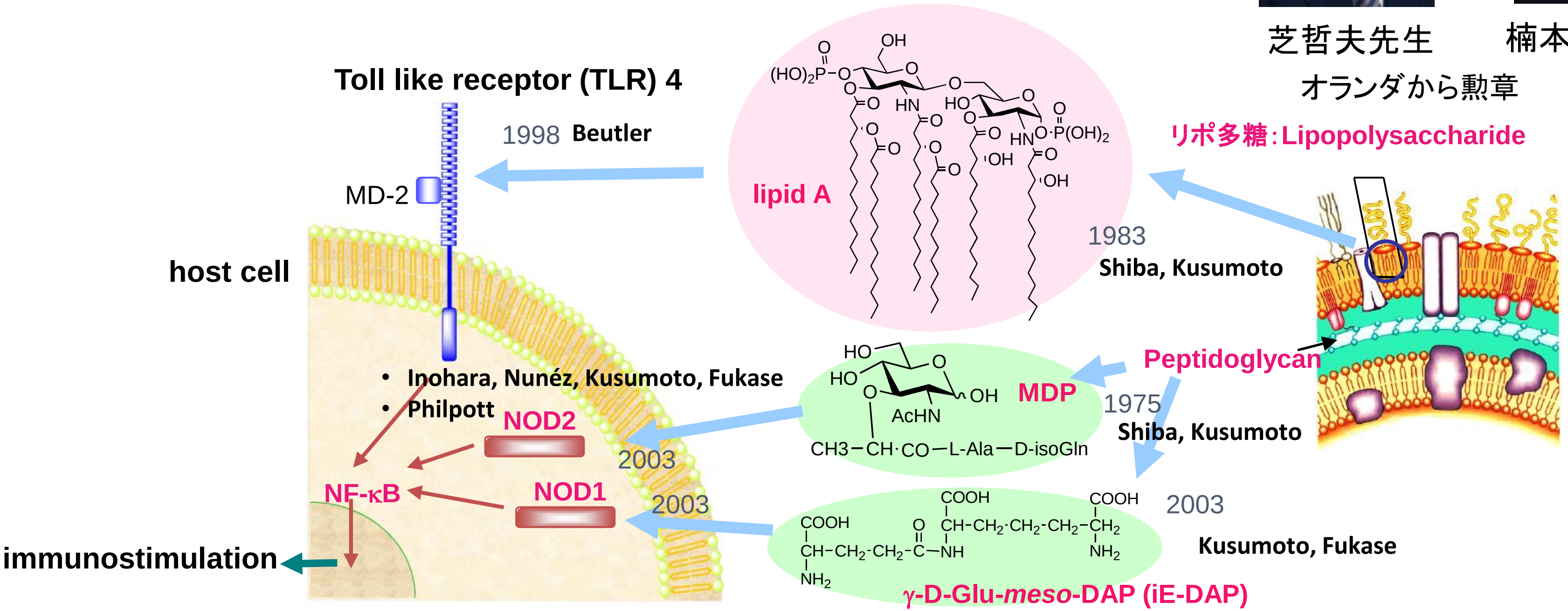
細菌複合糖質

不均一なポリマー
コンタミの可能性

活性本体や最小活性構造の決定
高分子の部分構造(低分子)によって活性が発現
→自然免疫研究に発展

芝哲夫先生
オランダから勲章

楠本正一先生
剣道7段



微生物由来分子による免疫活性化

自然免疫

Innate Immunity

センサーが自己と非自己を見分ける

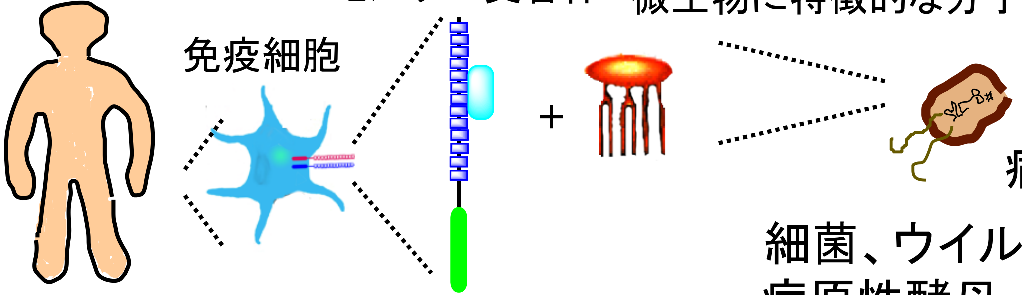
（構造の類似性と相違性に基づいて）

センサー受容体

微生物に特徴的な分子：リポタンパク質、リポペプチド

リポ多糖

アジュバントとして機能



細菌、ウイルス、かび、
病原性酵母、原生動物、
寄生虫、ダニ

2011年ノーベル生理学・医学賞
ホフマン教授
ボイトラー教授

免疫活性化
細菌殺傷

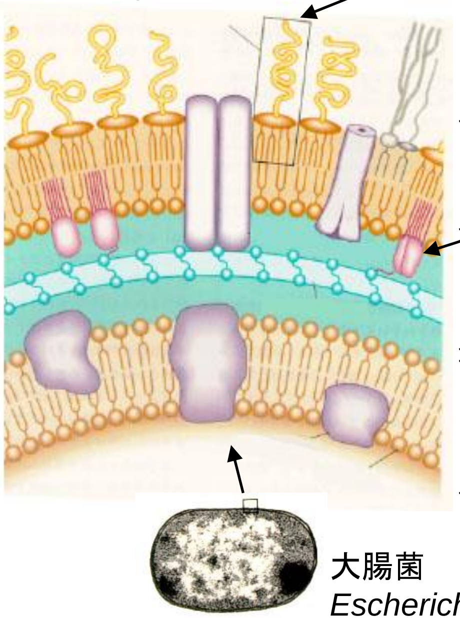
抗体産生
抗腫瘍作用

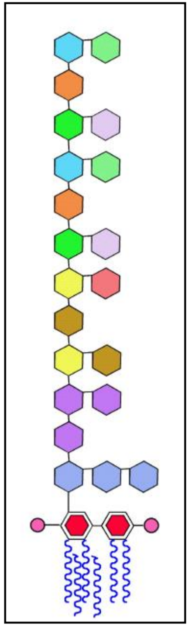
獲得免疫

微生物由来の糖鎖は自然免疫を活性化し、抗体産生を促す

細菌細胞壁ペプチドグリカン、グラム陰性菌リポ多糖、かび由来のβ-グルカンなど

抗腫瘍β-グルカン：シイタケ由来のレンチナン、スエヒロタケ由来のシゾフィランなど





Brief history of endotoxin (内毒素) & lipopolysaccharide (リポ多糖)

- Pfeiffer 1892, discovery of two toxins from *Vibrio cholerae*

  Heat unstable exotoxin

  Heat stable endotoxin
- Westphal 1945, isolation of lipopolysaccharide (LPS), LPS is endotoxin
- Westphal 1957, lipid A is endotoxic principle

- Shiba, Kusumoto, Lüderitz, Galanos, Brade, Zähringer, Seydel, Lindner, Rietschel, 1983~1985,

  Correct structure of lipid A
- Shiba, Kusumoto, 1983~1985,

  Total synthesis of lipid A

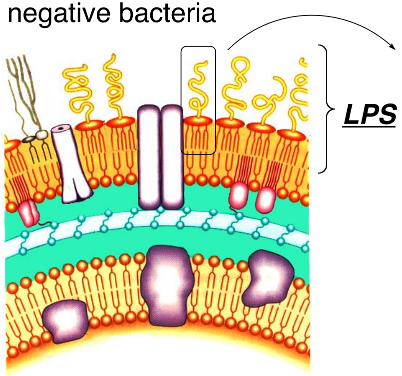
リポ多糖 (LPS), = 内毒素(Endotoxin)

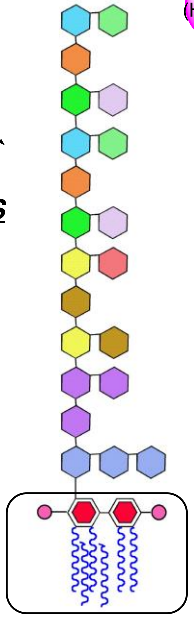
Immunostimulating activity, Antitumor activity, Lethal toxicity

Richard Pfeiffer identified endotoxin in 1892.

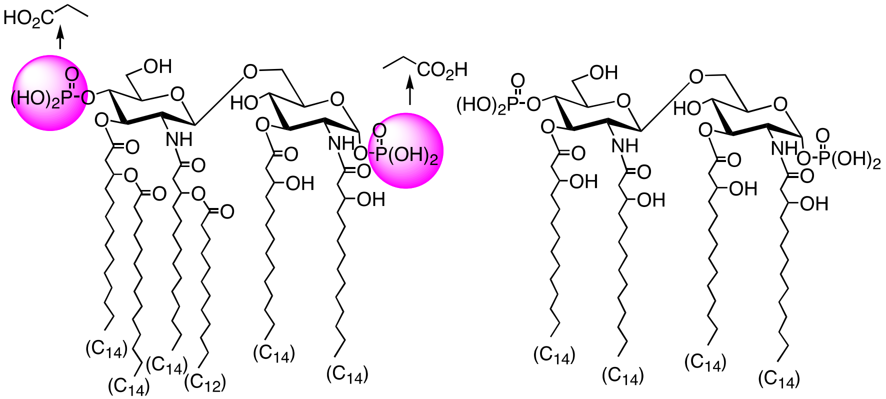
Lüderitz O, Westphal O,
Phenol-water extraction

Cell surface of Gram-negative bacteria





Lipid A



Escherichia coli lipid A

Highly inflammatory
Potent immunostimulant
Endotoxic principle

Biosynthetic precursor Ia
(lipid IVa)

human: antagonistic
mouse: endotoxic

1983 Total Synthesis, Kusumoto, Shiba
1984, 1985 Activity, Galanos

Tetsuo Shiba

Shoichi Kusumoto

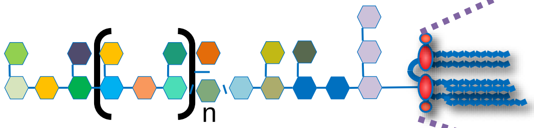
Ernst Th. Rietschel

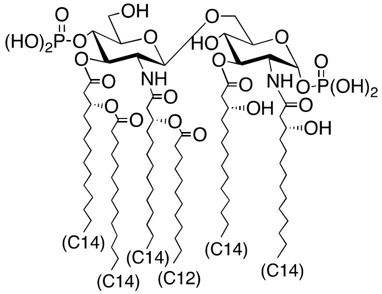
Otto Westphal

Improved synthesis was required for various bio-functional studies.

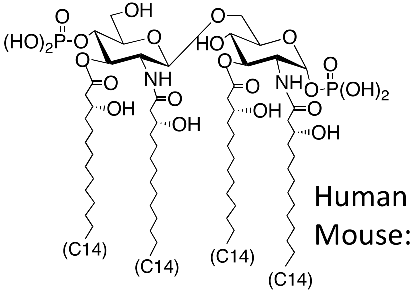
TLR4/MD-2 complex is receptor for LPS and Lipid A

LPS: potent immunostimulatnts
highly inflammatory, lethal toxicity





E. coli Lipid A:
Endotoxic principle

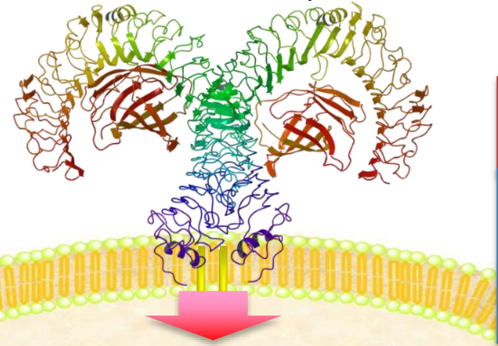


Human: antagonist
Mouse: agonist

Biosynthetic precursor, Lipid IVa
Key molecule in endotoxin study
Species specific response

Dr. Yoshizaki, Dr. Liu, Dr. Oikawa, Dr. Suda

TLR4/MD-2 complex



- Induction of pro-inflammatory cytokines
- Activation of immunity
- Enhancement of antibody production

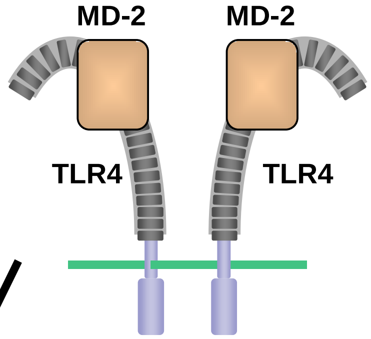
1998 TLR4: Beutler (Nobel Prize 2011)
1999 MD-2: Miyake

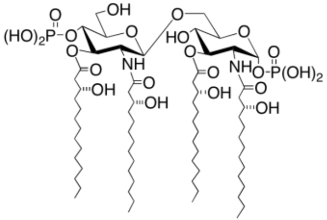
2000 Species specific response with Lipid IVa via TLR4:Golenbock
2001 Species specific response with Lipid IVa via MD-2: Miyake
2003 Direct binding of MD-2 with Lipid A and Lipid IVa: Miyake
2007 3D-structure of MD-2/Lipid IVa complex: Ohto

2009 3-D structure of Human TLR4/MD-2/LPS complex: Lee

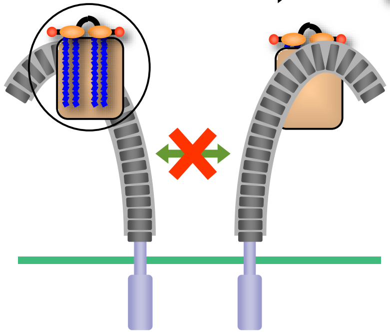
2012 3-D structure of Mouse TLR4/MD-2/Lipid IVa: Ohto

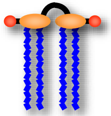
Recognition of LPS/lipid A with TLR4/MD-2



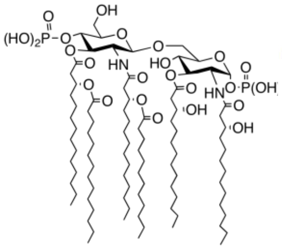


Lipid IVa
(*Yersinia pestis* lipid A)
antagonist

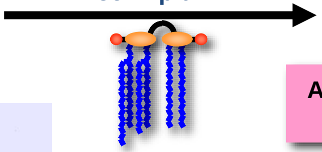




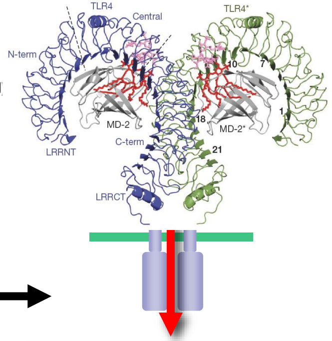
Inhibition of dimerization
No immunostimulation



***E.coli* lipid A**

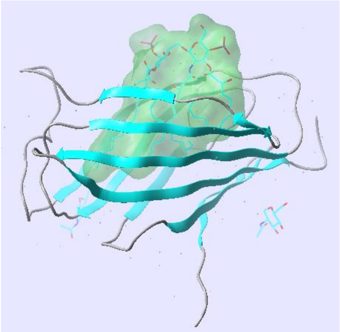


Lee, J-O. *Nature* 2009



Activation of TLR4/MD-2
Signal transduction

Miyake, Fukase, Kusumoto
***J Endotoxin Res* 2004, *10*, 257.**
***International Immunol.* 2004, *16*, 961.**

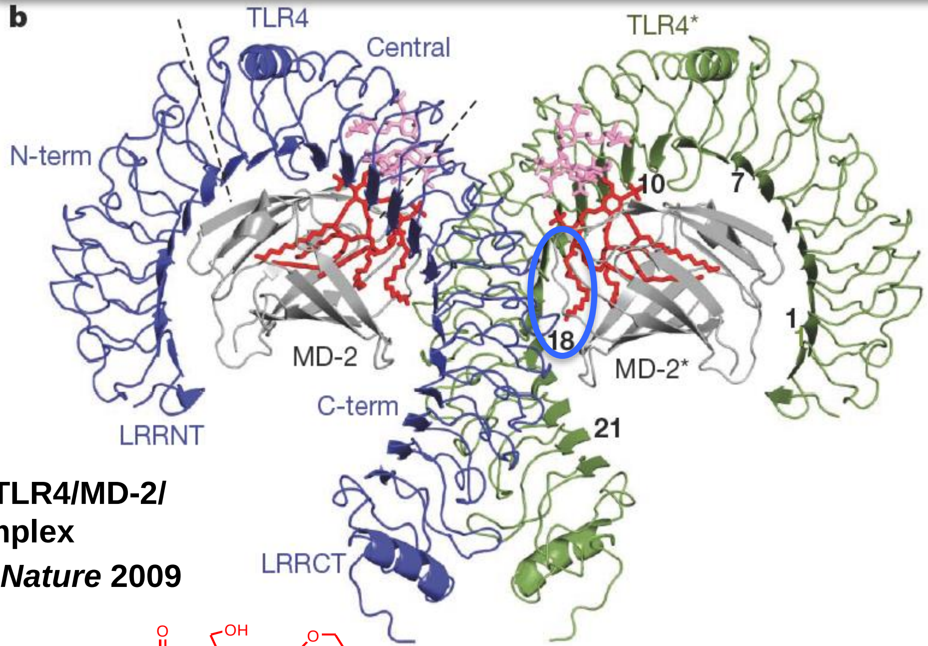


MD−2/lipid VIa complex
Ohto, Fukase, Miyake, Satow,
***Science*, 2007, *316*,1632.**

***Y. pestis* LPS does not activate TLR4/MD-2 and paralyzes immune response to determine the pathogenicity.**

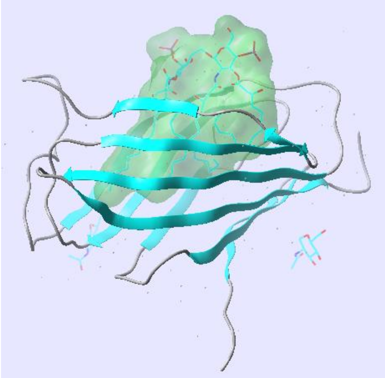
Lien *et al*., *Nature Immunol.* 2006, 7, 1066.

Uncontainable acyl group induces the dimerization



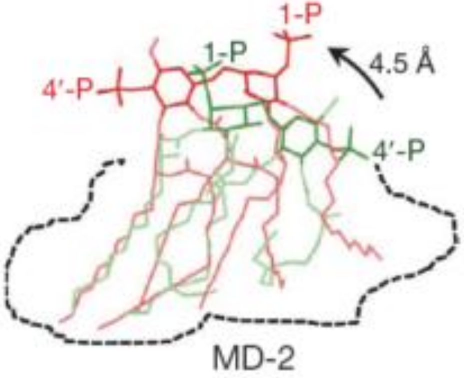
Human TLR4/MD-2/ LPS complex

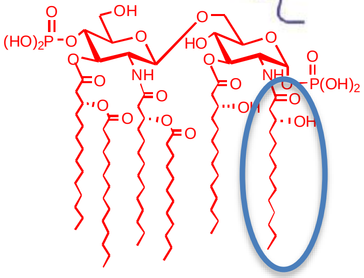
Lee, J-O. *Nature* 2009



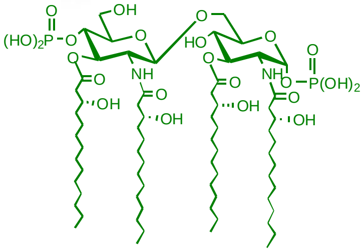
MD-2/lipid IVa complex

Ohto, Fukase, Miyake, Satow, *Science*, 2007, *316*,1632.



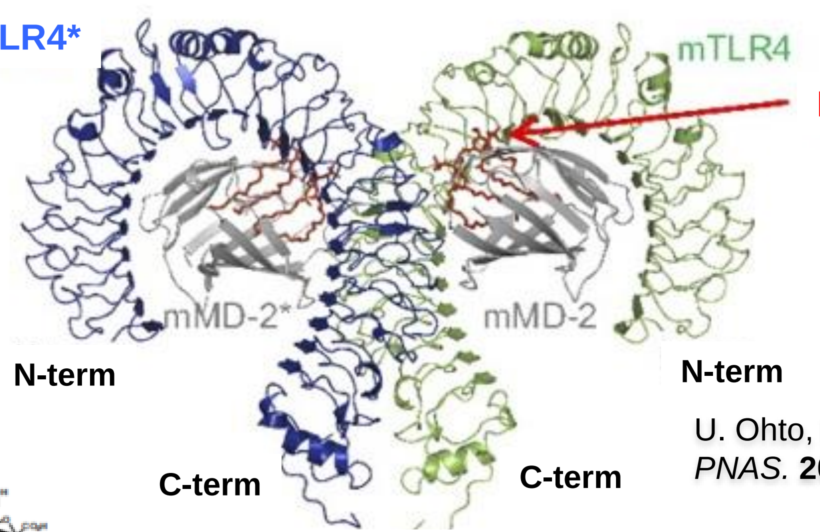


agonist



antagonist in human

Mouse TLR4/MD2/lipid IVa: Crystal structures are almost identical with TLR4/MD2/Re-LPS but dimerization dynamics is different.

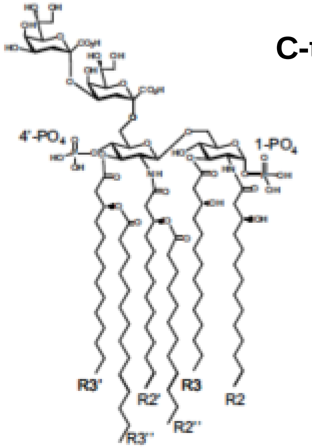


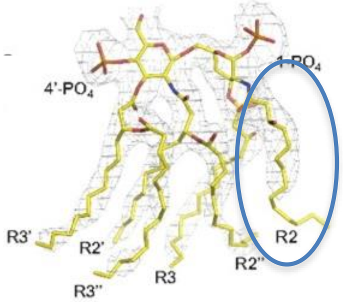
Lipid IVa

Modest agonist in mouse
Very low dimerization of TLR4/MD-2

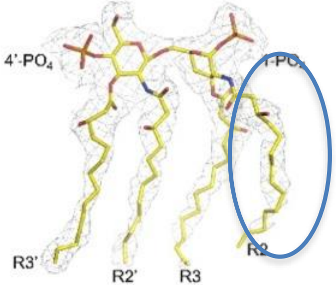
結晶化の条件と生体反応の条件は異なる

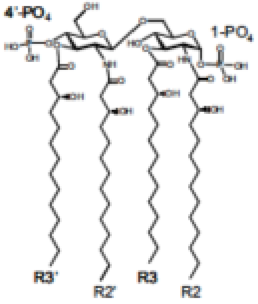
U. Ohto, K. Fukase, K. Miyake, T. Shimizu, *PNAS.* **2012**, *109*, 7421





Re-LPS: strong agonist, high dimerization





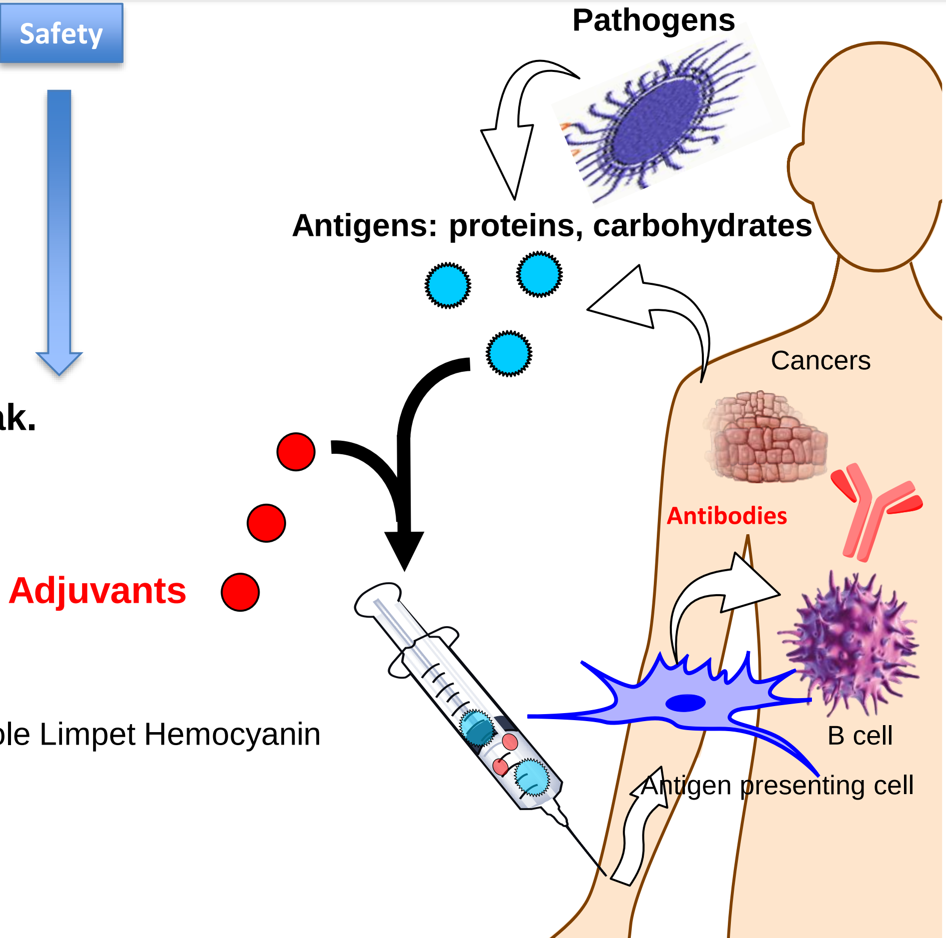
Lipid IVa

Vaccine therapy

Live attenuate vaccines

Killed vaccines (bacteria),
Inactivated vaccines (virus)

Purified antigen vaccines



Issue: Immunogenicity of pure antigens is weak.

i) Addition of adjuvants

Adjuvants enhance the immune response to the antigens.

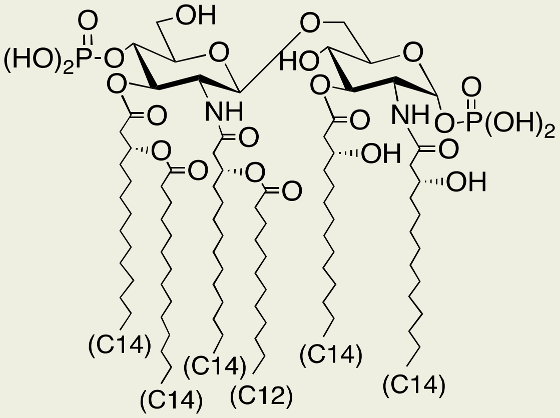
ii) Conjugation with carrier proteins such as Keyhole Limpet Hemocyanin (KLH), Tetanus toxoid

iii) Conjugation with adjuvants

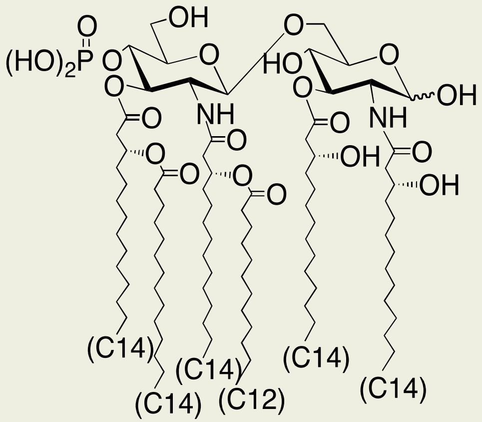
FDA approved adjuvants

Brand names	Composition	Applicable diseases	Mode of action
Alum (1924)	$AlPO_4$ or $Al(OH)_3$	Diphtheria Tetanus Pertussis, etc.	Antigen insolubilization Innate immune activation (NALP3?, TLR9)
AS04 (GSK：2005)	MPL, $Al(OH)_3$	Hepatitis B Human papilloma	Innate immune activation (TLR4)
MF59 (Novartis：1997)	Squalene，Tween 80，Span 86 (lipid, surfactant)	Influenza	Promotion of antigen uptake (oil-in-water emulsion)
AS03 (GSK：2009)	Squalene，Tween 80，α-tocopherol (lipid, surfactant)	Influenza	Promotion of antigen uptake (oil-in-water emulsion)
Virosomes (Berna Biotech：2000)	Lipids hemagglutinin	Influenza Hepatitis A	Promotion of antigen uptake (Virus particles)
AS01B （GSK：2017）	MPL, QS-21（saponin from soap bark tree）, liposomal formulation	Herpes simplex	Innate immune activation (TLR4)
CpG 1018 （Dynavax Technologies：2017）	CpG motif (DNA)	Hepatitis B	Innate immune activation (TLR9)

Development of lipid A analogs with reduced toxicity

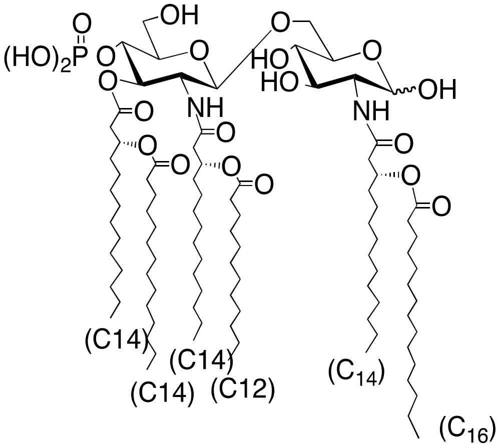


E. coli lipid A
Highly inflammatory, toxic



Monophosphoryl lipid A (MPL)
Mild immunostimulation

１位リン酸基の除去で、活性は弱くなるが、炎症性、毒性は大きく低減

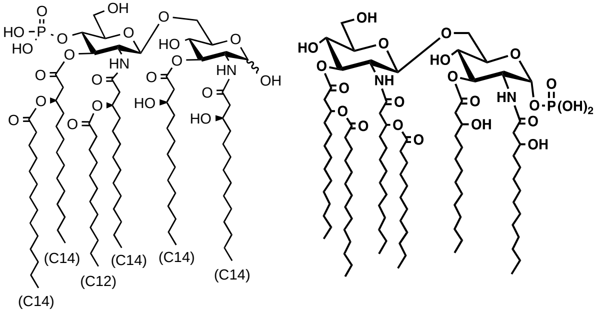


3D-MPL (GSK)
Selective Activation of Antiviral Signaling
Adjuvant for anti-viral vaccines
Hepatitis C Virus Vaccine
Human Papillomavirus Vaccine
(Cervical Cancer Preventive Vaccine)

抗ウイルス応答に偏る

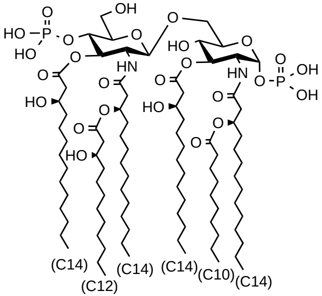
Adjuvant candidates in our group: appropriate immunoactivation with low inflammatory responses

TLR4 ligands



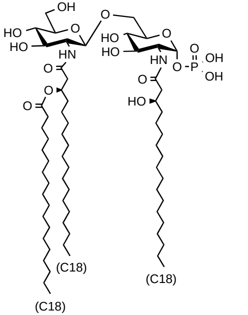
Monophosphoryl lipid A (*E. coli*)

- **Low inflammatory**



Symbiotic bacteria (*Alcaligenes faecalis*)

- **Low inflammatory**
- **Mild immunostimulation**
- **Th1, Th2, and Th17**

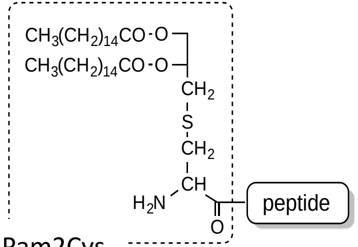


Parasitic bacteria (*H. pylori*)

- **Suppression of acute inflammation**
- **Chronic inflammation**
- **Induction of Th1 response**

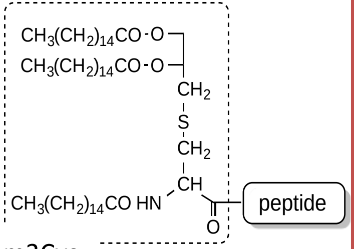
TLR2 ligands

TLR2-TLR6



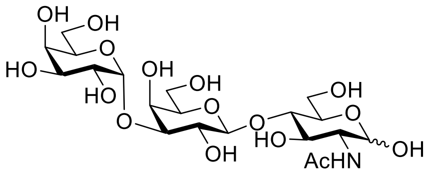
Pam2Cys

TLR2-TLR1



Pam3Cys

Natural antibody ligands



α-gal epitope

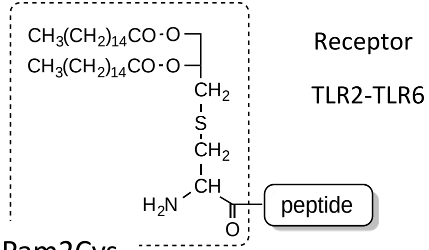
TLR2 agonists as safe and effective adjuvants

Trumenba: lipoprotein having triacylated Cys (TLR2/TLR1 agonist), self-adjuvanting vaccine against *Neisseria meningitidis* sero group B.

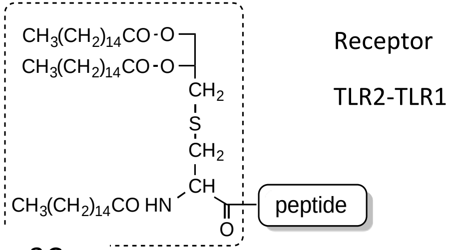
Luo Y. et al., AAPS J. 2016, 18, 1562.

Lipopeptides can stimulate Th1 and antitumor responses via TLR2/TLR1 or TLR2/TLR6.

Robust immunoresposnse induced by three component vaccine: Boons et al., *Nat Chem Biol*. 2007, 3, 663.
MUC1 glycopeptide-lipopeptide conjugate: Kunz, Li et al., *Angew. Chem. Int. Ed*. 2010, 10, 49, 3688
NK activation: Seya, Fujimoto, Fukase, et al., *Microbes Infect*. 2011, 13, 350.
CTL activation: Seya, Hashimoto. Fujimoto, Fukase, et al., *Innate Immun*. 2018, doi: 10.1177/1753425918777598.
Promote Fcγ receptor expression: Shah, et al., *J Biol Chem*. 2013, 288, 12345.
Promote antigen presentation and T Cell activation: Guo et al., *Front Immunol*. 2017, 8, 158.

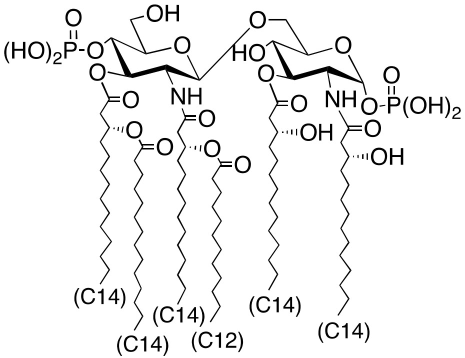


Pam2Cys

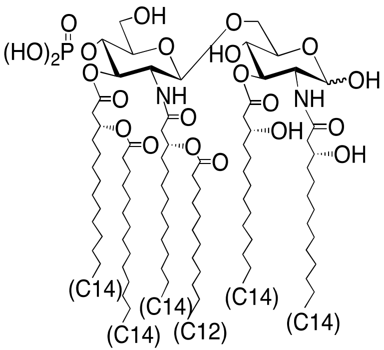


Pam3Cys

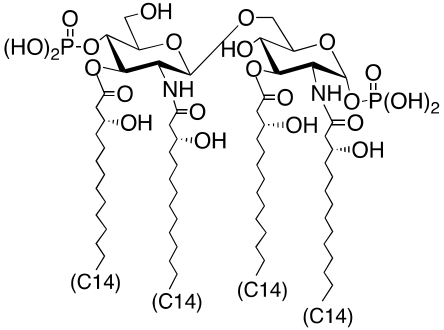
How to develop safe lipid A adjuvants?



E. coli lipid A:
Strong agonist
TLR4 dimerization
Highly inflammatory
High toxicity
Antibody production
Anti-viral response
Anti-bacterial response
Anti-tumor response



MPL:
Mild agonist
Very low dimerization
Low inflammatory
Low toxicity
Antibody production
Anti-viral response



Biosynthetic precursor
Antagonist in human
Inhibit dimerization
Mild agonist in mouse
Very low dimerization

TLR4の温和な活性化：
1. リン酸基の除去
2. アシル基の数を減らす

生物種によって、TLR4のアシル基の認識はかなり異なる

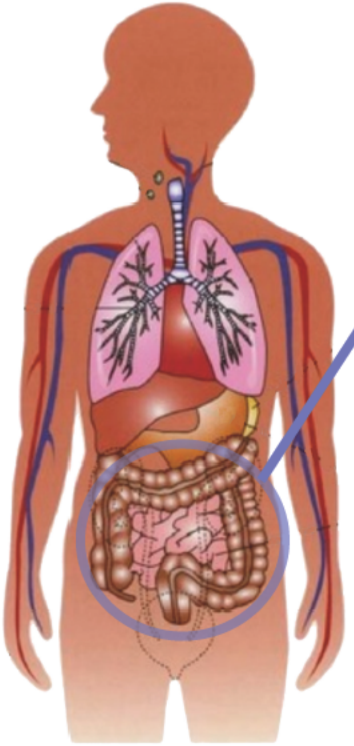
低毒性のリピドAを産生するバクテリアに着目

細菌との共生と防御

常在細菌　ヒト（標準的な大人）の場合…

重さにすると約 2 kg　の細菌を持っている。

自分自身の細胞数(60-70兆個）の 2 倍程度の数



主として

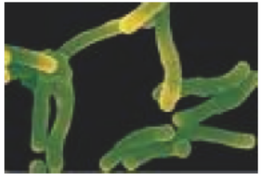
消化器官に存在（大腸他）

腸内細菌叢、細菌フローラ

（約 400 種類、100兆個）

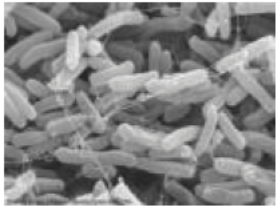
Science, **2005**, *308*, 1635

clostridium 属



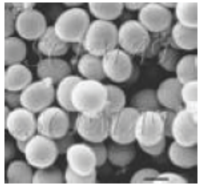
Bifidobacterium bifidum

ビフィズス菌



Escherichia coli

大腸菌



Staphylococcus aureus

黄色ブドウ球菌

細菌:

防御機構が必要な相手

であると同時に

恒常性維持に不可欠な存在

体内に寄生・共生する細菌に着目

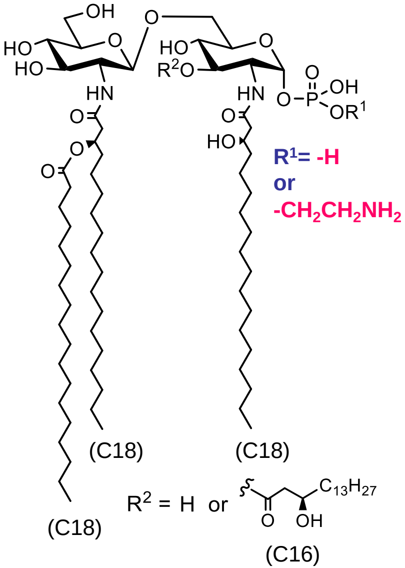
どこからが体内？

体外（消化器官内を含む）から体内（細胞・血管内）への侵入の検知と阻止

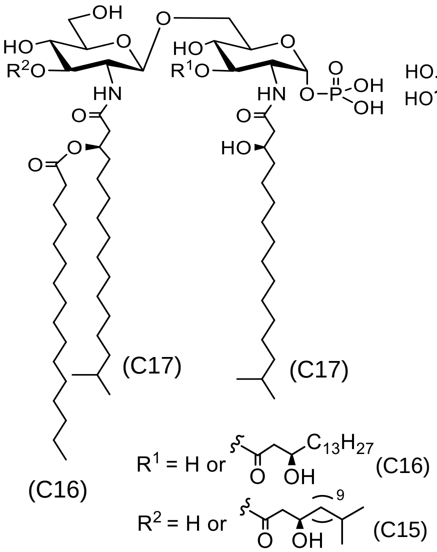
Low toxic Lipid As 1

Dr. Shimoyama

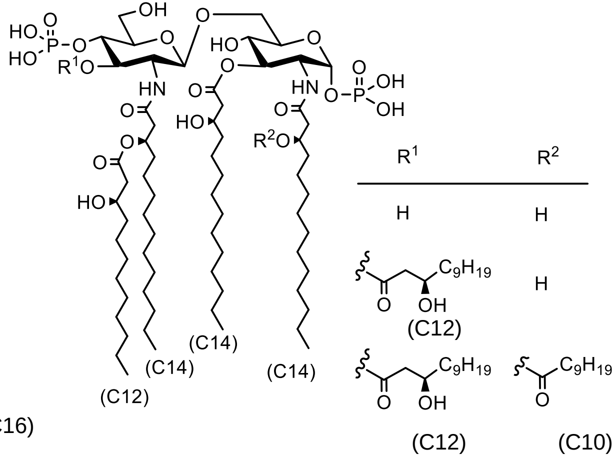
Prof. Fujimoto



Helicobacter pylori
Lipid A 寄生菌



Porphyromonas gingivalis
Lipid A 寄生菌



Alcaligenes faecalis
Lipid A 共生菌

TLR4アンタゴニスト 慢性炎症シグナル IL-12, IL-18

急性炎症を惹起しない
殺菌シグナルを抑制

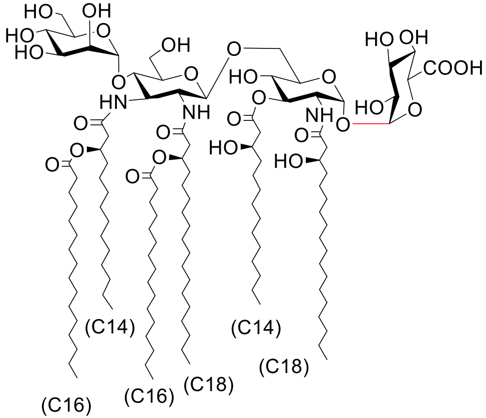
Th1

TNF-α, IL-6, IL-17

Th17

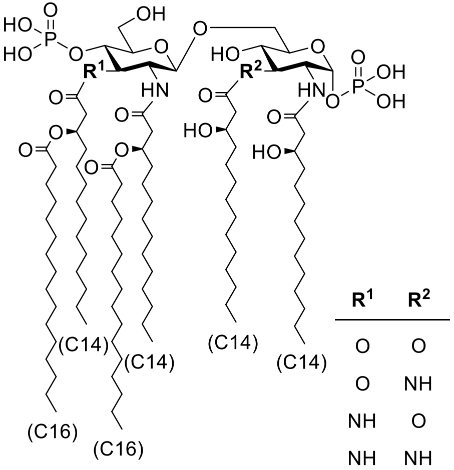
Low toxic Lipid As 2

*



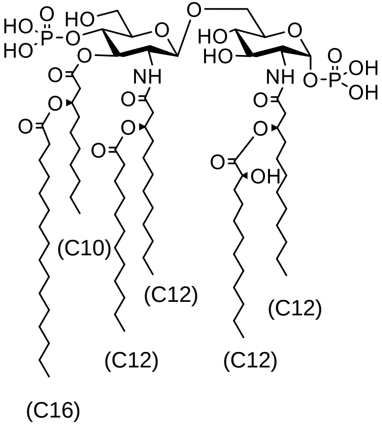
Acetobacter pasteurianus
Lipid A

黒酢に含まれる**



Campylobacter jejuni
Lipid A

Dr. Nakagawa
Dr. Matsuura
Mr. Fujie
Mr. Kanaoka

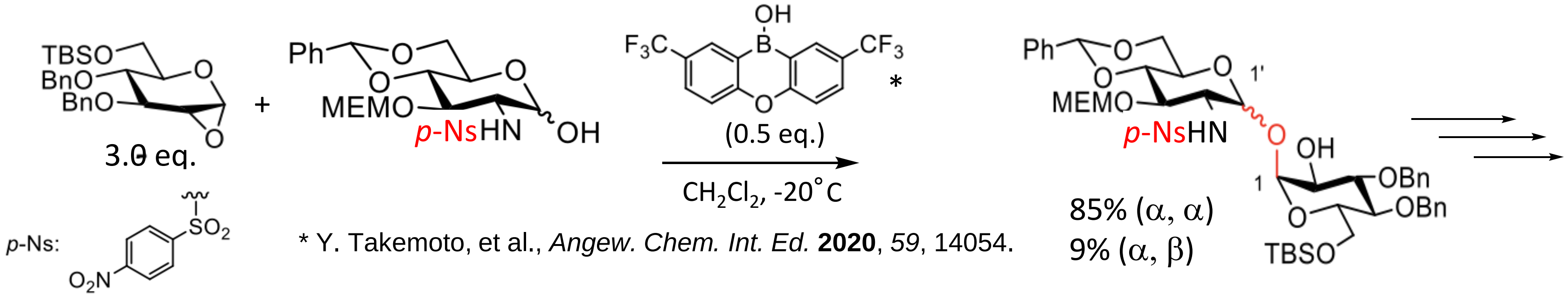


Pseudomonas aeruginosa
Lipid A

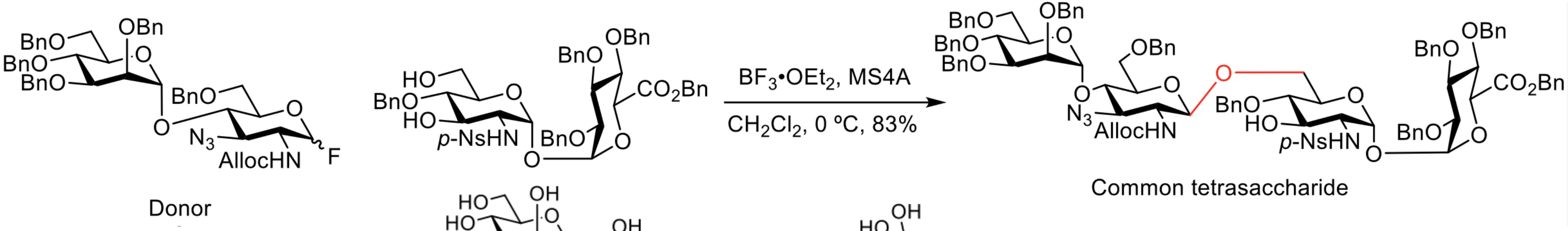
Mr. Oku

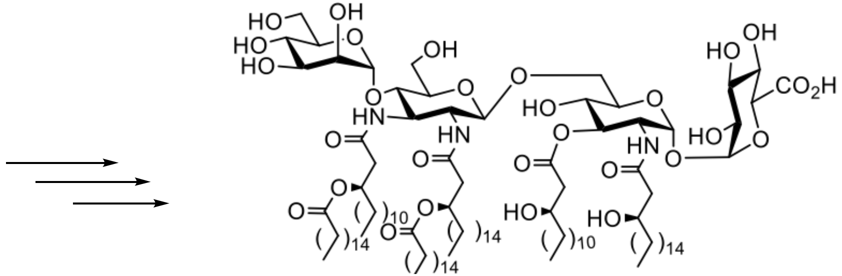
*Structural determination: Hashimoto, Suda, Fukase, Fujimoto et al., *J. Biol. Chem*. **2016**, *291*, 21184.
Hashimoto, Suda, Fukase, Fujimoto, Shigehisa et al., *J. Biosci. Bioeng.* **2013, *116*, 688.

Toral synthesis of *Acetobacter pasteurianus* lipid A



* Y. Takemoto, et al., *Angew. Chem. Int. Ed.* **2020**, *59*, 14054.





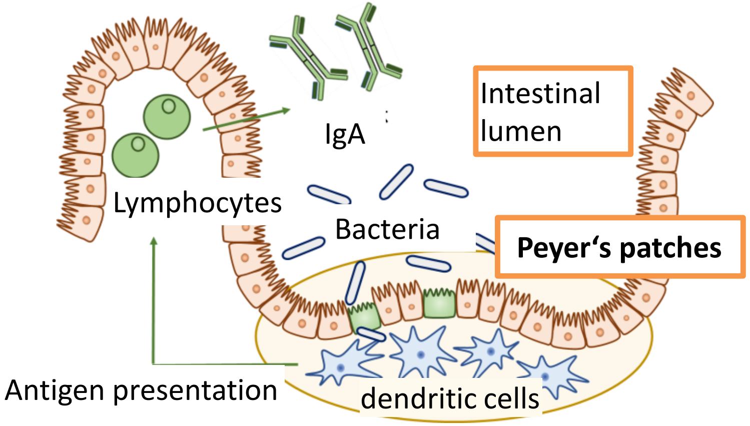
Dr. Yamaura

***Acetobacter pasteurianus* Hexaacylated lipid A**

酢酸菌リピドA:
- 温和な免疫増強作用
- 耐酸性
- 経口アジュバントの可能性

Yamaura H, Shimoyama A, Hosomi K, Kabayama K, Kunisawa J, Fukase K. *Angew Chem Int Ed Engl.* **2024**, 63, e202402922.

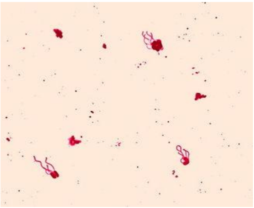
Gut symbiotic bacteria（腸内共生細菌）*Alcaligenes faecalis*



Peyer‘s patches（パイエル板）

- lymphoid tissue of the intestine（腸のリンパ組織）

腸管に侵入する細菌を捕捉し、それらを抗原提示細胞に届ける
パイエル板のリンパ球は、特異的な抗原に対するIgAを腸管腔内
に分泌し、恒常性を維持する



Alcaligenes faecalis

- パイエル板の樹状細胞（DC）内に生息
- パイエル板の*A. faecalis*は、致死毒性などの有害な作用を有さない

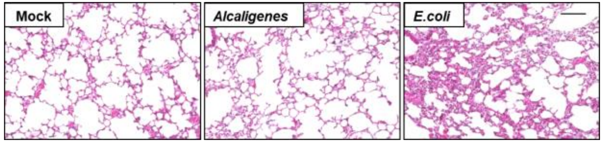
（Kiyono, Kunisawa, *et al.*, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 7419）

清野宏教授
Prof. Kiyono

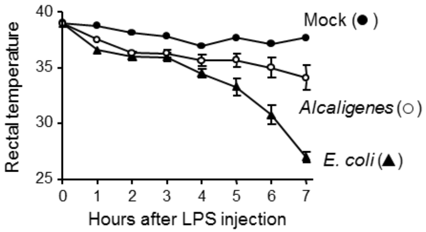
國澤純教授
Prof. Kunisawa

***A. faecalis* LPS has no lethal toxicity.**

Inflammatory symptoms in the lung (7 h after injection)



Mice rectal temperature



Dr. Shimoyama

抗体誘導能は優れている

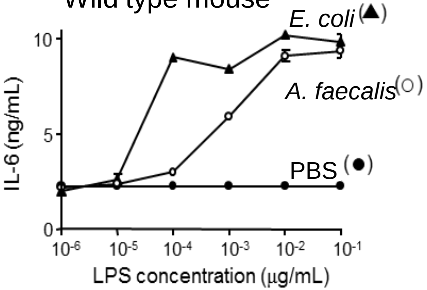
Immunological Function of *Alcaligenes faecalis* LPS

Immunostimulation (TLR4 dependent)

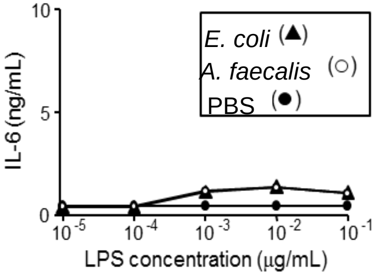
E. coli LPS > *A. faecalis* LPS

Moderate immune activation

Wild type mouse



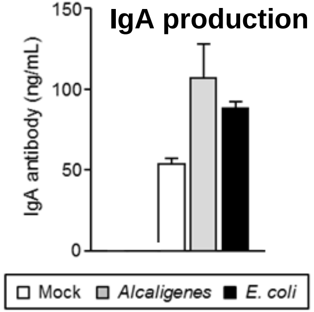
TLR4 KO mouse



Promotion of antibody production

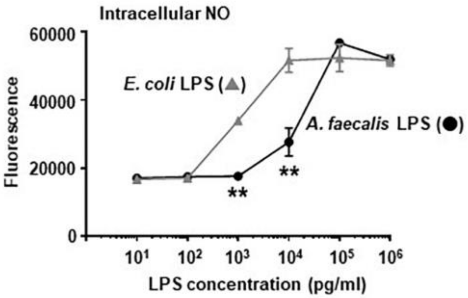
E.coli LPS ≅ *A. faecalis* LPS

Potent adjuvant effect

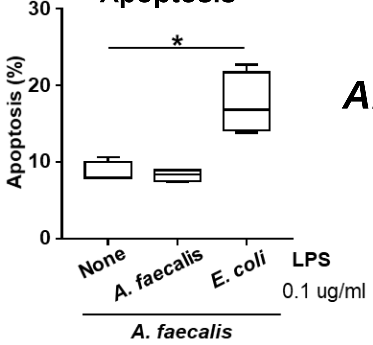


N. Shibata, J. Kunisawa, A. Shimoyama, K. Fukase, H. Kiyono *et al. Mucosal Immunology* **2018**,*11*, 693-702.

Intracellular NO production (mBMDCs)



Apoptosis



A. faecals **LPS does not induce cell death**

K. Hosomi, A. Shimoyama, K. Fukase, J. Kunisawa, et al. *Frontiers in Microbiology* **2020**, *11*, 561005.

Structural determination of *A. faecalis* LOS and synthesis of lipid A

Alcaligenes faecalis dried cell

↓

Pure LOS (Lipooligosaccharide)

↓

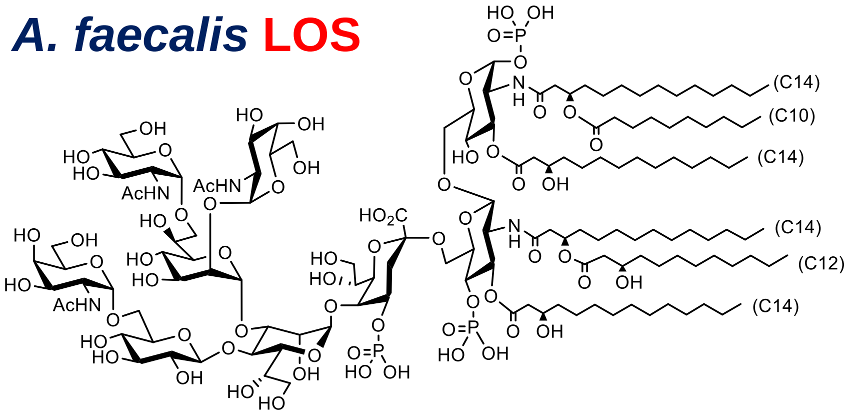
Structural determination by NMR, GCMS, MSMS

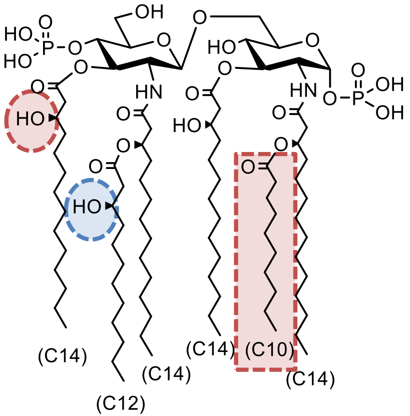
Università degli Studi di Napoli Federico II

Dr. Molinaro

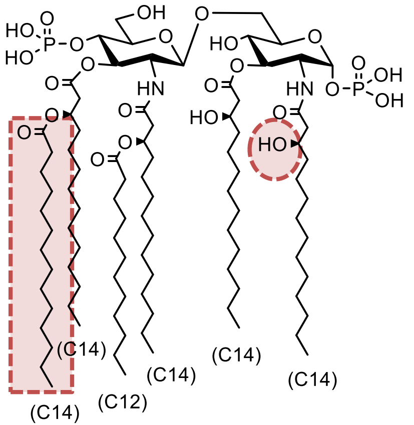
Dr. Di Lorenzo Dr. Silipo

A. faecalis LOS





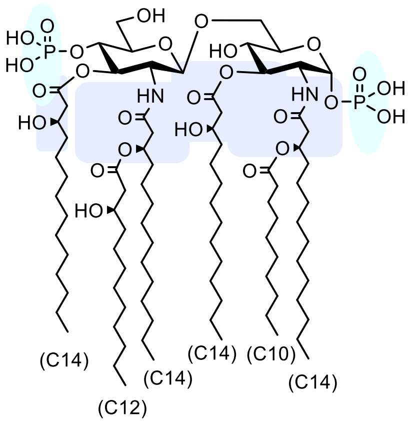
***A. faecalis* Lipid A**



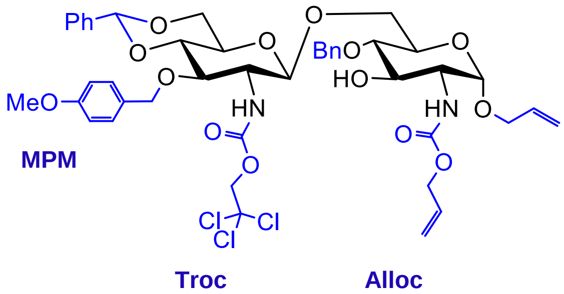
***E. coli* Lipid A**

Shimoyama, Di Lorenzo, Yamaura, Mizote, Palmigiano, Pither , Speciale, Uto, Masui , Sturiale, Garozzo , Hosomi , Shibata, Kabayama, Fujimoto, Silipo, Kunisawa, Kiyono , Molinaro, Fukase, *Angew. Chem. Int. Ed Engl.* **2021**, **60**, 10023.

Synthetic Strategy

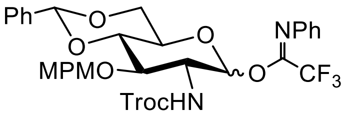


A. faecalis Lipid A

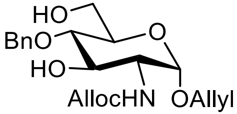


Key Intermediate

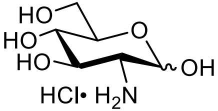
Shimoyama, A., *et al.*, *Chem. Eur. J.* **2011**, *17*, 14464-74.



Glycosyl Donor



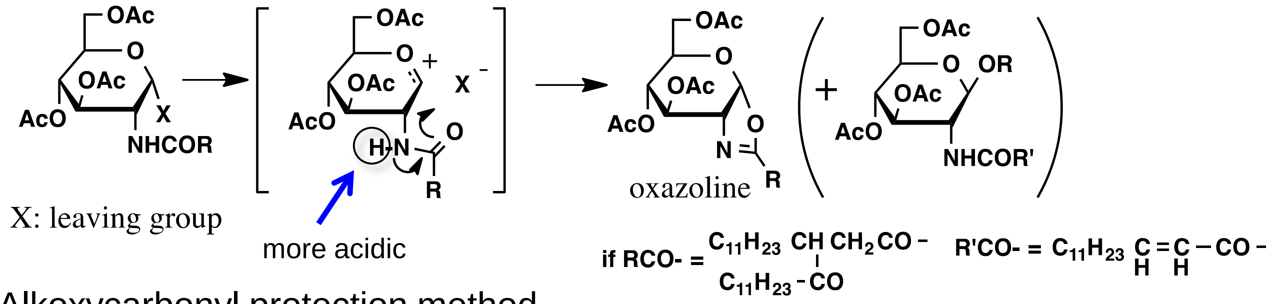
Glycosyl Acceptor



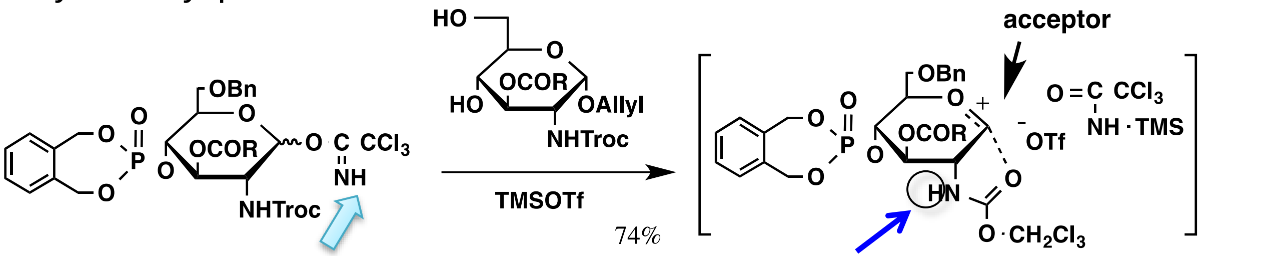
Glucosamine

β-Selective glycosylation with glucosaminyl donor

Oxazoline method

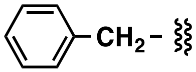


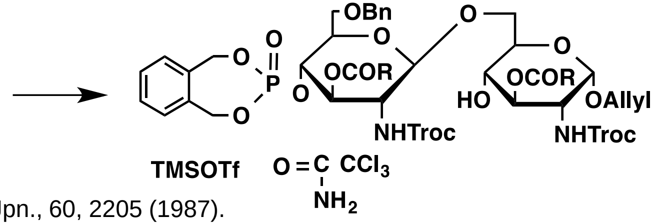
N-Alkoxycarbonyl protection method

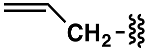


Schmidt's trichloroacetimidate

Troc: Cl_3CH_2OCO-

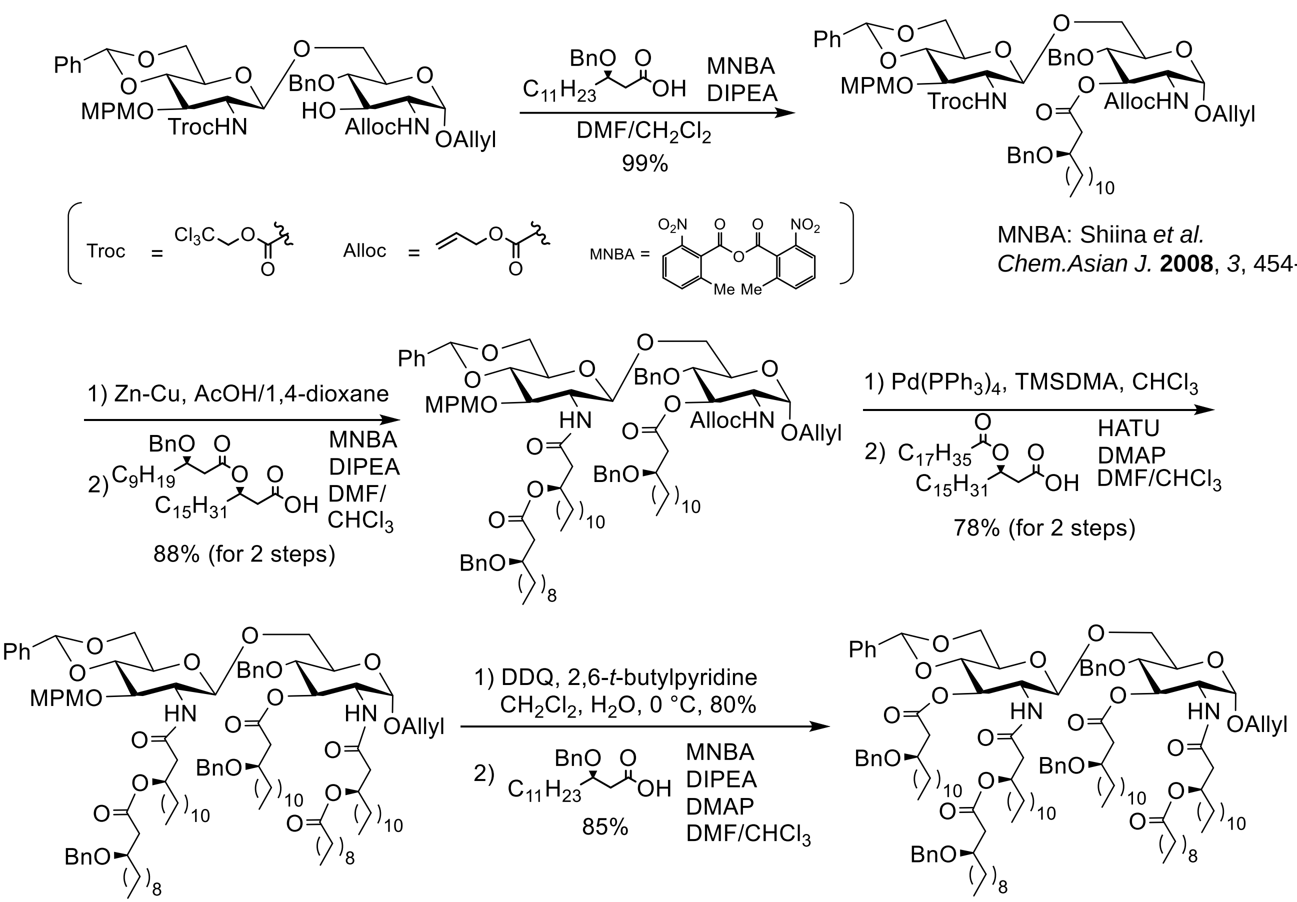
Bn: benzyl 



Allyl: 

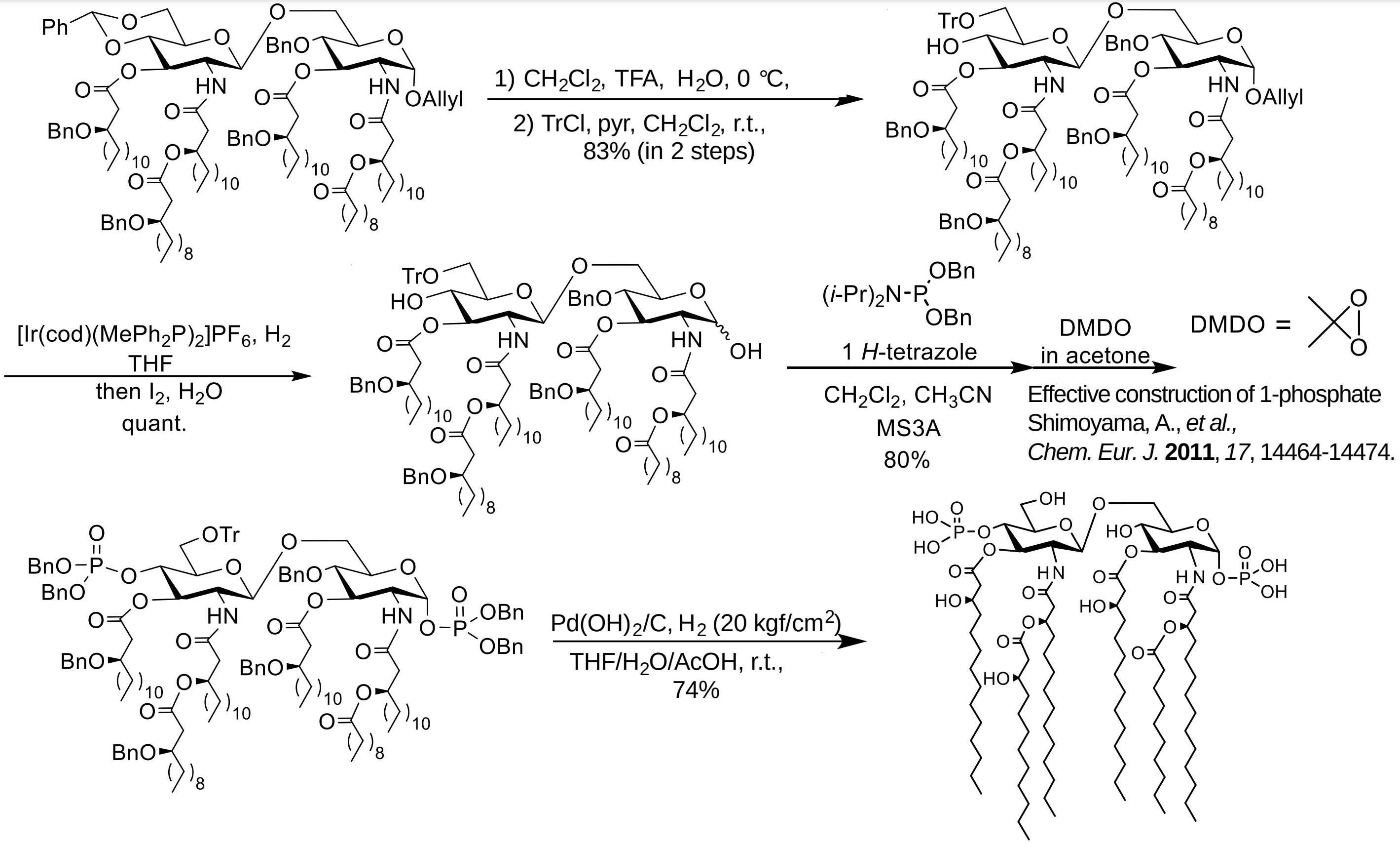
M. Imoto et al., Bull. Chem. Soc. Jpn., 60, 2205 (1987).
K. Fukase, et. al., Tetrahedron Lett., 36, 7455 (1995).

Synthesis of *Alcaligenes faecalis* lipid A (1)



MNBA: Shiina *et al.*
Chem.Asian J. **2008**, *3*, 454-461.

Synthesis of *Alcaligenes faecalis* lipid A (2)



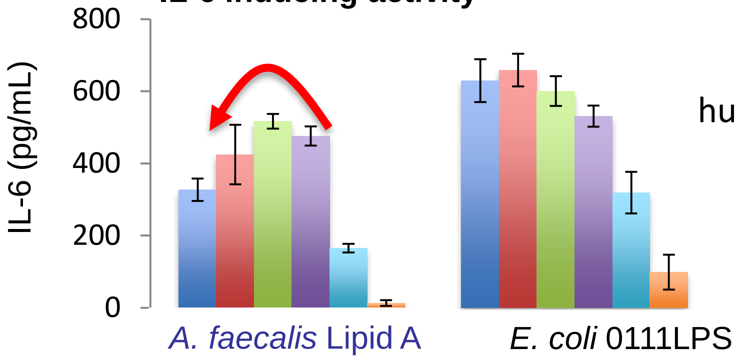
Patent application: JP2017-30179「Lipid A」

***A. faecalis* Lipid A**

12% from glucosamine (for 18 steps)

Synthetic *A. faecalis* lipid A is a mild TLR4 agonist

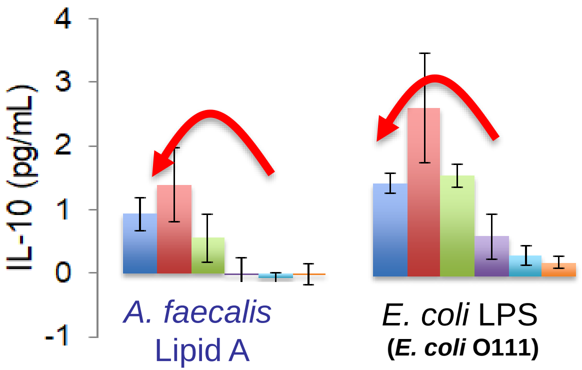
▪IL-6 inducing activity

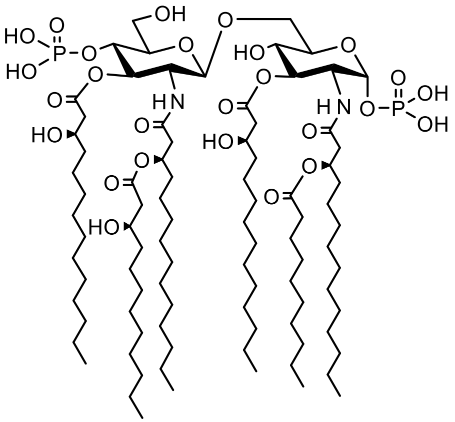


human macrophages derived from THP-1 cells

10 μg/mL 1 μg/ml 100 ng/mL
10 ng/mL 1 ng/mL 100 pg/mL

▪IL-10 inducing activity (anti-inflammatory effect)

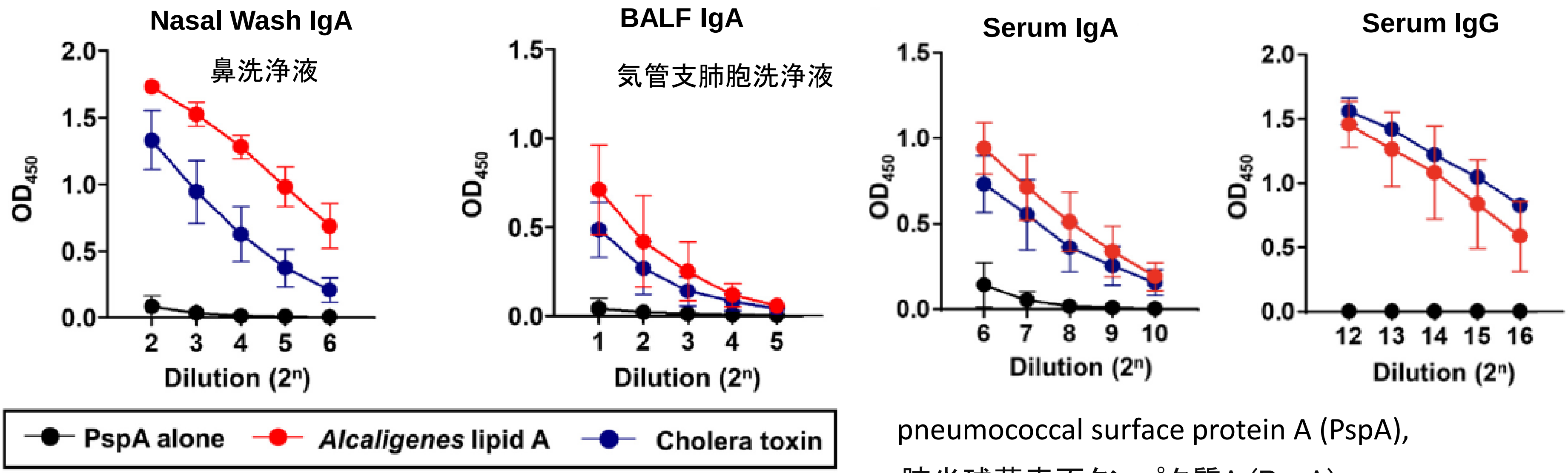




Development of a vaccine against *Streptococcus pneumoniae* using *A. faecalis* lipid A as a novel intranasal vaccine adjuvant

Chemically Synthesized Alcaligenes Lipid A Shows a Potent and Safe Nasal Vaccine Adjuvant Activity for the Induction of Streptococcus pneumoniae-Specific IgA and Th17 Mediated Protective Immunity

IgA: 粘膜で機能する抗体



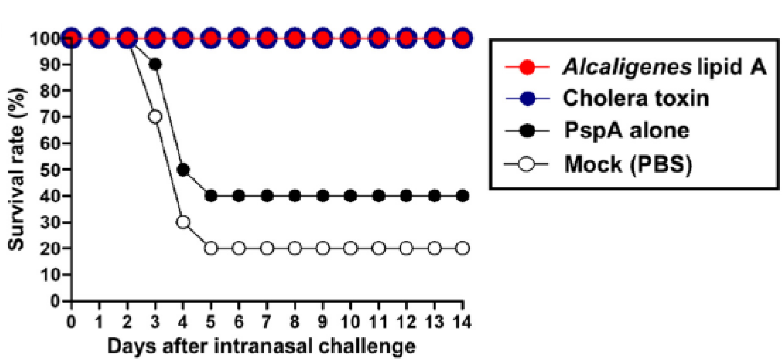
pneumococcal surface protein A (PspA),
肺炎球菌表面タンパク質A (PspA)

K. Yoshii, K. Hosomi, A. Shimoyama, Y. Wang, H. Yamaura, T. Nagatake, H. Suzuki, H. Lan, H. Kiyono, K. Fukase, J. Kunisawa
Microorganisms 2020, 8(8), 1102; https://doi.org/10.3390/microorganisms8081102

Development of a intranasal vaccine against *Streptococcus pneumoniae*

Nasal vaccination with PspA, together with *A. faecalis* lipid A protects against *S. pneumoniae* infection.

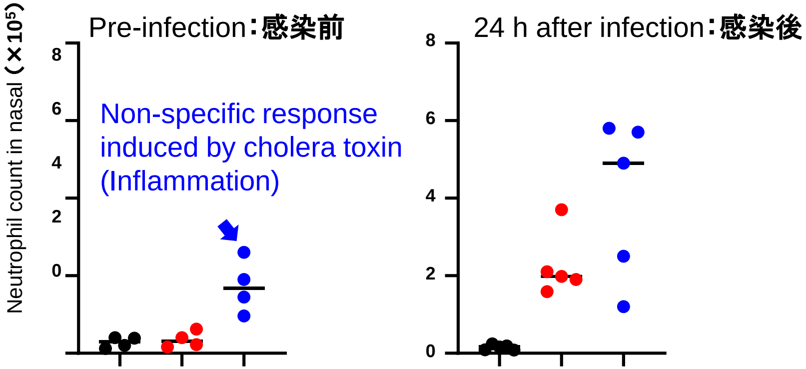
ワクチンの感染予防効果



- *A. faecalis*リピドAの感染予防効果は、コレラ毒素と同等
- *A. faecalis*リピドAの粘膜免疫活性化能は、コレラ毒素よりも優れる
- *A. faecalis*リピドAは、より低い炎症性を示し、抗炎症性サイトカイン IL−10を効率的に誘導
- *A. faecalis*リピドAは体内に存在（共生細菌由来）、高い安全性

Induction of specific neutrophil responses only after infection

好中球の応答

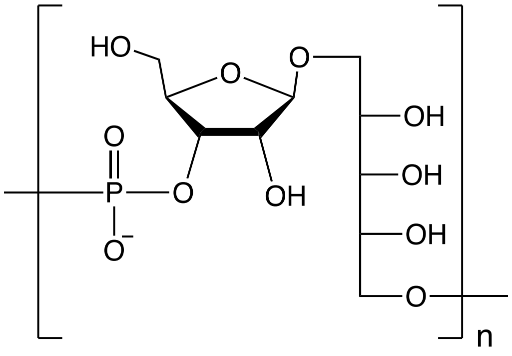


Unlike cholera toxin, *A. faecalis* lipid A did not elicit local inflammation, but induced a rapid response only upon infection.

コレラ毒素とは異なり、*A. faecalis*リピドAは局所炎症を引き起こさず、感染時にのみ迅速な反応を誘導

The effect on ActHIB by A. faecalis lipid A

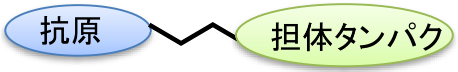
ActHIB®: vaccine against *Haemophilus influenzae* type b (Sanofi) for use in children
2 months through 5 years of age
Haemophilus b PRP–tetanus toxoid (TT) conjugate vaccine



Hib capsular polysaccharide PRP

Immunization to mice
three immunizations at 1-week intervals
The serum was collected after 1 week.

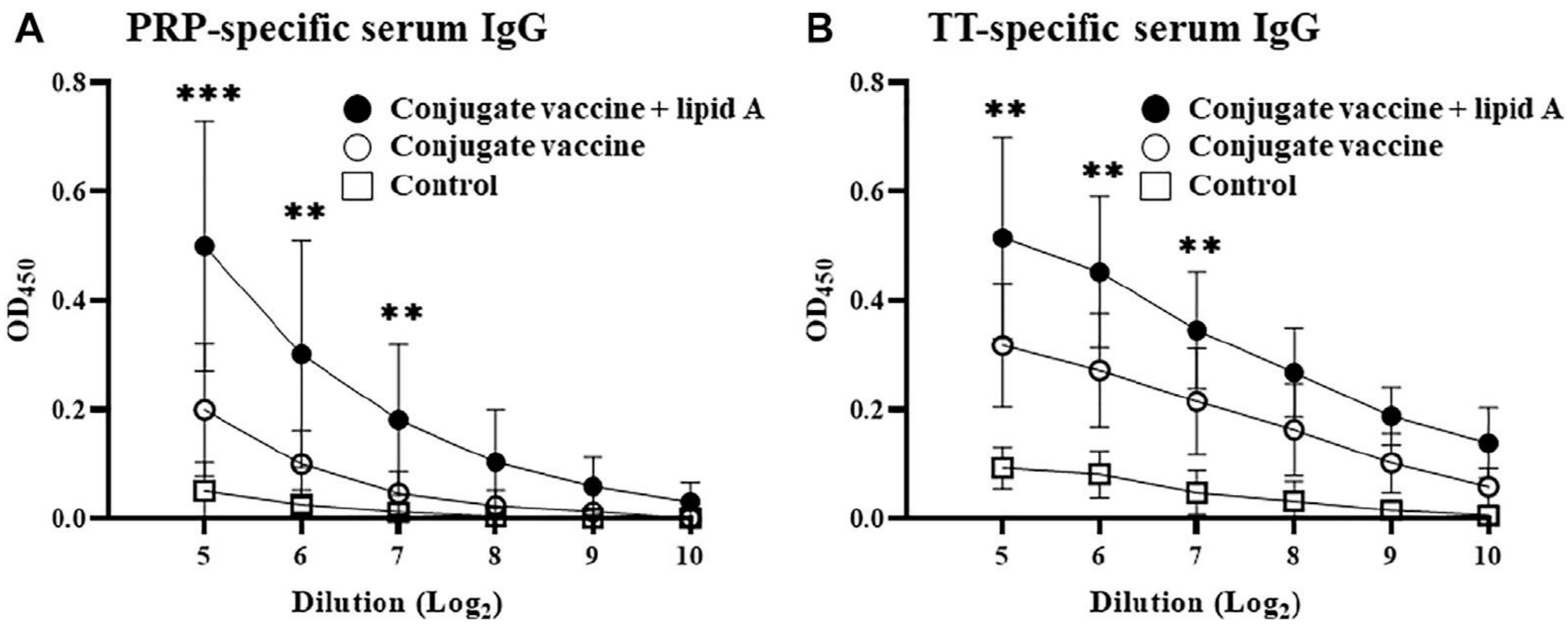
Chemically Synthesized Alcaligenes Lipid A as an Adjuvant to Augment Immune Responses to *Haemophilus Influenzae* Type B Conjugate Vaccine
Liu, Shimoyma, Kiyono, Fukase, Kunisawa et al.,
Front. Pharmacol., 22 October 2021
https://doi.org/10.3389/fphar.2021.763657



＋アジュバント（Adjuvant） （ミョウバンアジュバントなど）

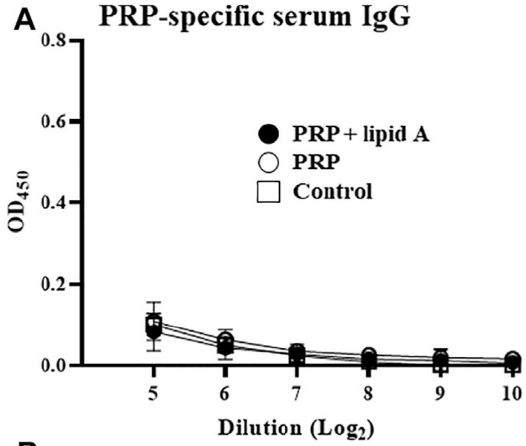
（ActHIB®には含まれていない）

Alcaligenes lipid A enhanced antigen-specific IgG production in *Haemophilus* B conjugate vaccination.



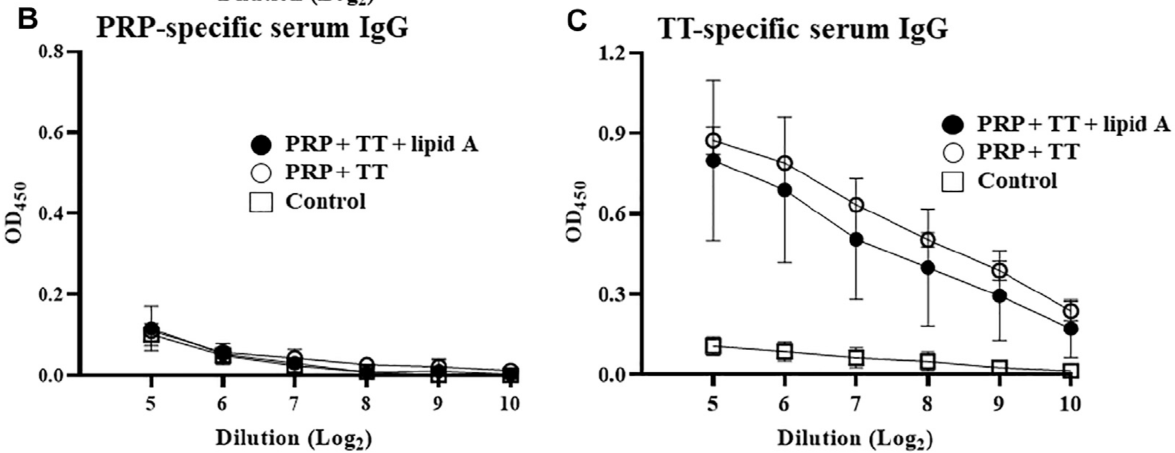
Mice were immunized subcutaneously with PBS (control group) or *Haemophilus* b conjugate vaccine containing 0.01 µg of PRP with or without 1 µg of *Alcaligenes* lipid A.

Conjugation is essential for enhancement of PRP-specific IgG production by *Alcaligenes* lipid A.



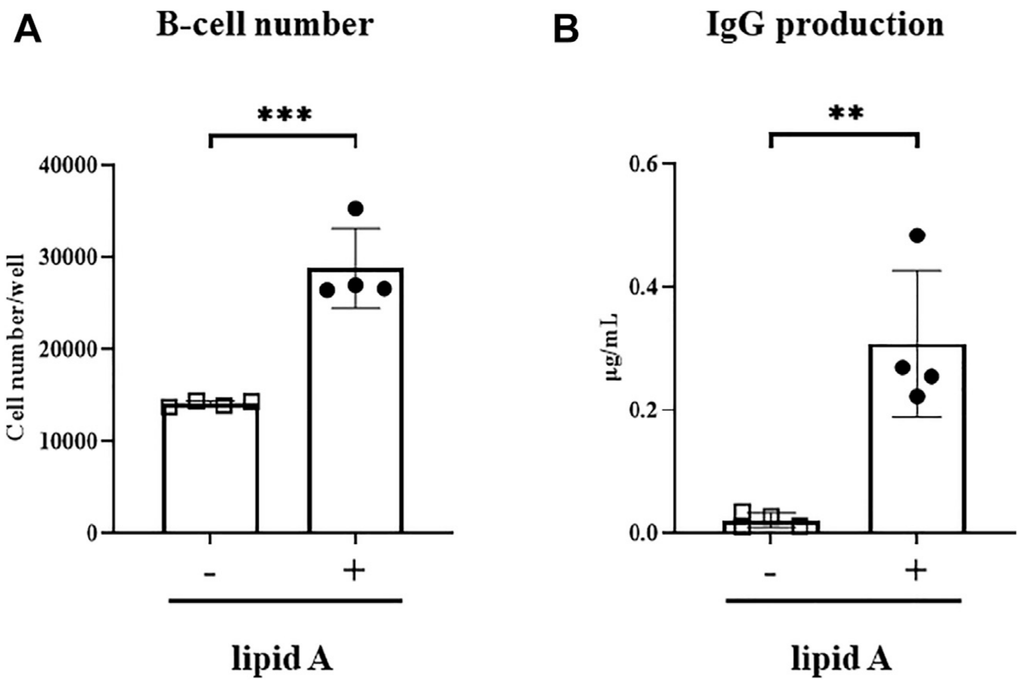
Conjugation is necessary for lipid A dependency for IgG production to both PRP and TT.

担体となる破傷風トキソイドは、抗原性の向上とT細胞の活性化の両方に重要（破傷風トキソイドはヘルパーT細胞エピトープを含む）



Alcaligenes lipid A directly activates B cells.

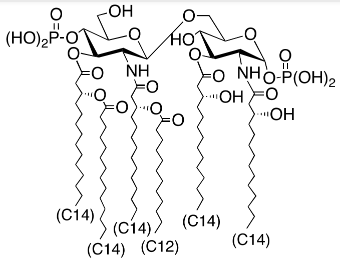
Alcaligenes lipid A はB細胞を直接活性化する（B細胞はTLR4を発現）



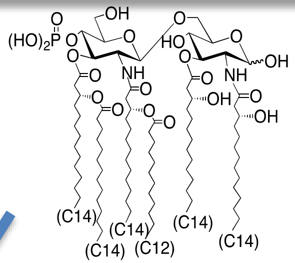
Splenic B220+ cells were isolated from naive mice. After 4 days of culture with (+) or without (–) *Alcaligenes* lipid A.

なおリピドAはT細胞を直接は活性化しない

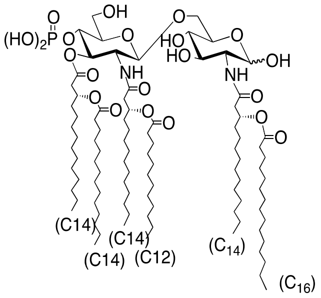
Receptor dynamics is critical for activity of lipid A.



***E. coli* Lipid A:**
Strong agonist
TLR4 dimerization
CD14 dependent
Internalization

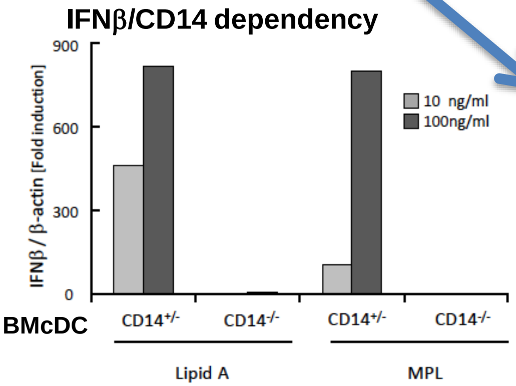


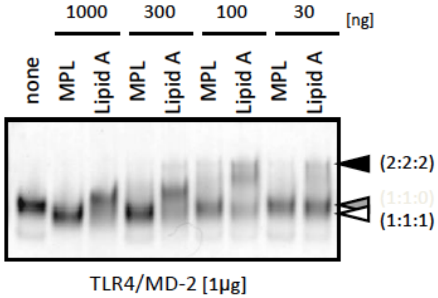
***E. coli* MPL:**
Mild agonist
Very low dimerization
CD14 independent
LBP dependent
Internalization

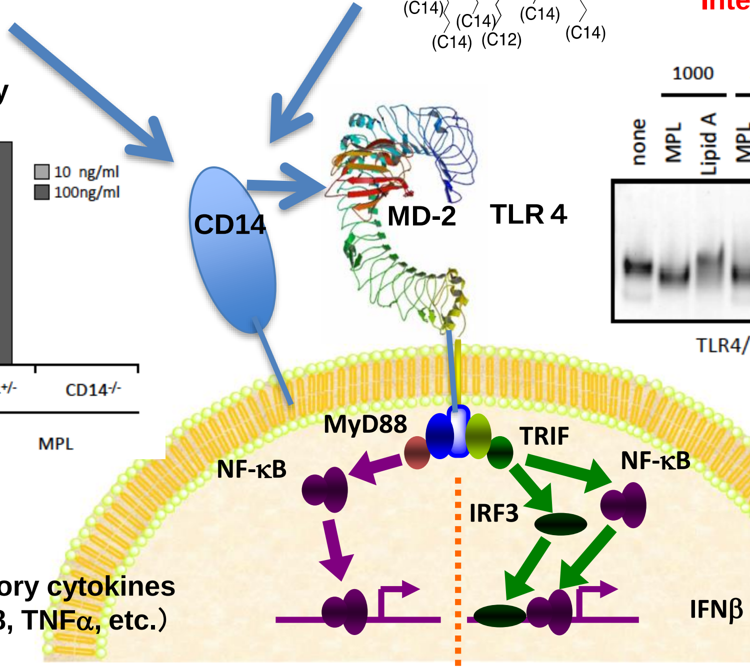


MPLA-SM
(3D-MPL)

E. coli MPL よりも、
TRIF選択的である





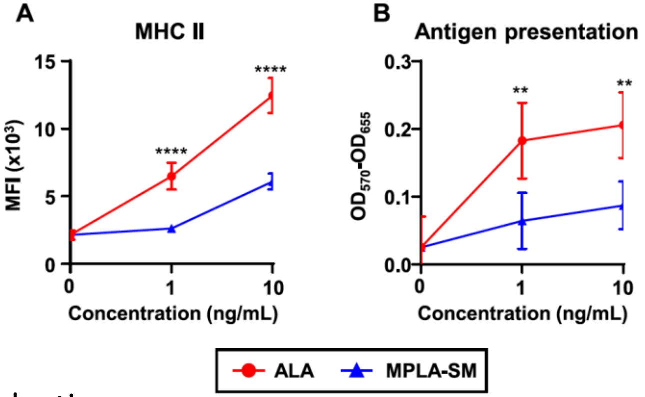


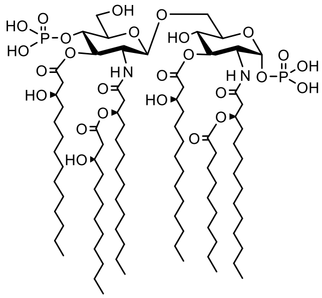
Salmonella minnesota Re595
Lipid A (L-0774),
MPL (L-6895), Sigma.

Int Immunol. 2014, 26:307-314. Tanimura N, Saitoh S, Ohto U, Akashi-Takamura S, Fujimoto Y, Fukase K, Shimizu T, Miyake K

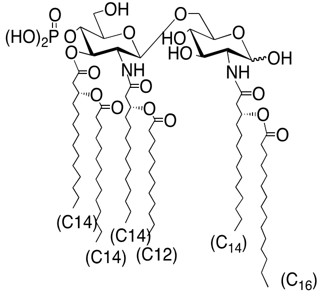
A. faecalis lipid A elicited broad immune responses

A. faecalis lipid A (ALA) upregulated the expression of MHC II and antigen presentation on T cells.





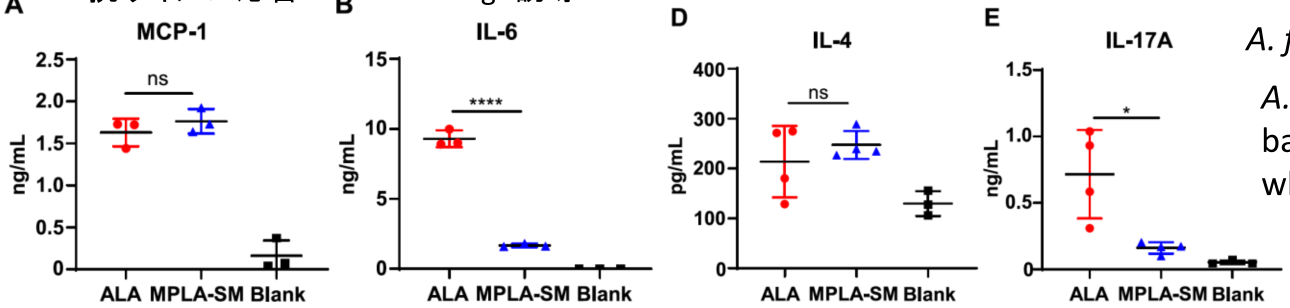
ALA



MPLA-SM

Cytokine productions

抗ウイルス応答 IgA誘導 抗原提示細胞の増殖・分化 細菌感染防御

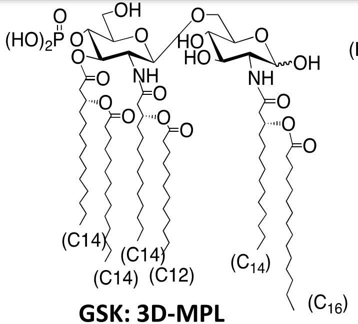


A. faecalis lipid A is a universal adjuvant.

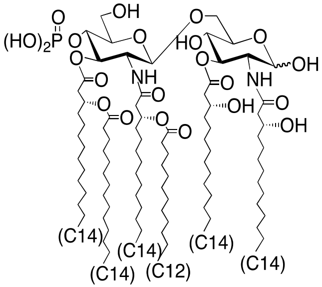
A. faecalis lipid A activates both anti-bacterial and anti-viral immunity, while MPL skews anti-viral response.

Sun, Hosomi, Shimoyama, Fukase, Kunisawa, et al.,Int. Immunopharmacol. 2023, 117:109852.

リピドAの構造により、炎症シグナルの制御が可能

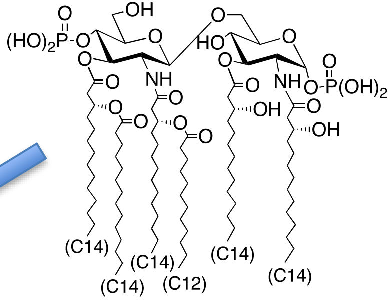


GSK: 3D-MPL

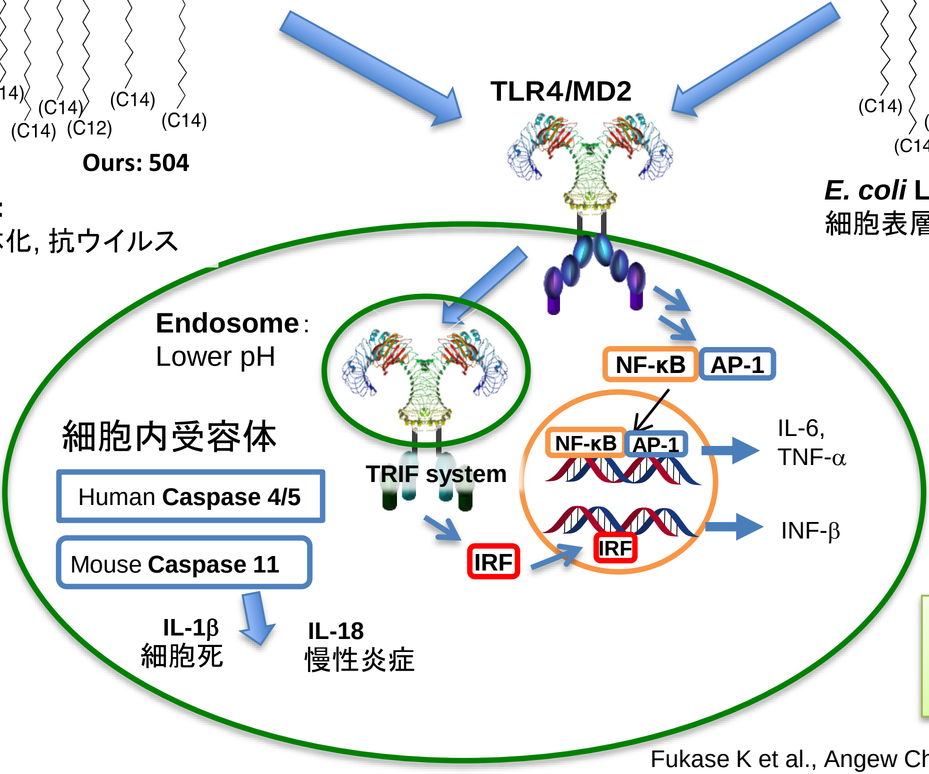


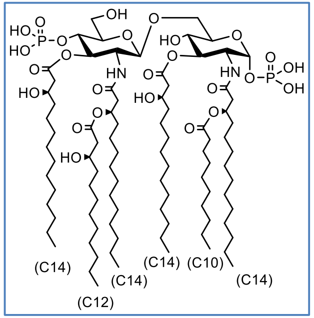
Ours: 504

Monophosphoryl lipid As (MPLs):
弱毒性, 細胞内でTLR4/MD-2 二量体化, 抗ウイルス

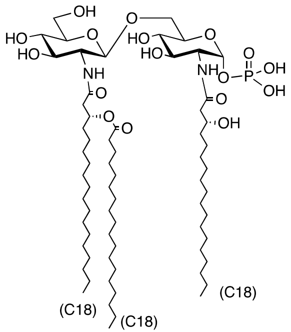


***E. coli* LPS, lipid A, 高い炎症性**
細胞表層でTLR4/MD2を二量体化





***A. faecalis* LPS, lipid A**
毒性なし、細胞死を誘導しない
高い抗体産生能



***H. pylori* lipid A**
R = H: antagonistic to NF-kB system,
R= $CH_2CH_2NH_2$: very weak agonist
IL-18, IL-12、慢性炎症シグナル

Fukase K et al., Angew Chem Int Ed Engl. 2021, 60, 10023.

自己アジュバント化ワクチン:複合化による高次機能の創製

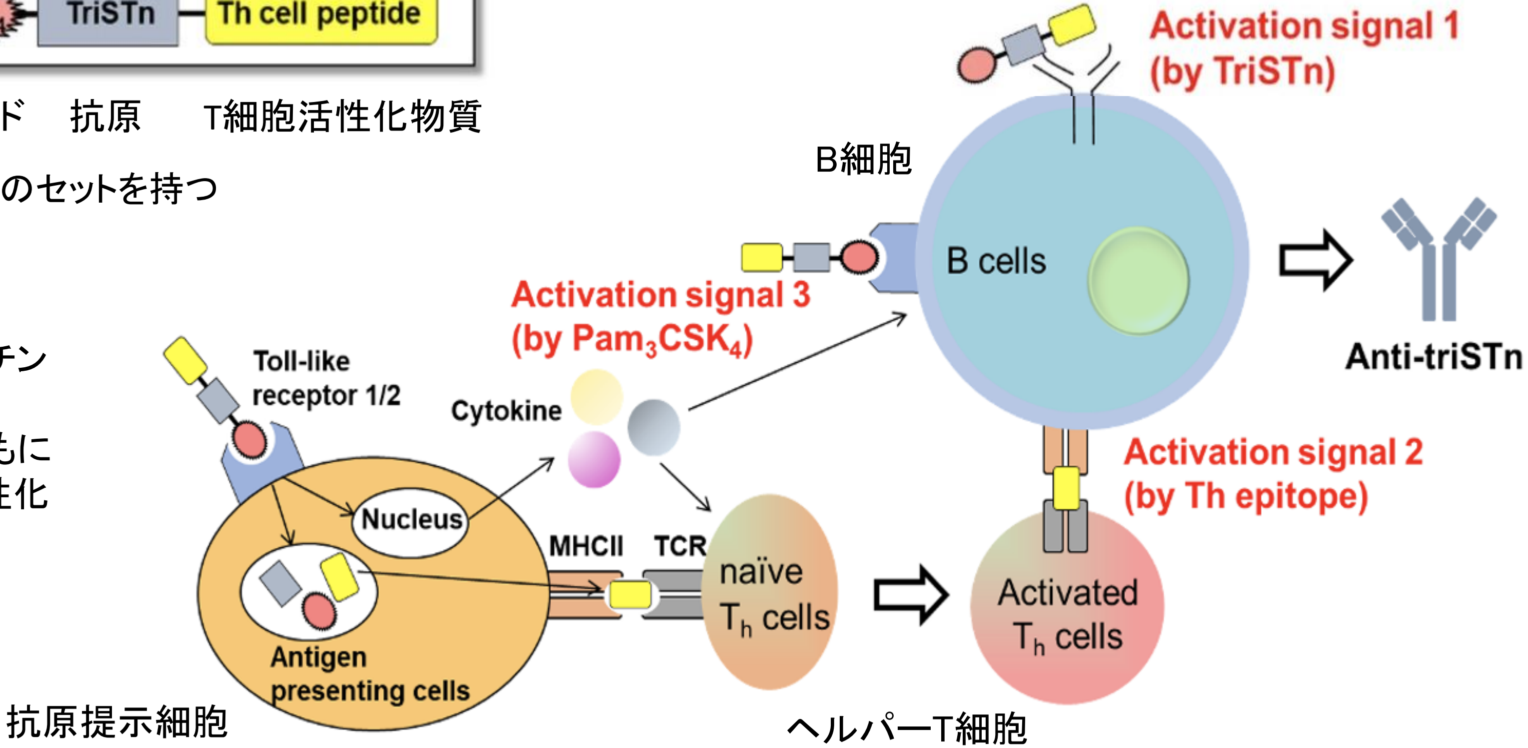
自己アジュバント化ワクチンの開発

自然免疫リガンド　抗原　T細胞活性化物質

病原体は必ずこのセットを持つ

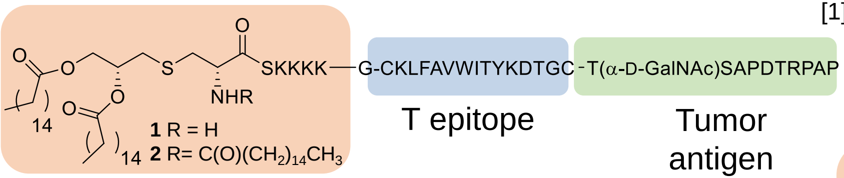
抗がんワクチン、
抗ウイルスワクチン

抗体産生とともに
細胞性免疫活性化
が望ましい



Self-adjuvanting vaccines

[1]

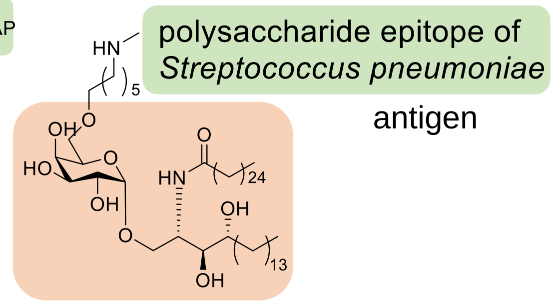


T epitope

Tumor antigen

Adjuvant (Pam$_3$CSK$_4$)

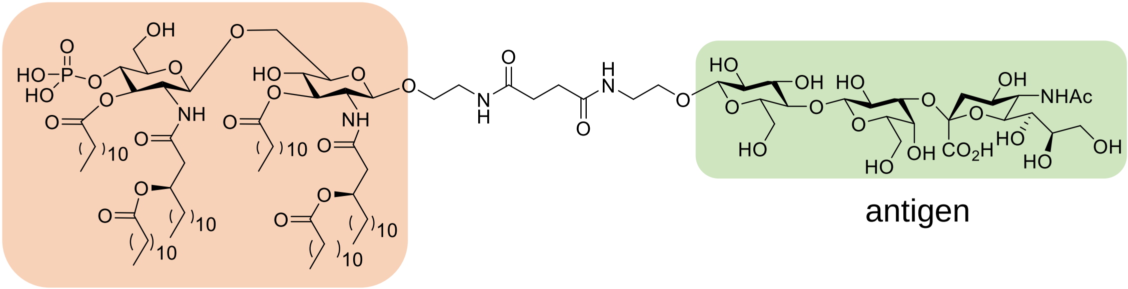
[2]



antigen

Adjuvant (α-GalCer)

[3]



antigen

Adjuvant (MPL)

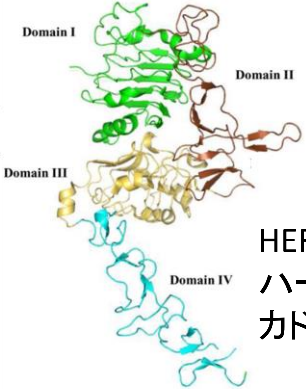
[1] Ingale, S., Wolfert, M. A., Gaekwad, J., Buskas, T., Boons, G. *J. Nat. Chem. Biol*. **2007**, 3(10), 663–667.
[2] Cavallari, M., Stallforth, P., Kalinichenko, A. *et al. Nat Chem Biol.* **2014**, 10, 950–956
[3] Wang, Q., Zhou, Z., Tang, S., Guo, Z., *ACS Chem. Biol.* **2012**, 7, 235-240.

Various cancer antigens (様々ながん抗原)

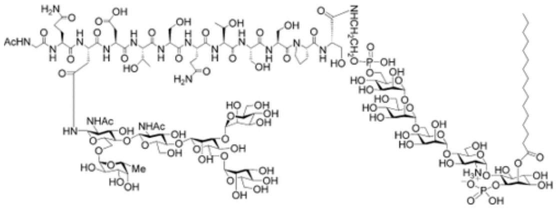
Targets for antibody drugs （抗体医薬の標的）

https://onlinelibrary.wiley.com/doi/10.1002/ange.200353251

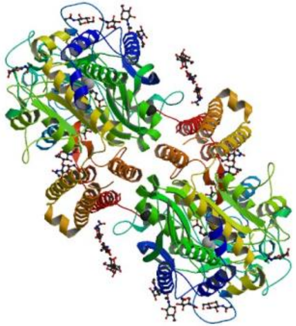
CD20 : B細胞リンパ腫
リツキサン
ゼヴァリン



HER2 : 乳がん
ハーセプチン
カドサイラ

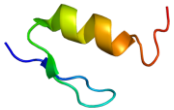


CD52 : B細胞性慢性リンパ性白血病
マブキャンパス

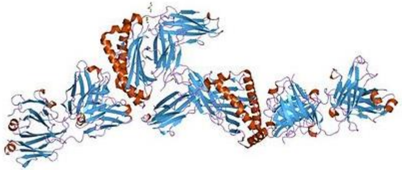


PSMA : 前立腺がん

低分子放射線医薬候補



Wilms' tumor protein (WT1)
（転写因子）:
ウィルムス腫瘍、腎芽腫



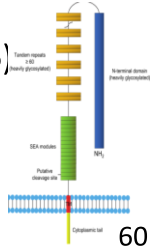
Melanoma-associated antigen 1
(MAGE-1) :
メラノーマ（悪性黒色腫）

Eukaryotic elongation factor 2
(eEF2):
卵巣がん

メソテリン:
卵巣がん

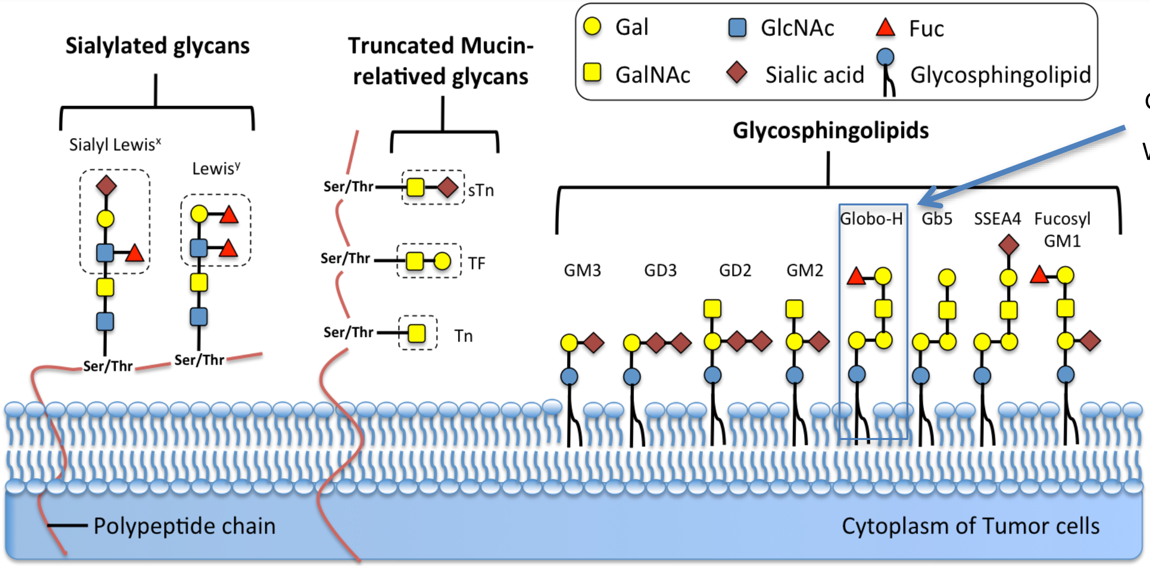
がん細胞では細胞表層の糖鎖構造が変わる。

CA125 (MUC16: ムチン16)
MUC1 (ムチン1）
など



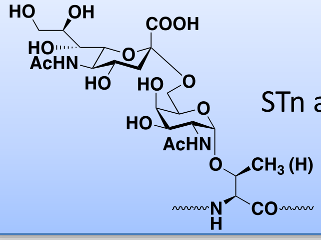
がんワクチンのターゲット

Tumor-Associated Carbohydrate Antigens (TACAs) 腫瘍関連糖鎖抗原

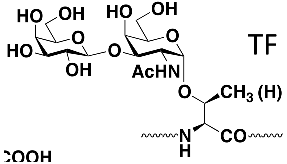


Globo H

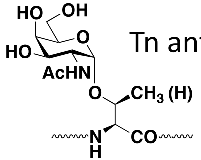
Wong et al,



STn antigen



TF antigen



Tn antigen

STn antigen is richly-expressed on a number of epithelial-related tumors, such as breast, lung, colorectal, gastric, pancreas, and ovarian cancer**, but rarely observed on normal tissues.**

Theratope vaccine (STn-KLH)

Phase III trial of STn keyhole-limpet hemocyanin (KLH) was completed in 1028 women with **metastatic breast cancer**. (By Biomira company)

1028 Patient (Metastatic breast cancer) Phase III clinical trial	STn-KLH	KLH (Control)
Average Survival time (months)	23.1	22.3

Table 3. Median antibody titers at week 12

Treatment	Anti-OSM IgM	Anti-OSM IgG	Anti-STn IgM	Anti-STn IgG	Anti-KLH IgM	Anti-KLH IgG
STn-KLH	1,280	320	10,240	20,480	80	20,480
KLH	0	0	0	0	1,280	81,920

Abbreviations: KLH, keyhole limpet hemocyanin; OSM, ovine submaxillary mucin; STn, sialyl-TN.

OSM: ovine submaxillary mucin

Theratope did not provide a survival benefit and longer time-to-progression to patients, though it did not appear to be detrimental.

Oncologist, **2011**, *16*, 1092-1100. *J. Cancer*, **2013**, *22*, 577-584.

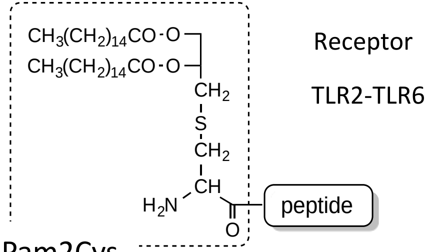
TLR2 agonists as safe and effective adjuvants

Trumenba: lipoprotein having triacylated Cys (TLR2/TLR1 agonist), self-adjuvanting vaccine against *Neisseria meningitidis* sero group B.

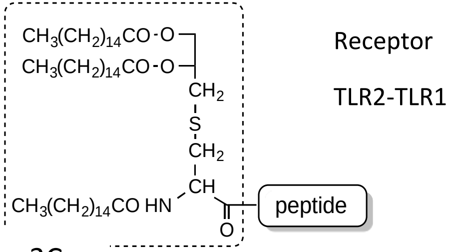
Luo Y. et al., AAPS J. 2016, 18, 1562.

Lipopeptides can stimulate Th1 and antitumor responses via TLR2/TLR1 or TLR2/TLR6.

Robust immunoresposnse induced by three component vaccine: Boons et al., *Nat Chem Biol*. 2007, 3, 663.
MUC1 glycopeptide-lipopeptide conjugate: Kunz, Li et al., *Angew. Chem. Int. Ed*. 2010, 10, 49, 3688
NK activation: Seya, Fujimoto, Fukase, et al., *Microbes Infect*. 2011, 13, 350.
CTL activation: Seya, Hashimoto. Fujimoto, Fukase, et al., *Innate Immun*. 2018, doi: 10.1177/1753425918777598.
Promote Fcγ receptor expression: Shah, et al., *J Biol Chem*. 2013, 288, 12345.
Promote antigen presentation and T Cell activation: Guo et al., *Front Immunol*. 2017, 8, 158.

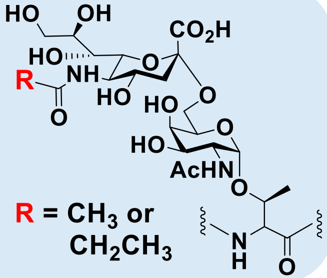


Pam2Cys



Pam3Cys

Self-adjuvanting vaccine candidates with TriSTn antigen

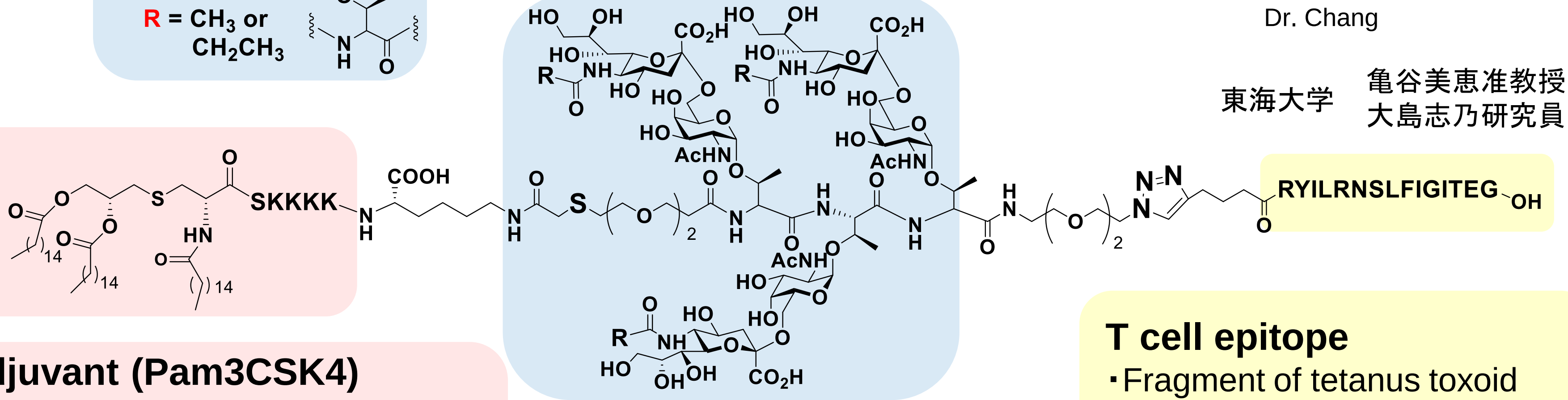


Tumor Antigen: Sialyl Tn (STn)

・C5-*N* modification (propyl modification) of sialic acid enhances the immunogenicity.

Dr. Chang

東海大学　亀谷美恵准教授
大島志乃研究員



Adjuvant (Pam3CSK4)

・Toll like receptor (TLR) 2 ligand
・activate innate immune responses and tumor immunity, and promote the antigen uptake

TriSTn
(clustered STn)

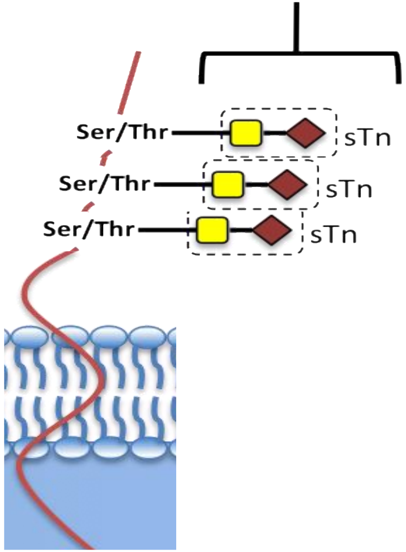
T cell epitope

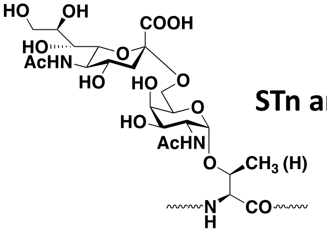
・Fragment of tetanus toxoid
・T cell activation to enhance the production of IgG

Chang TC, Manabe Y, Fujimoto Y, Ohshima S, Kametani Y, Kabayama K, Nimura Y, Lin CC, Fukase K. *Angew Chem Int Ed Engl.* 2018, 57, 8219-8224.

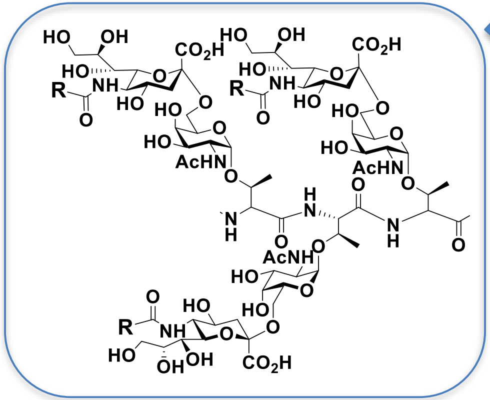
STn antigens are also expressed as clusters on cancer cells.

STn antigens are expressed as clusters on cancer cells.
Clustered STn antigens should be barely expressed on normal cells.





STn antigen



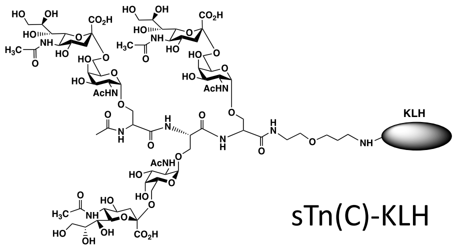
TriSTn antigen

MUC1: 131, 139, 140, 144 positions
MUC4: 558, 569, 579, 607, 618, 629, 640, 651, 662, 673 positions

Strategies to enhance immunogenicity of MUC antigens

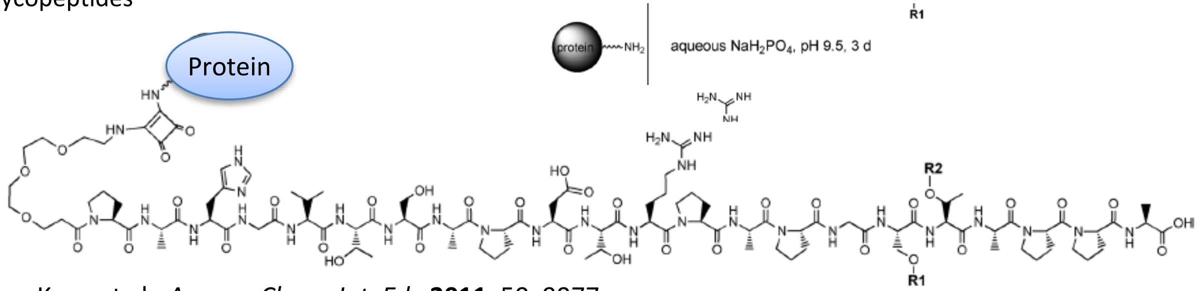
1) Clustered STn

Livingston et al., *Cancer Research*, **1995**, 55, 3364.



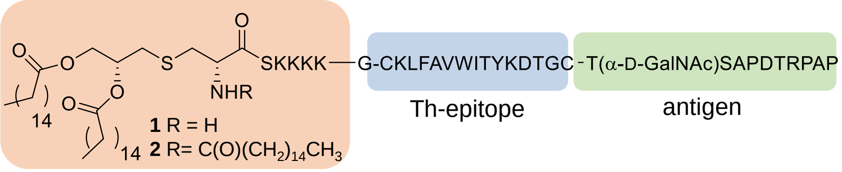
sTn(C)-KLH

2) STn glycopeptides



Kunz et al., *Angew. Chem. Int. Ed.*, **2011**, 50, 9977.

2) Self-adjuvanting vaccine



Tn antigen

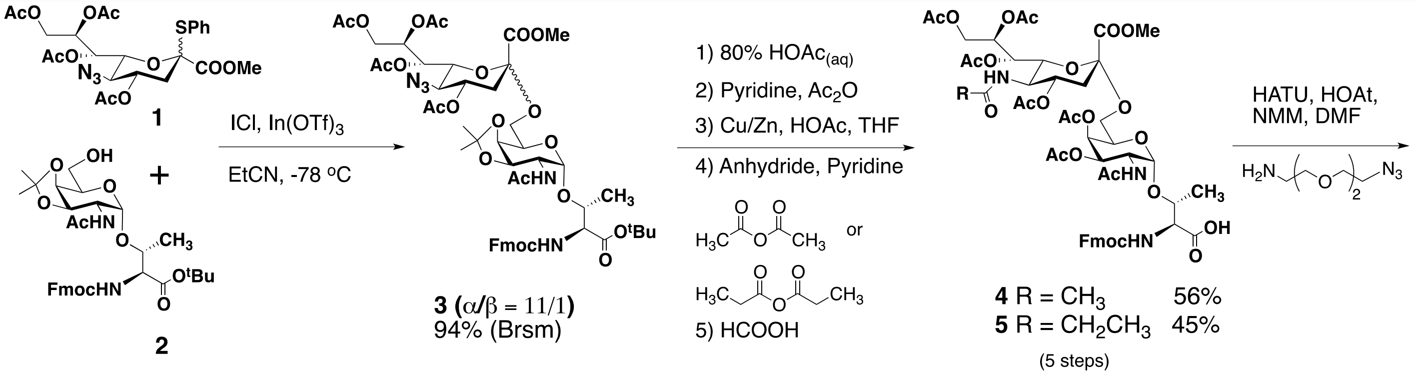
Th-epitope

antigen

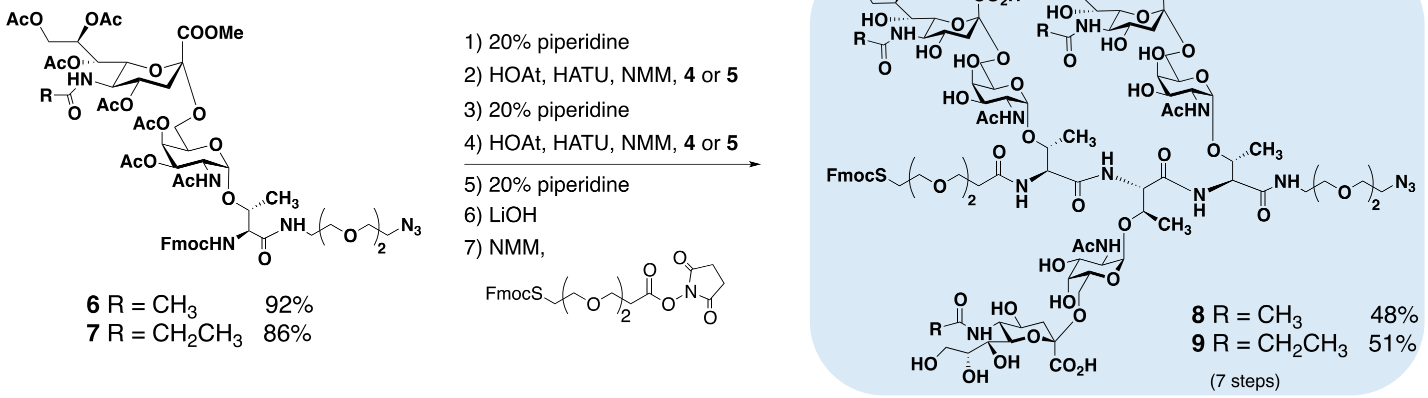
Adjuvant (Pam_3CSK_4)

Boons, G. et al., *J. Nat. Chem. Biol*. **2007**, 3(10), 663–667.

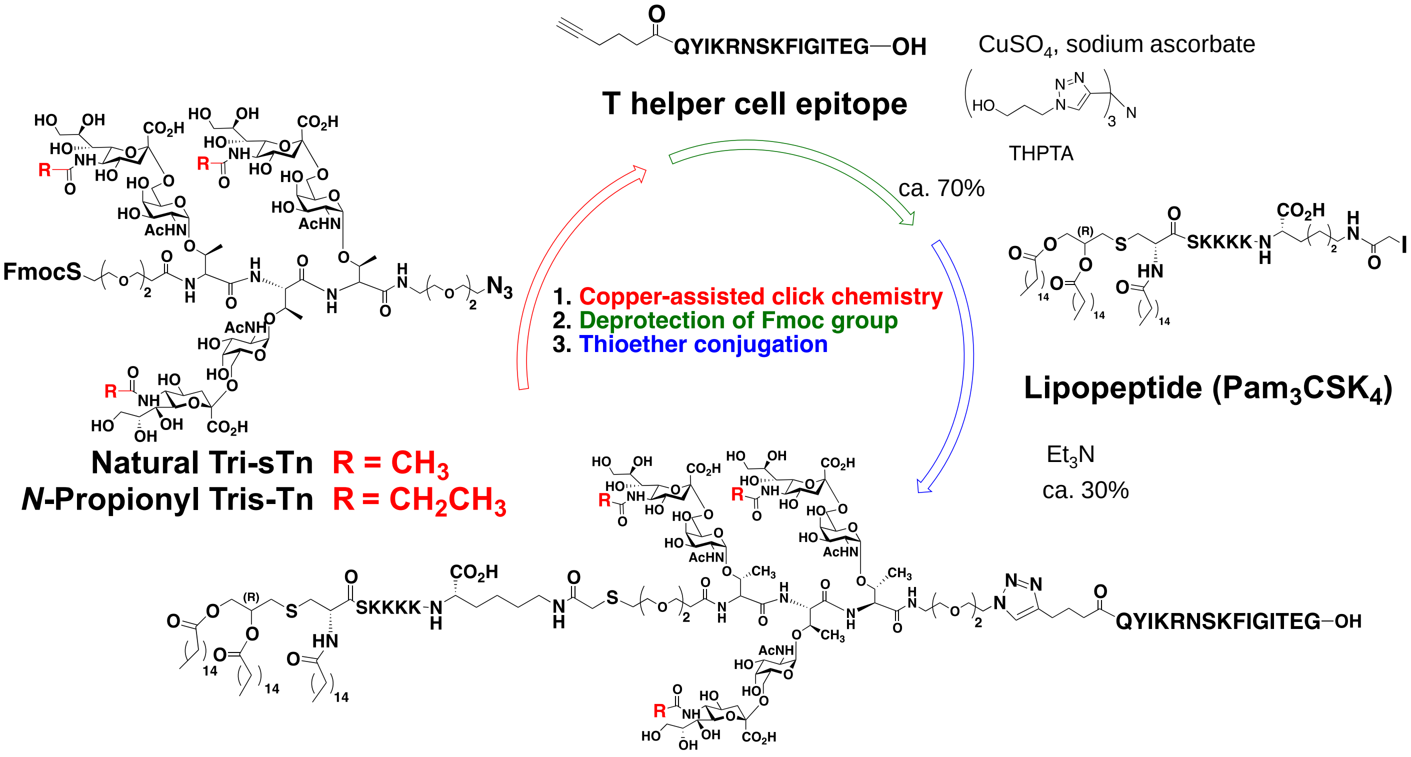
Synthesis of Tri-STn

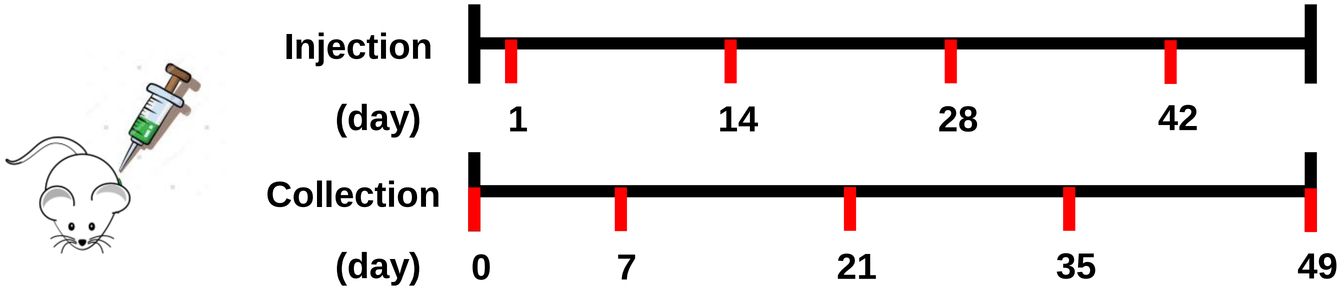


Salmasan, R. M., Manabe, Y., Kitawaki, Y., Chang, T.-C., Fukase, K. *Chem. Lett.*, **2014**, *43*, 956-958.



Construction of self-adjuvanting anticancer vaccine

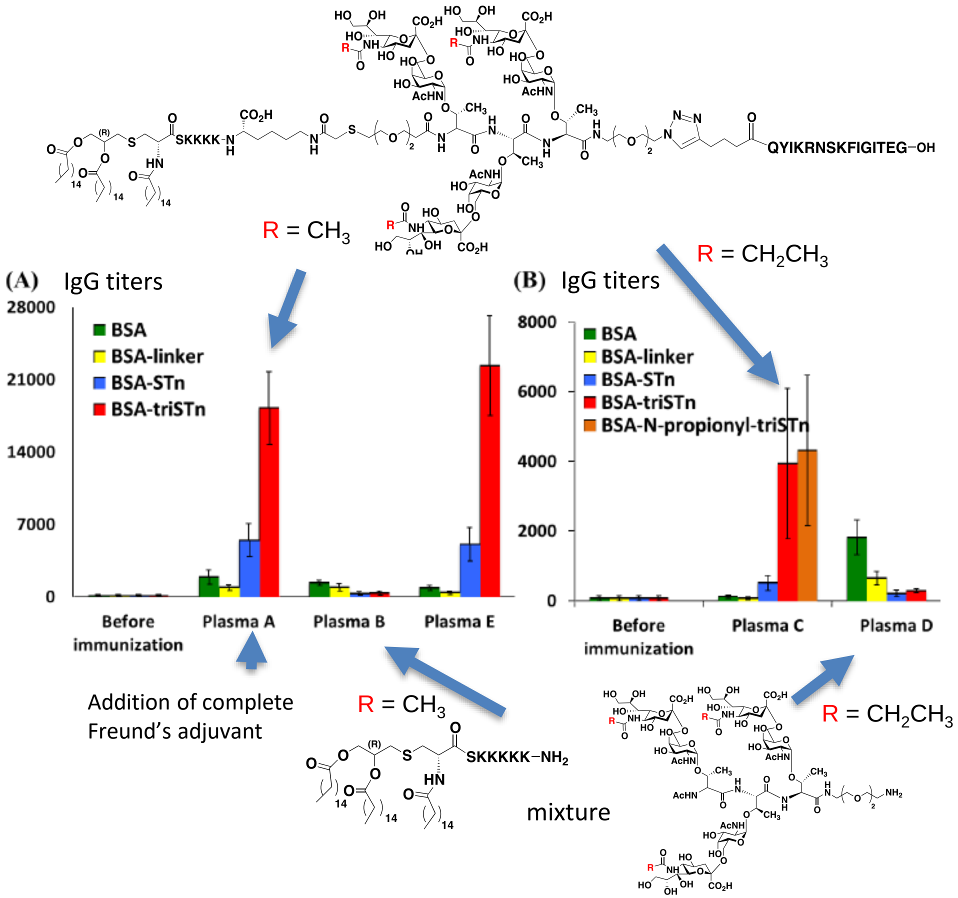




The **five BALB/c Mice (8-10 weeks age)** were immunized **intraperitoneal (i.p.) injection** with **20 µg** of the vaccines compounds on day 1.

Mouse blood samples were also collected prior to the initial immunization on day 0 (as negative control) and after immunization on day 7, 21, 35 and 49.

IgG antibody titers



Self-adjuvanting vaccines effectively induce antibodies.

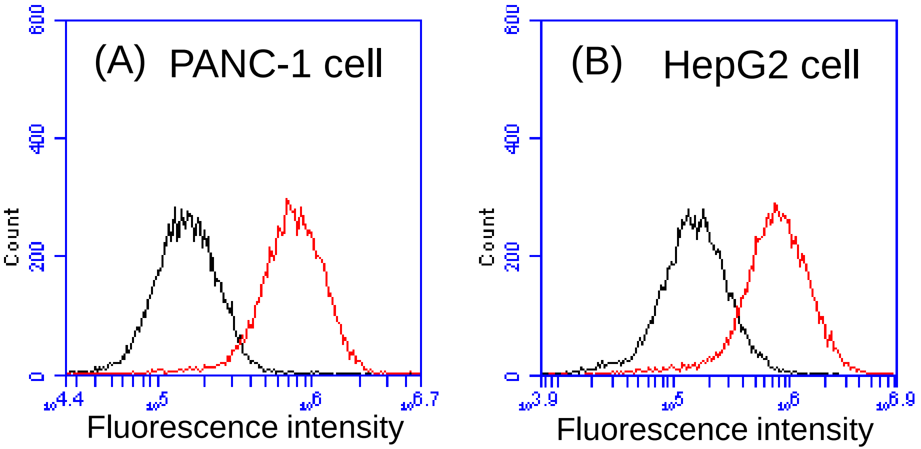
Antibodies recognize cancer cells (Next slide).

Additional adjuvant is not necessary.

Addition of complete Freund's adjuvant caused un-specific inflammation.

Three component conjugation is necessary.

Flow cytometry (Plasma A induced by TriSTn)



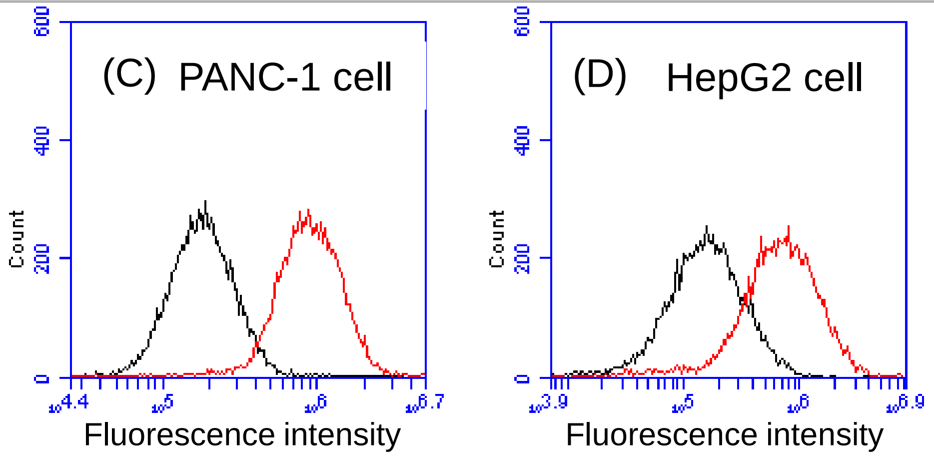
Control
(Before immunization)

Plasma A
(Mixture of all mice plasma)

PANC-1, Human pancreatic cancer cell line. MUC1

HepG2, Human hepatic cancer cell line. MUC15

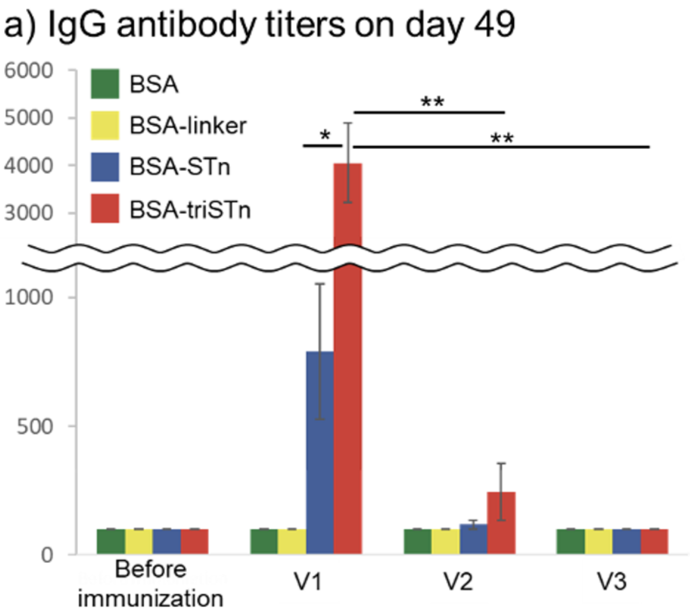
Flow cytometry (Plasma B induced by *N*-Propionyl TriSTn)

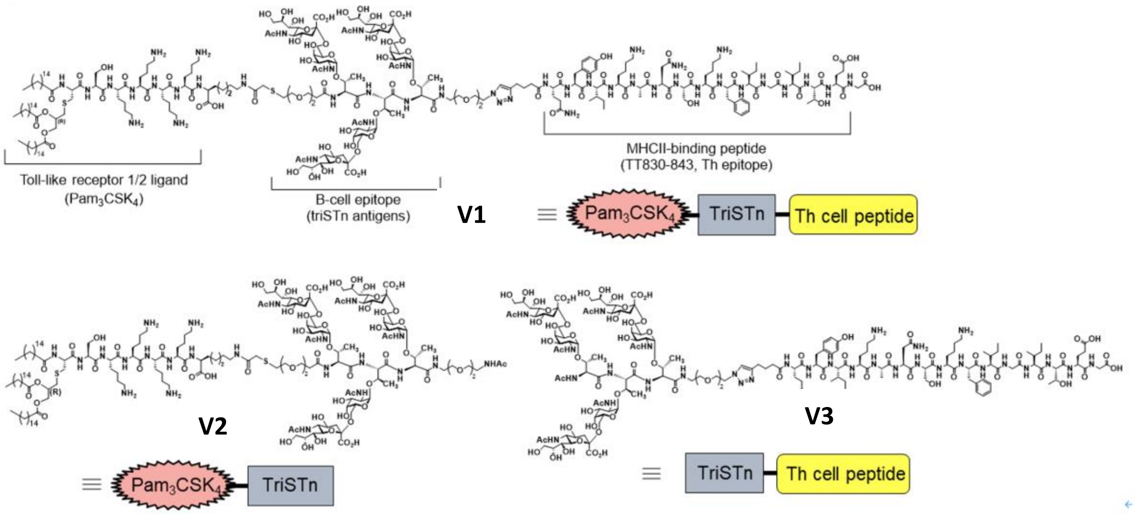


Control
(Before immunization)

Plasma B
(Mixture of all mice plasma)

Importance of conjugation of three-components

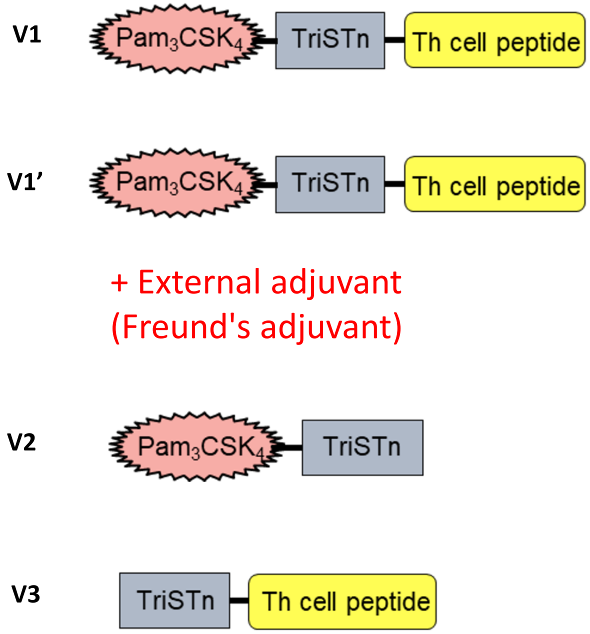


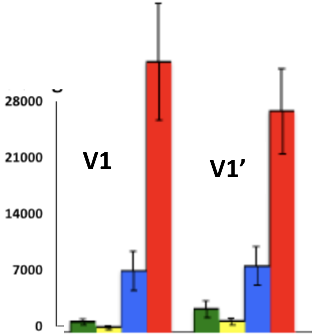


Simultaneous stimulation by the three components is necessary to induce antibody production against glycan antigen.

T.-C. Chang, Y. Manabe, K. Ito, R. Yamamoto, K. Kabayama, S. Ohshima, Y. Kametani, Y. Fujimoto, C.-C. Lin, K. Fukase, *RSC Advances*, **2022**, *12*, 18985.

Response of dendritic cells to a three-component conjugated vaccine



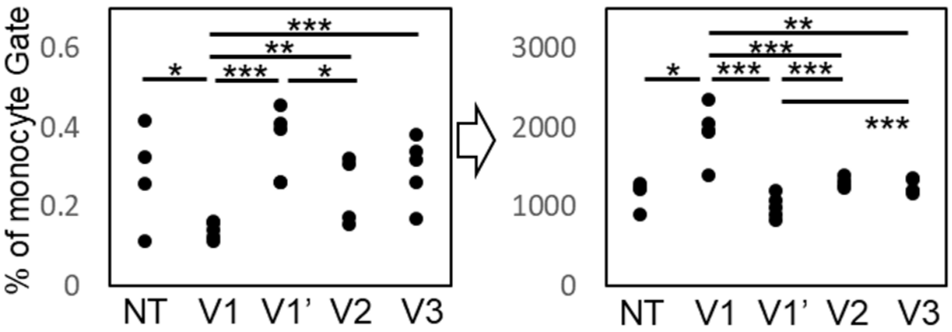


Additional adjuvant decreased the antibody production.

アジュバントの追加は抗体産生を下げる

Activated dendritic cells

- **CD11c+** dendritic cells
- **MFI of CD80+ (CD11c+)**



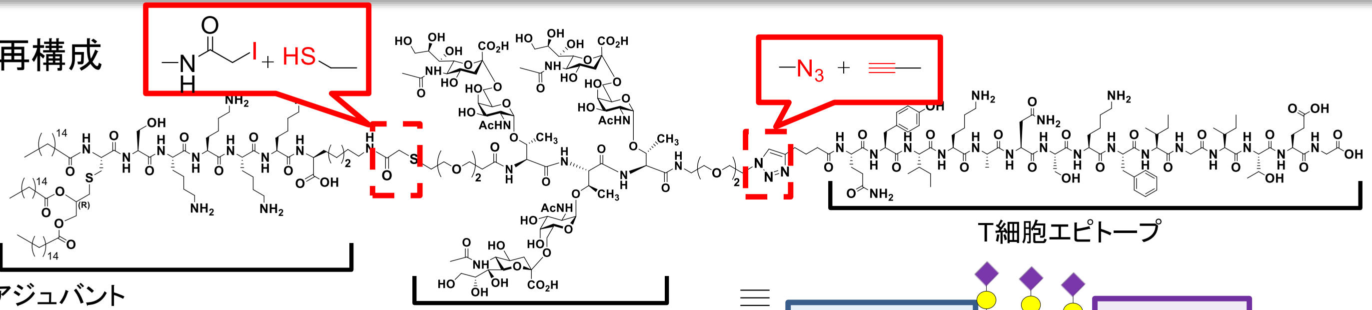
Mean fluorescence intensity (MFI)

External adjuvant induces systemic inflammation to increase dendritic cells.

アジュバントの追加は樹状細胞を非特異的に増加
自己アジュバント化ワクチンは効率的に樹状細胞を活性化

複合化によって機能を発現：セルフアジュバンティングワクチン

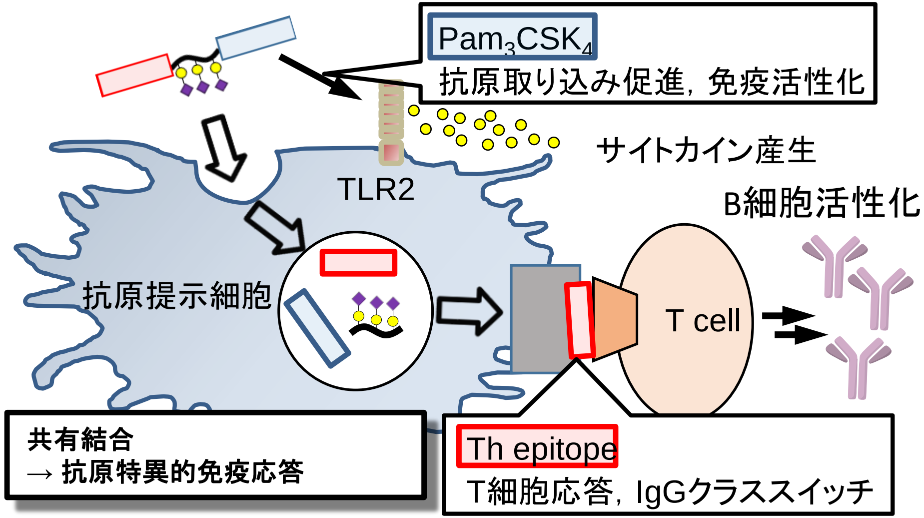
高次グリココードの再構成



アジュバント
免疫賦活化剤，Pam_3CSK_4）

triSTn（がん抗原）

≡



CD4+（ヘルパーT細胞）エピトープを持つが

CD8+（CTL) エピトープを持たない

CTL: 細胞傷害性T細胞

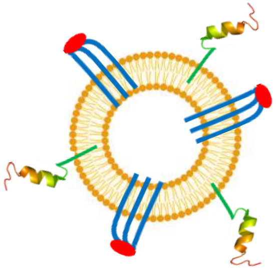
がんワクチンやウイルスワクチンには、B細胞エピトープ、CD4+ エピトープ、CD8+ エピトープを有するペプチドワクチンが有効

Chem. Asian. J. **2019**, *14*, 4268. *Angew. Chem. Int. Ed.* **2020**, *59*, 17705.

Self-adjuvanting Composite Vaccine （自己アジュバント化複合ワクチン）

複合ワクチン(細菌糖鎖ワクチンなど)

Th epitope
(Th-エピトープ)



Antigen(タンパク抗原)

Adjuvant
(アジュバント)

リポソーム・脂質微粒子型 (Liposome•LNP)

抗原 — 担体タンパク + アジュバント(Adjuvant)

Antigen　Carrier　(ミョウバンアジュバントなど)

複合体型 (Conjugated Vaccines)

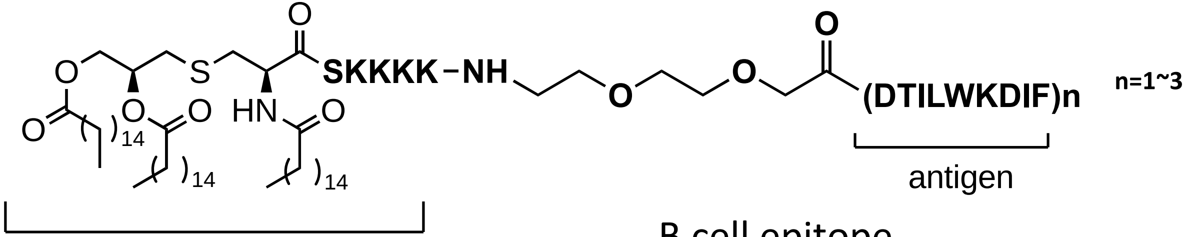
共有結合型
Self-adjuvanting vaccine

Adjuvant (アジュバント) — Antigen (抗原) — Th-epitope (Th-エピトープ)

抗原提示細胞・B細胞活性化　B細胞活性化　T細胞活性化

- アジュバント：免疫活性化、抗原を抗原提示細胞にリクルート
  抗原の取り込み促進（受容体を介した取り込み、凝集体のエンドサイトシス促進）
  アジュバントの投与量を減らせる→炎症作用の低減
- 抗原に対する特異的な免疫誘導（担体タンパク質が不要）
- 高純度ワクチンの合成、品質管理が容易

Self-assembling Self-adjuvaning Anti-breast Cancer Vaccine Candidates

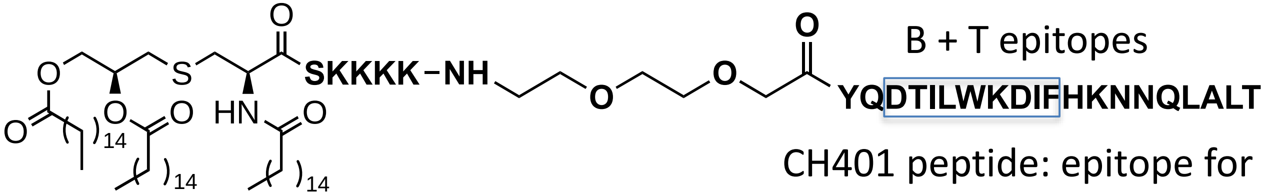


Pam_3CSK_4 (TLR2 ligand)

B cell epitope

9aa(N:165-173: DTILWKDIF)

Feng Q, Manabe Y, Kabayama K, Aiga T, Miyamoto A, Ohshima S, Kametani Y, Fukase K. *Chem Asian J.* **2019**, 14, 4268.



B + T epitopes

CH401 peptide: epitope for Her2

誘導された抗体価はこちらの方が高い

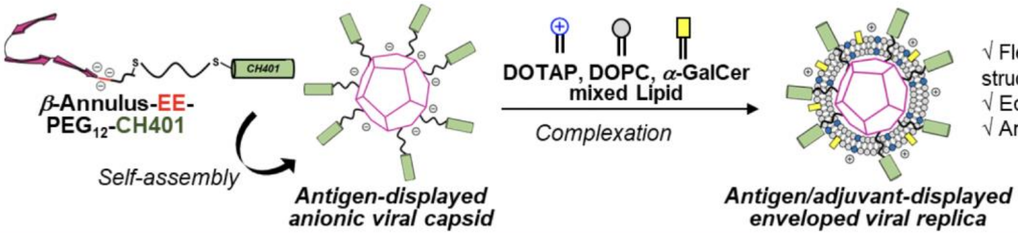
Her2:受容体型チロシンキナーゼ, 乳がんなど多くのがんに高発現

Aiga T, Manabe Y, Ito K, Chang TC, Kabayama K, Ohshima S, Kametani Y, Miura A, Furukawa H, Inaba H, Matsuura K, Fukase K. *Angew. Chem. Int. Ed. Engl*. **2020**, 59, 17705.

これらは、HER2を発現するがん細胞を認識する抗体を誘導した。

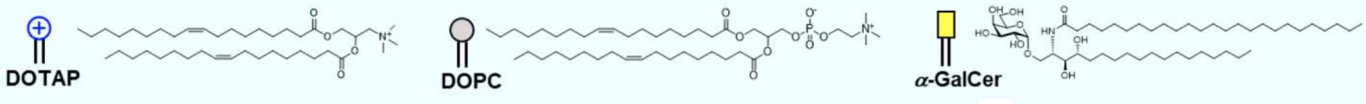
Antigen/adjuvant-displaying enveloped viral replica as a new LNP vaccine platform

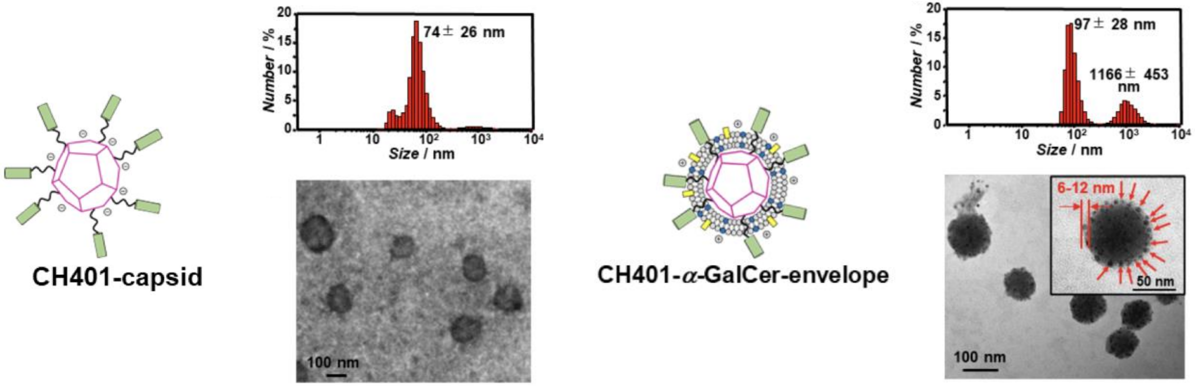
Enveloped viral replica vaccine



√ Flexible design with maintaining rigid structure
√ Equipment of multiple components
√ Antigen-specific immune responses

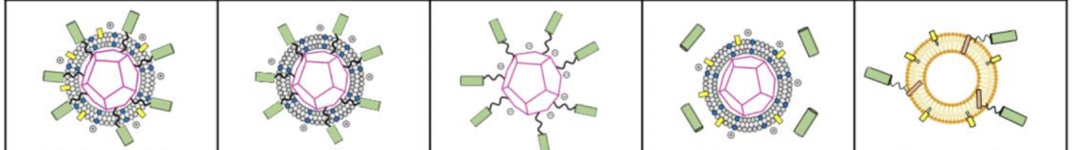
β-Annulus-EE: INHVGGTGGAIMAPVAVTRQLVGSEEGGGCG, CH401: CYQDTILWKDIFHKNNQLALT



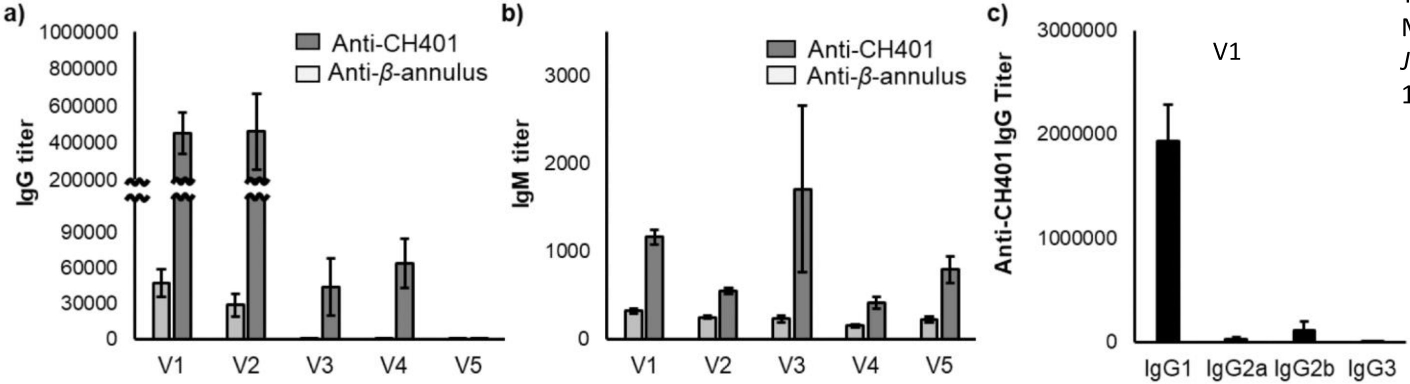


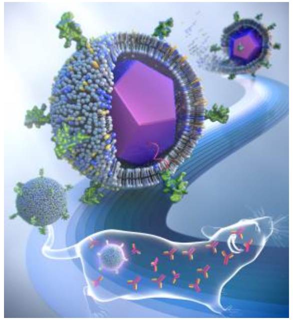
Collaborative Research with Professor Matsuura from Tottori University and Professor Kametani from Tokai University"

Antibody production by viral replica-LNP-type vaccines



V1	V2	V3	V4	V5
CH401-α-GalCer-envelop	**CH401-envelop**	**CH401-capsid**	**α-GalCer-envelop** +CH401 peptide	**CH401-α-GalCer-liposome**
[β-annulus-EE-PEG$_{12}$-CH401] = 3.5 μM [α-GalCer] = 3.5 μM [DOTAP] = 10.5 μM [DOPC] = 105 μM in PBS (pH 7.4)	[β-annulus-EE-PEG$_{12}$-CH401] = 3.5 μM [DOTAP] = 10.5 μM [DOPC] = 105 μM in PBS (pH 7.4)	[β-annulus-EE-PEG$_{12}$-CH401] = 3.5 μM in PBS (pH 7.4)	[β-annulus-EE] = 3.5 μM [Cys-CH401] = 3.5 μM [α-GalCer] = 3.5 μM [DOTAP] = 10.5 μM [DOPC] = 105 μM in PBS (pH 7.4)	[Pam-CH401] = 3.5 μM [α-GalCer] = 3.5 μM [DOTAP] = 219 μM [DSPC] = 44 μM [Cholesterol] = 169 μM [DMG-PEG] = 6.6 μM in PBS (pH 7.4)





Ito K, Furukawa H, Inaba H, Ohshima S, Kametani Y, Maeki M, Tokeshi M, Huang X, Kabayama K, Manabe Y, Fukase K, Matsuura K *J Am Chem Soc*. 2023 doi: 10.1021/jacs.3c02679..

天然物有機化学研究室における生物活性分子の機能研究
Functional studies of Bioactive Molecules in the Laboratory of Natural Product Chemistry

深瀬浩一、下山敦史助教、真鍋良幸助教
樺山一哉教授（放射線科学基盤機構
2024年2月1日～）

Prof. Antonio Molinaro
(University of Naples, Federico II)

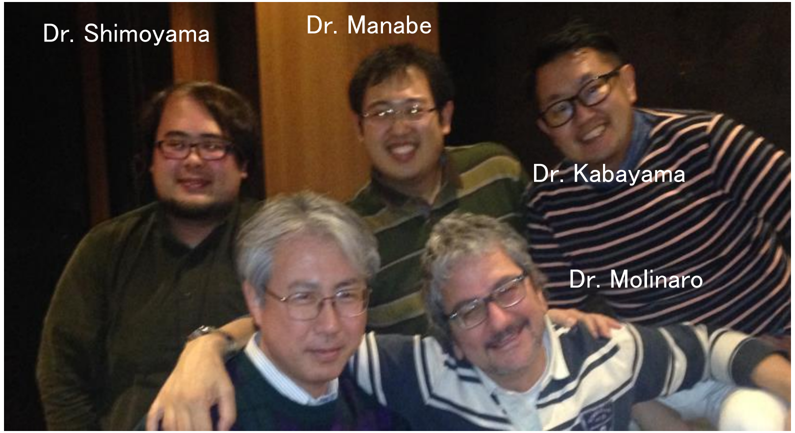
藤本ゆかり先生(2003~2014.3に在籍)
田中克典先生 (2005-2011に在籍)

Main research targets: glycans, glycoconjugates
Main research topics: chemical synthesis, biofunctional mechanism, bio-imaging

糖鎖の効率合成
鍵化合物の合成と供与（共同研究）
生物活性発現機構の解明
新規医薬や医療法への展開

世界最先端であることが重要
発明と発見のバランス
新しい概念の提唱

対等な共同研究



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“Middle molecular strategy” Koichi Fukase

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新学術領域研究
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CiDER: Center for Infectious Diseases Education and Research

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