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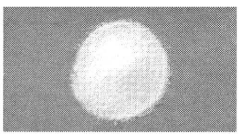
アラムアジュバントをふくむ粒子状物質の新規免疫学的メカニズム

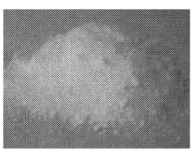
黒田悦史

産業医科大学　医学部　免疫学寄生虫学教室

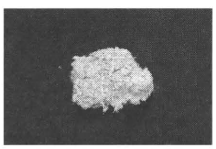
粒子状化学物質



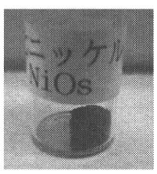
アラム



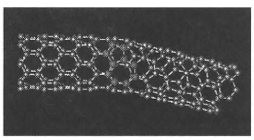
シリカ



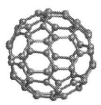
アスベスト



酸化ニッケル



カーボンナノチューブ

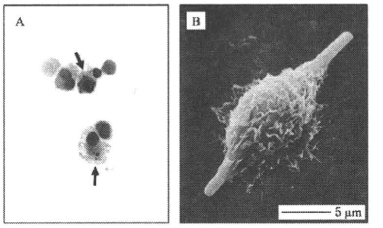


フラーレン

粒子状化学物質の特性と アジュバント効果

1）形状（粒子状　or　繊維状）

繊維状ではマクロファージによる
クリアランスが低下する
↓
永続的な炎症反応
↓
慢性炎症，腫瘍化



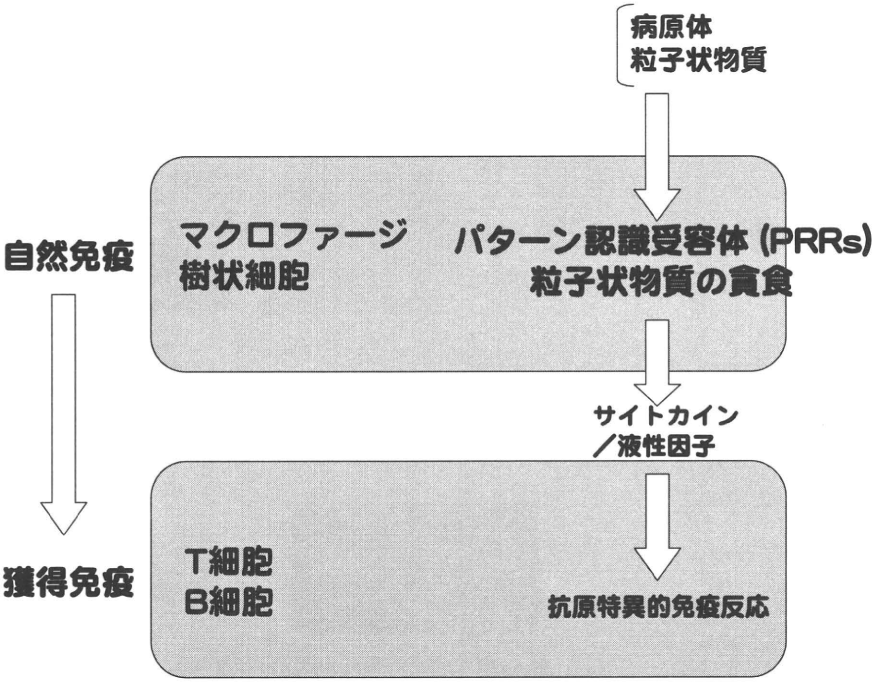
エアロゾル研究2009 森本ら

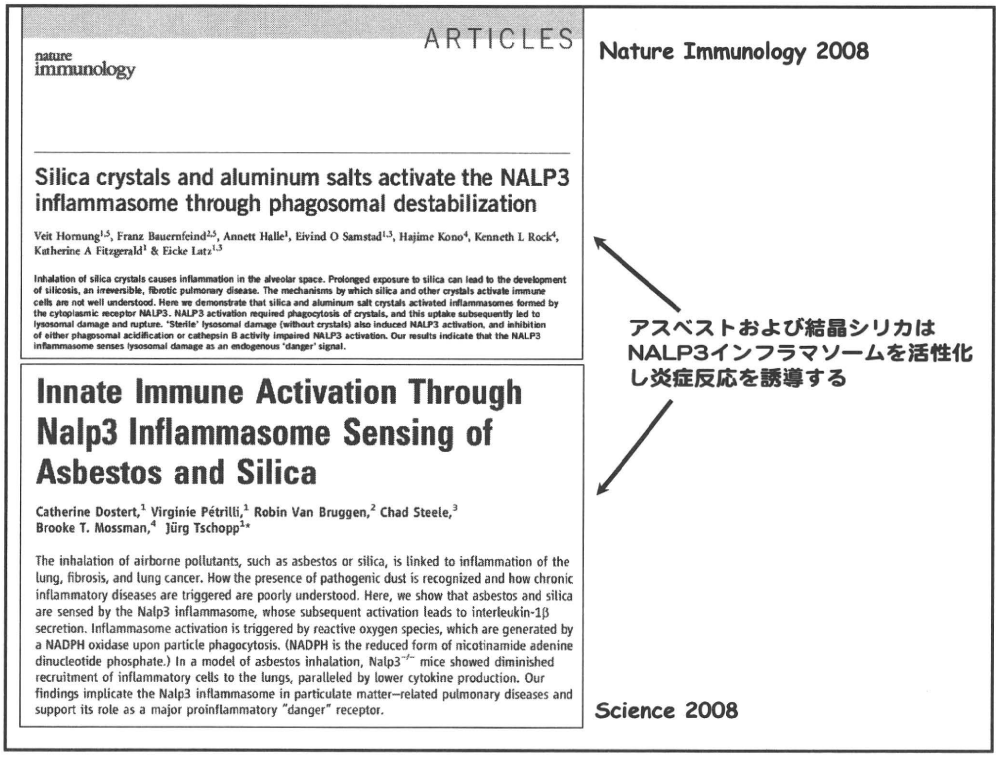
2）サイズ

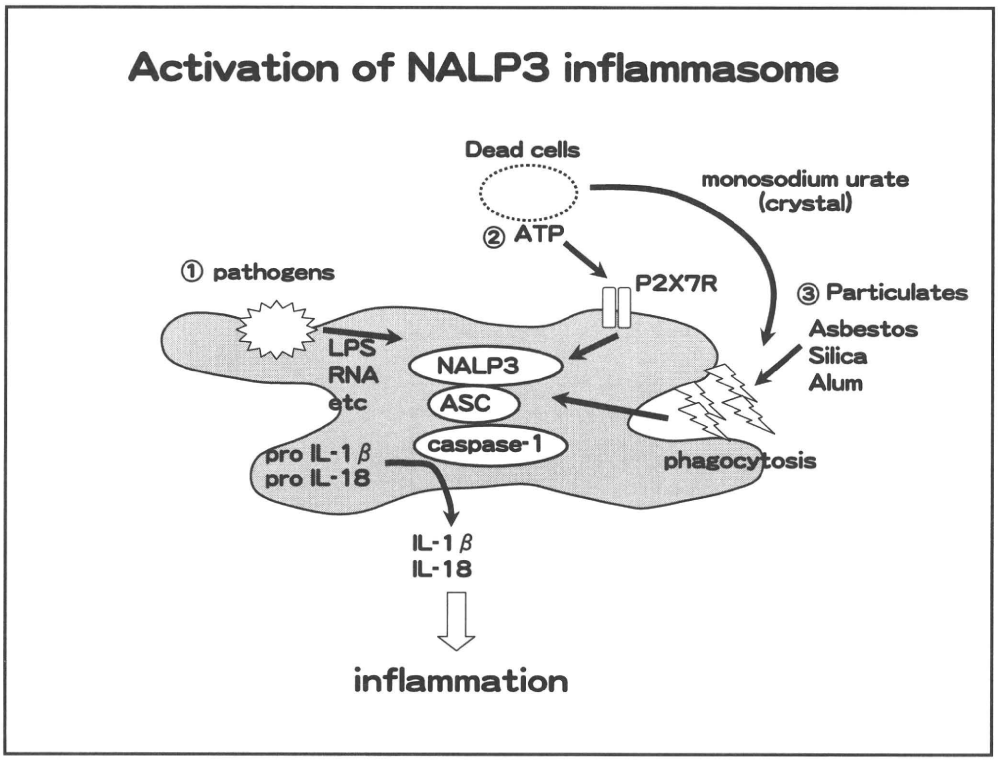
一般的に，マクロファージや樹状細胞は
５μmから数百nmの粒子を効率よく貪食する

3）粒子自体が有する刺激活性

自然免疫と獲得免疫を活性化するメカニズム

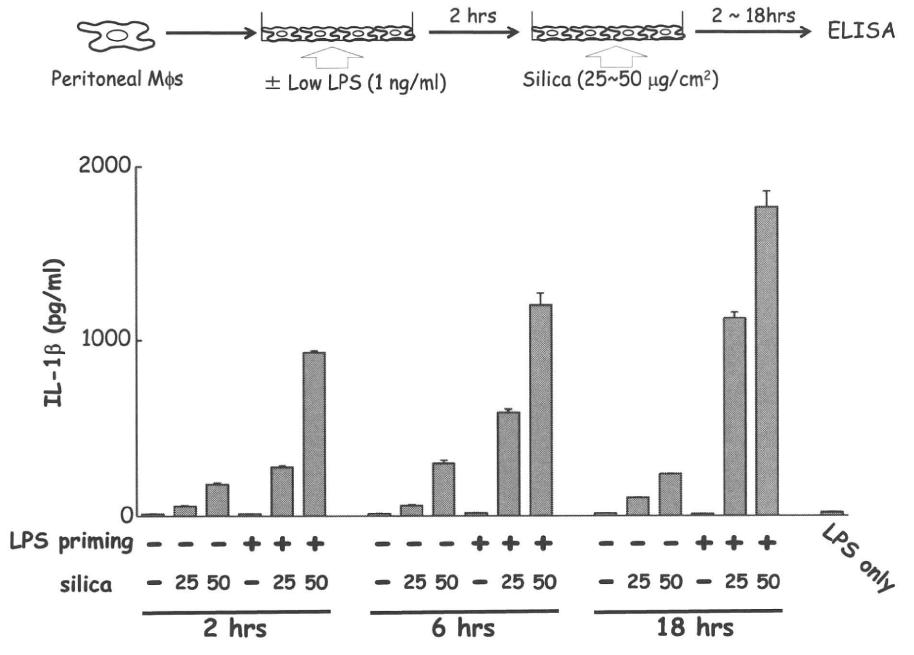



ARTICLES
nature immunology
Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization
Veit Hornung[1,3], Franz Bauernfeind[2,3], Annett Halle[1], Eivind O Samstad[1,3], Hajime Kono[4], Kenneth L Rock[4], Katherine A Fitzgerald[1] & Eicke Latz[1,3]
Inhalation of silica crystals causes inflammation in the alveolar space. Prolonged exposure to silica can lead to the development of silicosis, an irreversible, fibrotic pulmonary disease. The mechanisms by which silica and other crystals activate immune cells are not well understood. Here we demonstrate that silica and aluminum salt crystals activated inflammasomes formed by the cytoplasmic receptor NALP3. NALP3 activation required phagocytosis of crystals, and this uptake subsequently led to lysosomal damage and rupture. "Sterile" lysosomal damage (without crystals) also induced NALP3 activation, and inhibition of either phagosomal acidification or cathepsin B activity impaired NALP3 activation. Our results indicate that the NALP3 inflammasome senses lysosomal damage as an endogenous "danger" signal.
Nature Immunology 2008
アスベストおよび結晶シリカは
NALP3インフラマソームを活性化
し炎症反応を誘導する
Innate Immune Activation Through Nalp3 Inflammasome Sensing of Asbestos and Silica
Catherine Dostert,[1] Virginie Pétrilli,[1] Robin Van Bruggen,[2] Chad Steele,[3] Brooke T. Mossman,[4] Jürg Tschopp[1]*
The inhalation of airborne pollutants, such as asbestos or silica, is linked to inflammation of the lung, fibrosis, and lung cancer. How the presence of pathogenic dust is recognized and how chronic inflammatory diseases are triggered are poorly understood. Here, we show that asbestos and silica are sensed by the Nalp3 inflammasome, whose subsequent activation leads to interleukin-1β secretion. Inflammasome activation is triggered by reactive oxygen species, which are generated by a NADPH oxidase upon particle phagocytosis. (NADPH is the reduced form of nicotinamide adenine dinucleotide phosphate.) In a model of asbestos inhalation, Nalp3$^{-/-}$ mice showed diminished recruitment of inflammatory cells to the lungs, paralleled by lower cytokine production. Our findings implicate the Nalp3 inflammasome in particulate matter–related pulmonary diseases and support its role as a major proinflammatory "danger" receptor.
Science 2008

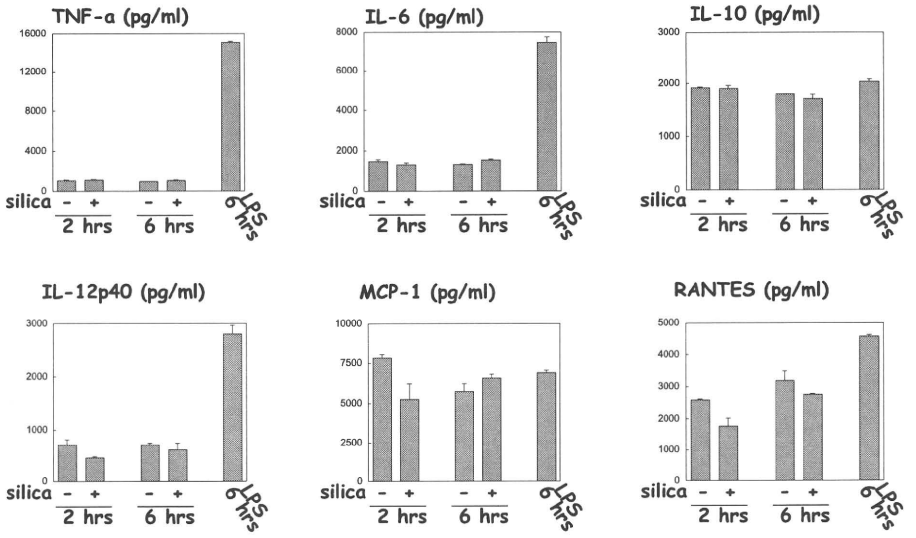

Activation of NALP3 inflammasome
Dead cells
monosodium urate
(crystal)
② ATP
① pathogens
P2X7R
③ Particulates
Asbestos
Silica
Alum
LPS
RNA
etc
NALP3
ASC
caspase-1
pro IL-1β
pro IL-18
phagocytosis
IL-1β
IL-18
inflammation

Today's Topics

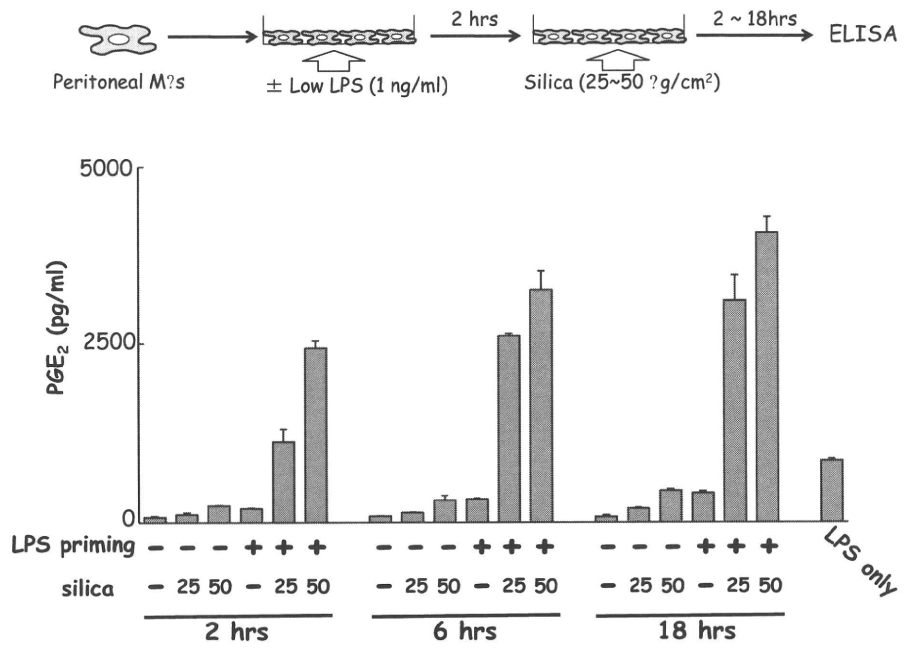
- シリカ以外の粒子状化学物質によりインフラマソームが活性化されるか？
- インフラマソーム依存性の炎症性サイトカイン以外に液性因子は産生されるのか？
- 粒子状化学物質により誘導される液性因子とそれによって活性化される免疫反応の解析
- 粒子状化学物質によって活性化されるシグナル伝達体



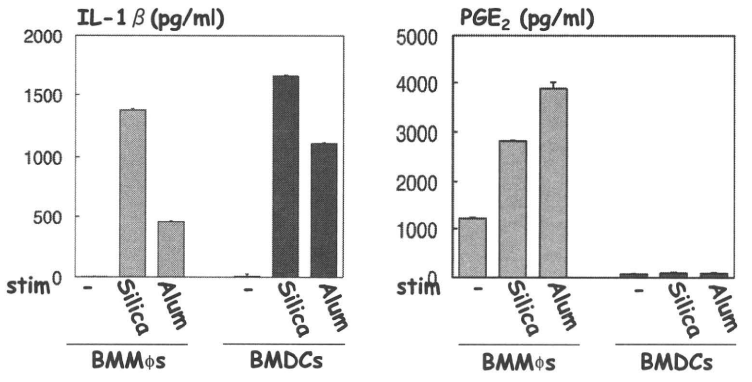
シリカはインフラマソーム依存的にマクロファージからのIL-1β産生を誘導する



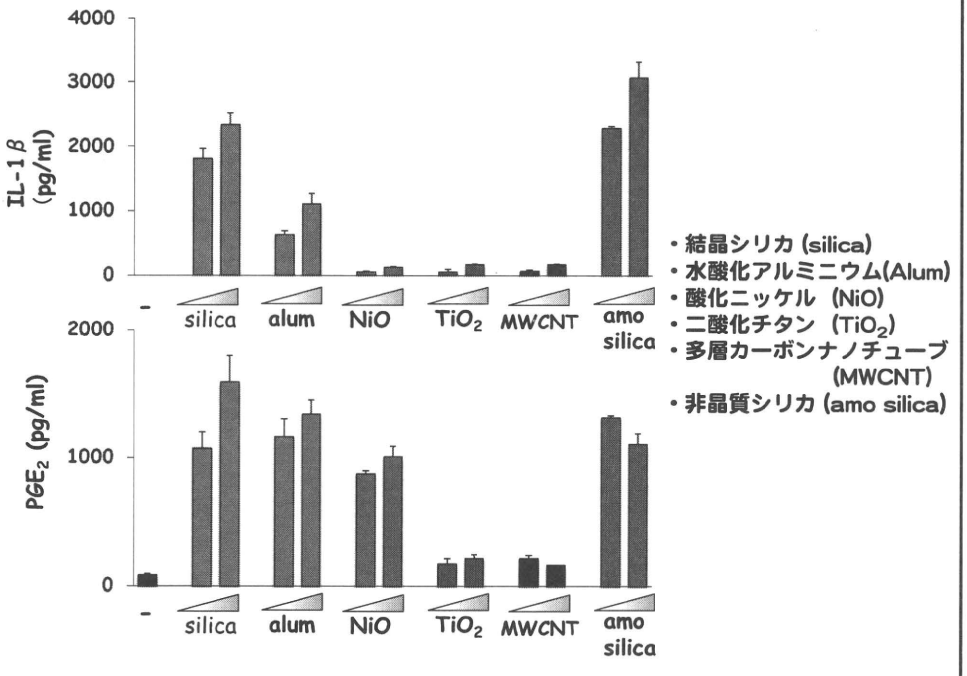
シリカはその他の炎症性サイトカインやケモカインを誘導しない



シリカは脂質メディエータであるプロスタグランジンE2の産生を誘導する

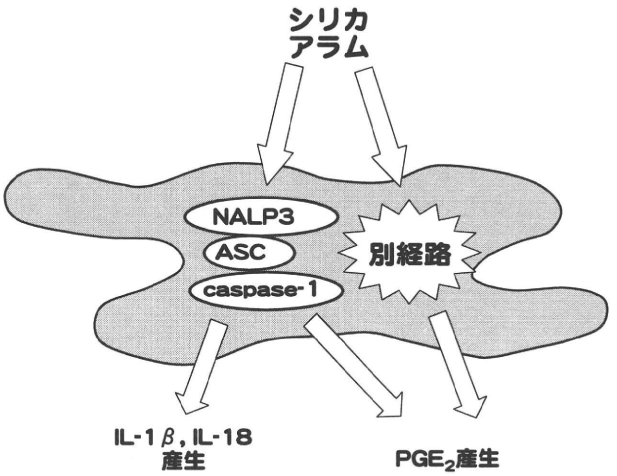


マクロファージはシリカやアラムの刺激により多量のPGE_2を産生する



種々の粒子状化学物質によるマクロファージの活性化とIL-1β、PGE_2産生

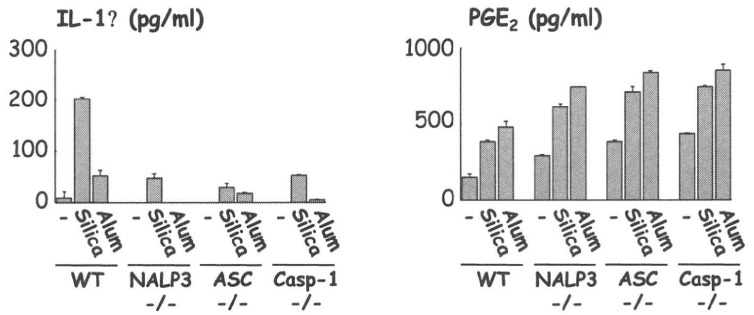
粒子状化学物質によるPGE_2産生のメカニズムとは？



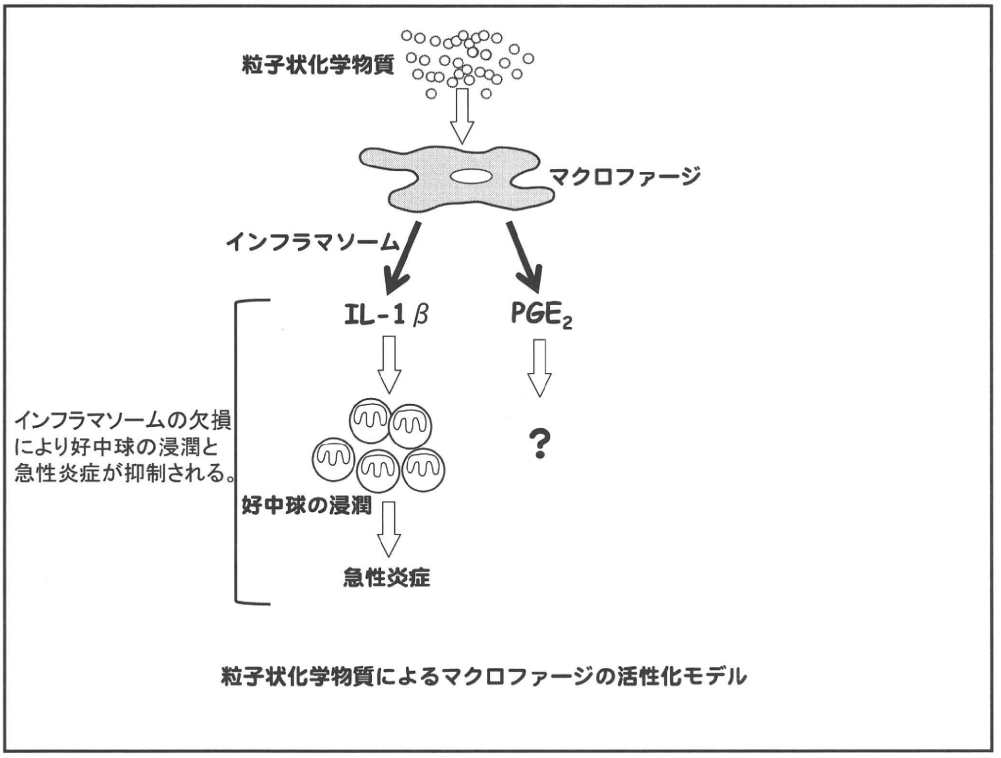
これまでの報告と同じくインフラマソーム依存性？？

インフラマソームを介さない別経路？？

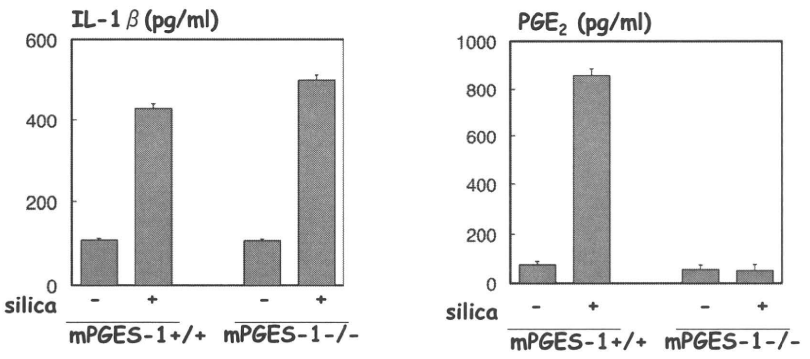
NALP3
ASC
Caspase-1
→ 欠損 → インフラマソームが活性化されない



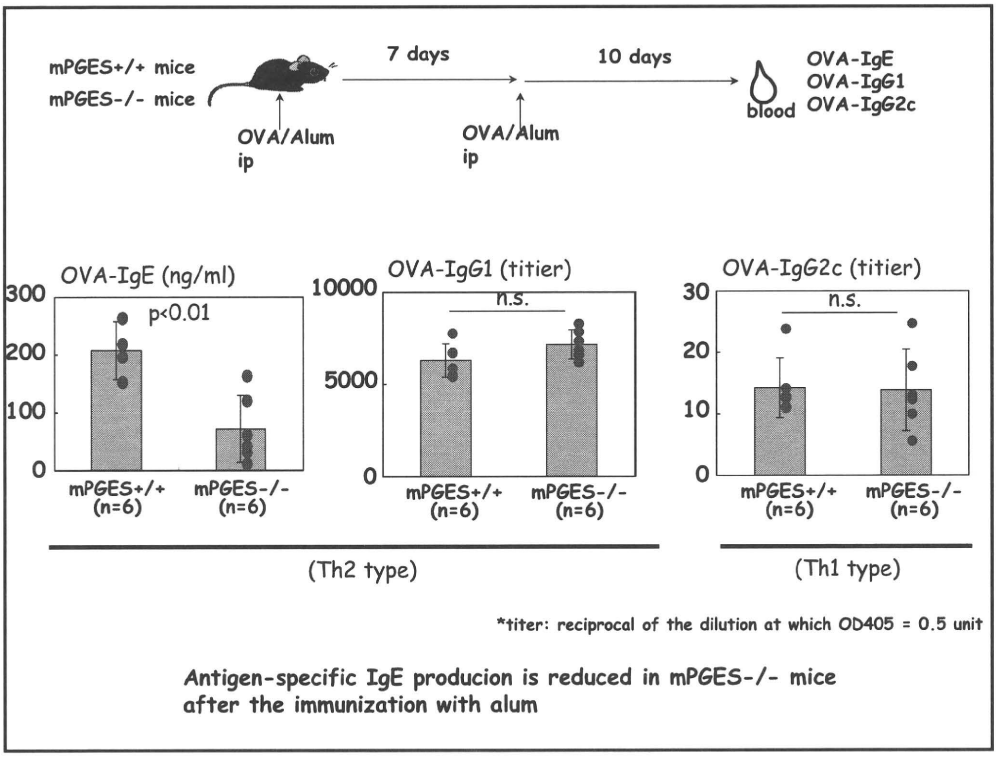
シリカおよびアラムによるマクロファージからのPGE_2産生は
インフラマソーム非依存性に産生される



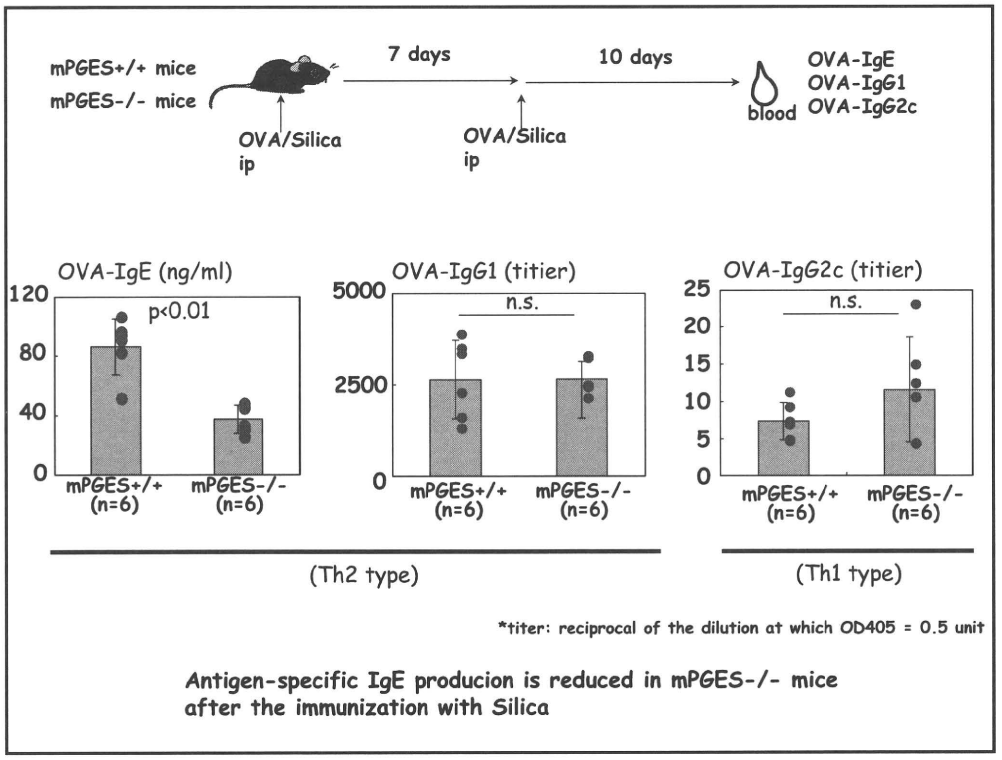
粒子状化学物質によるマクロファージの活性化モデル



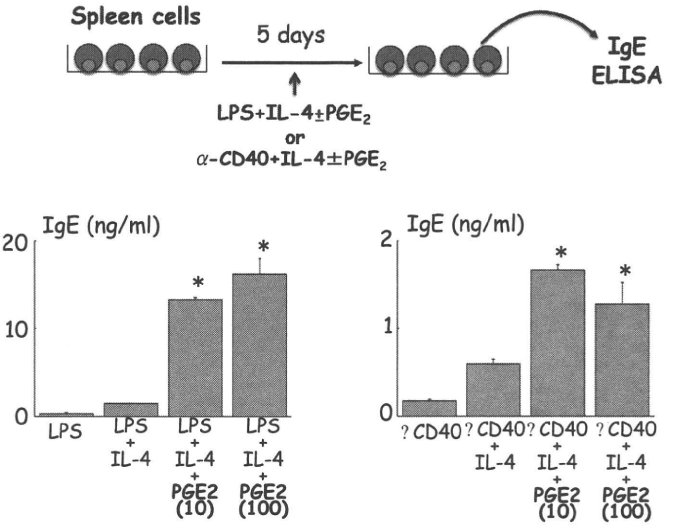
mPGES-1欠損マウス由来マクロファージではシリカによる
PGE_2産生が低下する



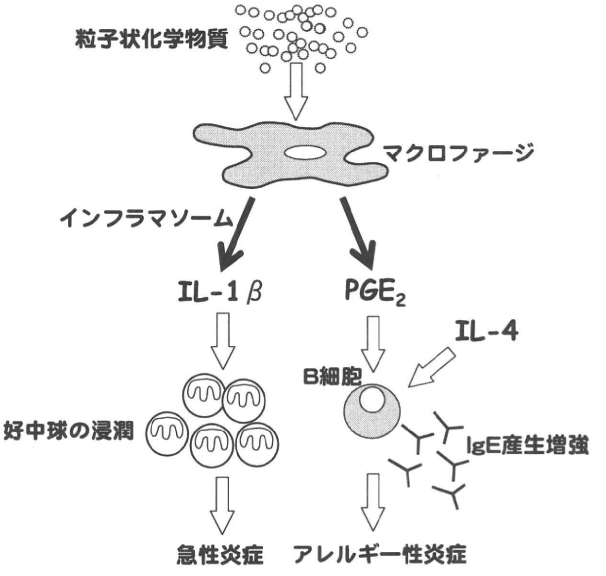
Antigen-specific IgE producion is reduced in mPGES-/- mice after the immunization with alum



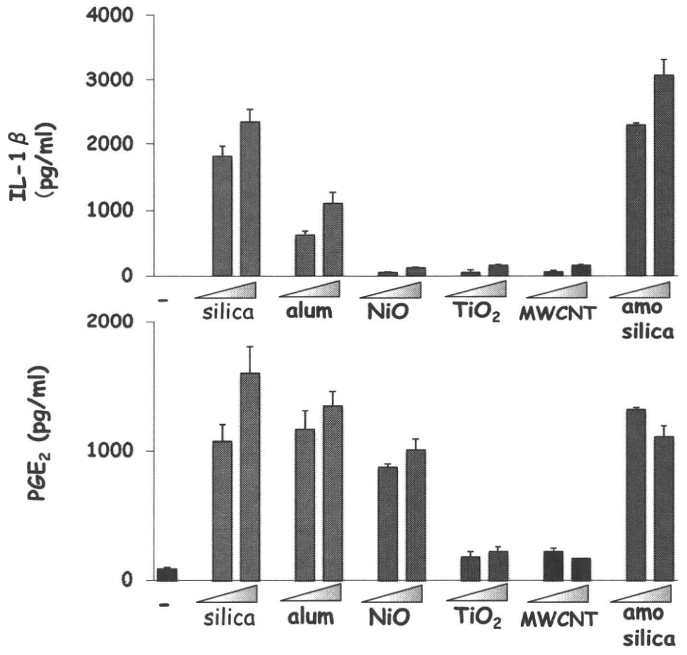
Antigen-specific IgE producion is reduced in mPGES-/- mice after the immunization with Silica



PGE_2はin vitroにおいてB細胞からのIgE産生を促進する

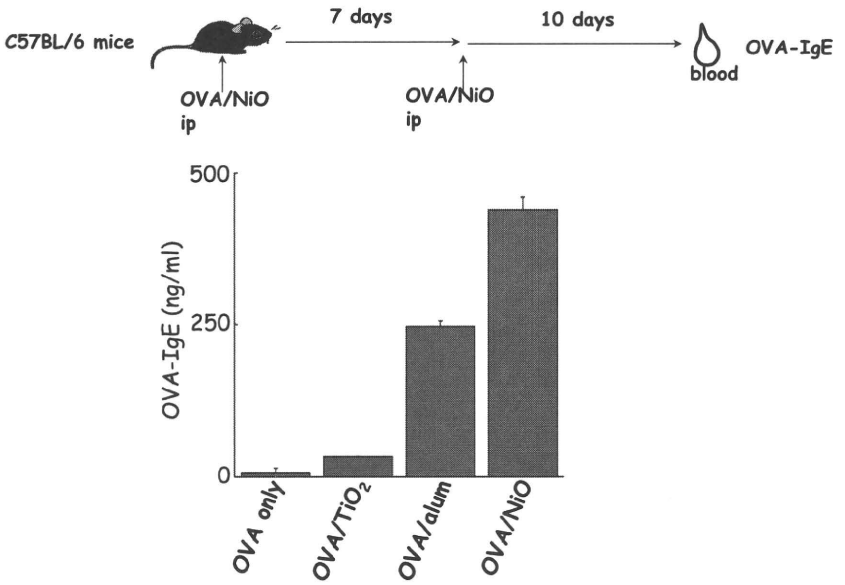


粒子状化学物質によるマクロファージの活性化と急性炎症およびアレルギー性炎症の誘導モデル

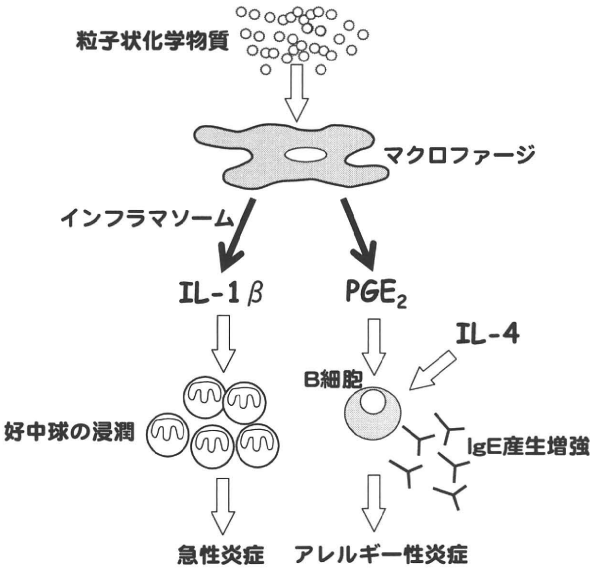


酸化ニッケル（NiO）はPGE$_2$産生のみ誘導する！！

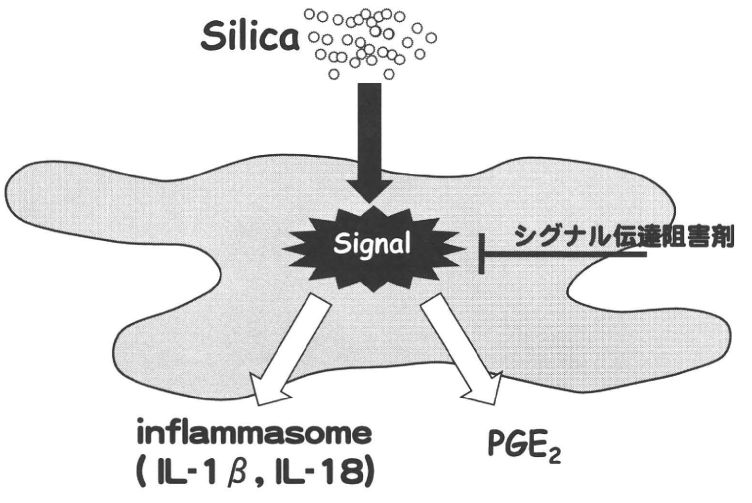
酸化ニッケル（NiO）はIgEを誘導するか？



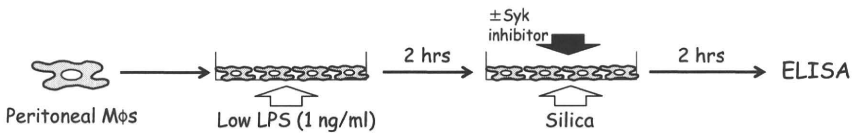
粒子状化学物質による血清IgE抗体価の増強

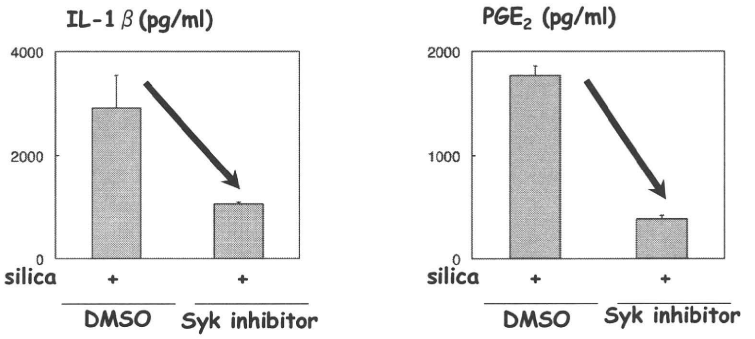


粒子状化学物質によるマクロファージの活性化と急性炎症およびアレルギー性炎症の誘導モデル

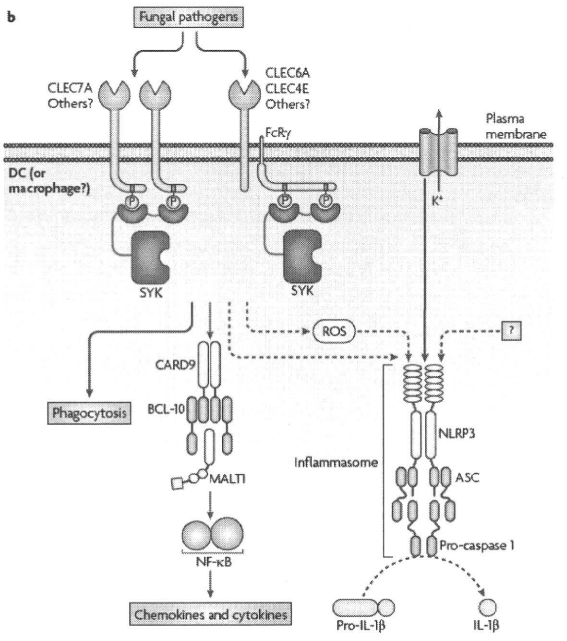


どのようなシグナル伝達経路がinflammasome活性化とPGE_2産生誘導に関与しているのか？



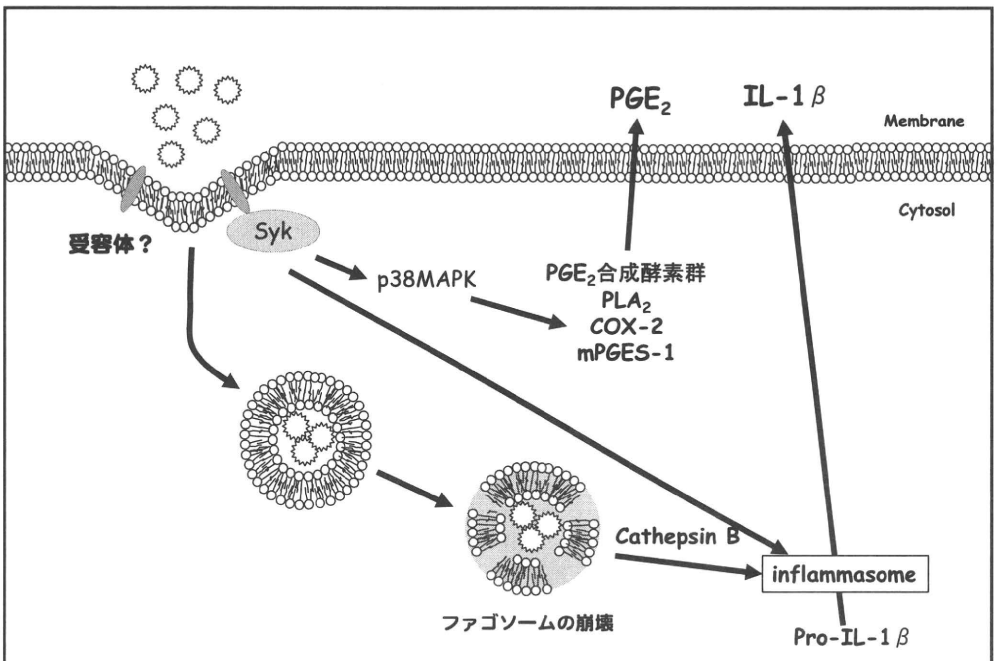


Sykはinflammasome活性化とPGE$_2$産生誘導の両方に関与するマスターレギュレーターである



Syk：
Fc受容体，補体受容体，Dectin-1などのPRRsのITAMに結合し，下流にシグナルを伝える分子

Nature reviews in Immunology 2010 Mocsai et al.



粒子状化学物質によるinflammasome活性化とPGE$_2$産生誘導を制御するシグナル伝達経路

Collaborators

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Department of Occupational Pneumology
Dr. Yasuo Morimoto
Dr. Lee Byeonwoo
Masami Hirohashi

National Institute of Biomedical Innovation
Dr. Ken J Ishii

Osaka University
Dr. Sizuo Akira
Dr. Satoshi Uematsu

Vaccine Forum 2010

アジュバント・ワークショップ

「α-GalCerアジュバントの免疫制御メカニズムと臨床応用」

石井 保之
独立行政法人理化学研究所
免疫・アレルギー科学総合研究センター(RCAI)
ワクチンデザイン研究チーム

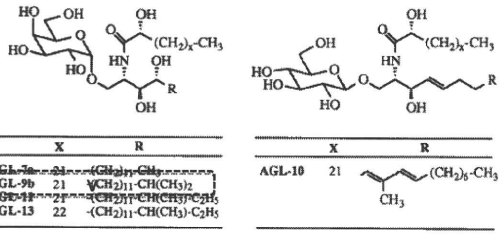
α-GalCer(α-galactosylceramide)の発見

- 旧キリンビール医薬探索研究所のチームが、胆癌マウスを用いたin vivoアッセイで、抗腫瘍効果を示す天然物化合物アゲラスフィン類(α-GalCer)を沖縄海域に生息する海綿から単離同定した。
- その後、様々な誘導体を合成し、最も抗腫瘍効果が期待できるα-GalCer (KRN7000)を選択した。

- 1993: Natori T, Koezuka Y, Higa T. Agelasphins, "Novel α-galactosylceramides from the marine sponge agelas mauritianus". *Tetrahedron letters*, 34:5591.
- 1994: Natori T, Morita M, Akimoto K, Koezuka Y. "Agelasphins, novel antitumor and immunostimulatory cerebrosides from the sponge *Agelas mauritanus*." *Tetrahedron*, 50:2771.
- 1995: Morita M, Natori T, Akimoto K, Osawa T, Fukushima H, Koezuka Y. "Syntheses of a-, b-monoglycosylceramide and four diastereomers of an α-galactosylceramide." *Bioorganic & Medical Chemistry Letters*, 5:699.
- 1995: Kobayashi E, Motoki K, Uchida T, Fukushima H, Koezuka Y. "KRN7000, a novel immunomodulator, and its antitumor activities." *Oncol Res*, 7:529.
- 1995: Morita M, Motoki K, Akimoto K, et al. "Structure-activity relationship of α-galactosylceramides against B16-bearing mice." *J Med Chem* , 38:2176.

α-GalCerの構造と活性

天然物



	X	R
AGL-9b	21	$-(CH_2)_{11}-CH(CH_3)_2$
AGL-13	22	$-(CH_2)_{11}-CH(CH_3)-C_2H_5$

	X	R
AGL-10	21	$-(CH_2)_6-CH_3$ (CH3)

Fig. 1. Structures of AGL-7a, AGL-9b, AGL-11, AGL-13, and AGL-10.
Natori et al. 1994 *Tetrahedron*

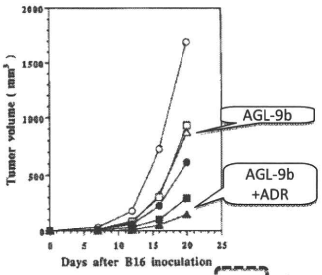
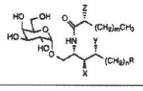


Figure 1. Tumor growth inhibitory effects of AGL-9b and AGL-502 in combination with or without adriamycin on mice subcutaneously inoculated with B16 cells. B16 cells (1×10^6) were subcutaneously inculated into female BDF$_1$ mice on day 0. AGL-9b and AGL-502 were intravenously administered on days 1, 5, and 9, and ADR was intraperitoneally administered on day 1. The mean of six mice is shown here: ○, control; △, AGL-9b (100 μg/kg); □, AGL-502 (100 μg/kg); ●, ADR (10 mg/kg); ▲, ADR + AGL-9b; ■, ADR + AGL-502.
Morita et al. 1995 *J Med Chem*

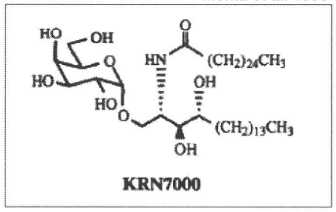
Table 3. Tumor Growth Inhibitory Effects of AGL Analogues on Mice Subcutaneously Inoculated with B16 Cells

化学合成物



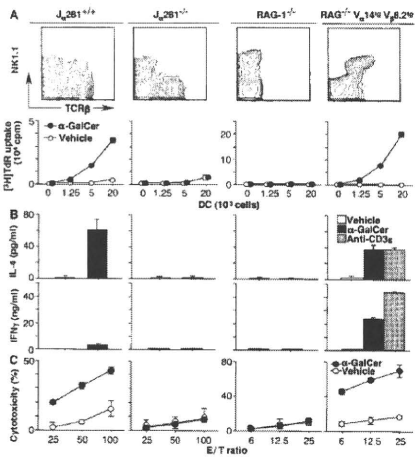
compd	X	Y	Z	R	m	n	maximum TGIR (%) single	MMC	AGLs + MMC
AGL-502	OH	OH	OH	$CH(CH_3)_2$	21	11	86.8	61.5	94.6
AGL-519	OH	OH	OH	CH_3	21	12	82.4	61.5	90.6
AGL-509	OH	OH	OH	CH_3	21	5	46.2	46.5	85.1
AGL-510	OH	OH	OH	CH_3	21	10	94.1	46.5	77.4
AGL-512	OH	OH	OH	CH_3	21	13	57.9	64.0	91.9
AGL-548	OH	OH	OH	CH_3	23	13	92.6	57.3	97.7
AGL-549	OH	OH	OH	CH_3	23	14	72.3	57.3	97.1
AGL-550	OH	OH	OH	CH_3	23	15	92.6	57.3	94.3
AGL-512	OH	OH	OH	CH_3	21	13	57.9	64.0	91.9
AGL-525	OH	OH	H	CH_3	21	13	65.0	64.8	90.8
AGL-506	OH	H	OH	CH_3	21	13	51.3	70.7	98.6
AGL-514	OH	H	H	CH_3	21	13	68.9	64.0	99.1
AGL-535[a]	H	H	H	CH_3	21	13	40.7	80.0	91.4
AGL-517	OH	H	H	CH_3	11	13	54.2	64.1	54.4
AGL-536	OH	H	H	CH_3	15	13	75.1	64.1	80.3
AGL-544	OH	H	H	CH_3	17	13	83.1	39.4	90.4
AGL-543	OH	H	H	CH_3	19	13	56.9	39.4	85.0
AGL-514	OH	H	H	CH_3	21	13	68.9	64.0	99.1
AGL-562	OH	OH	H	CH_3	23	13	53.8	67.6	96.7[b]

[a] C2 epimeric mixture. [b] AGL-548 and AGL562 were administered at days 6, 10, and 14. Tumor volume of each mouse was measured on days 8, 12, 16, and 20, and maximum TGIRs are shown in this table.

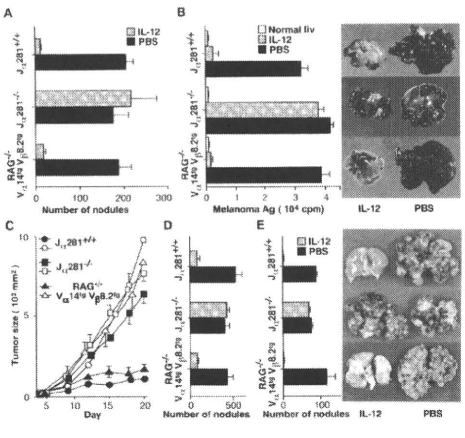


α-GalCerはVα14 iNKT細胞のリガンドであった

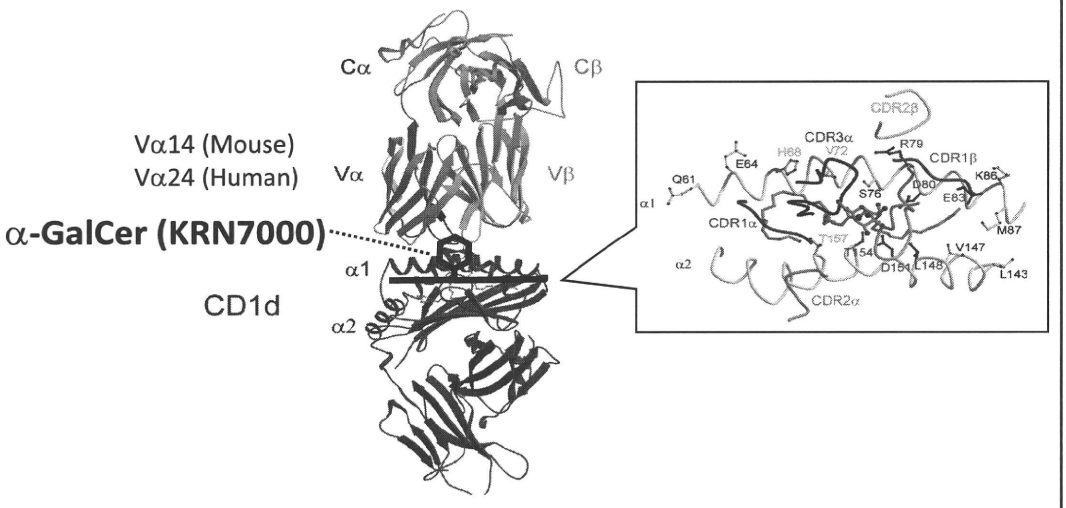
- 1997: Kawano T, Cui J, Koezuka Y, et al. "CD1d-restricted and TCR-mediated activation of Vα14 NKT cells by glycosylceramides." *Science* , 278:1626.



- 1997: Cui J, Shin T, Kawano T, Sato H, et al. "Requirement for Vα14 NKT cells in IL-12-mediated rejection of tumors." *Science* , 278:1623.



α-GalCerはCD1d分子提示され、invariant TCRα 鎖を発現する iNKT細胞を刺激する



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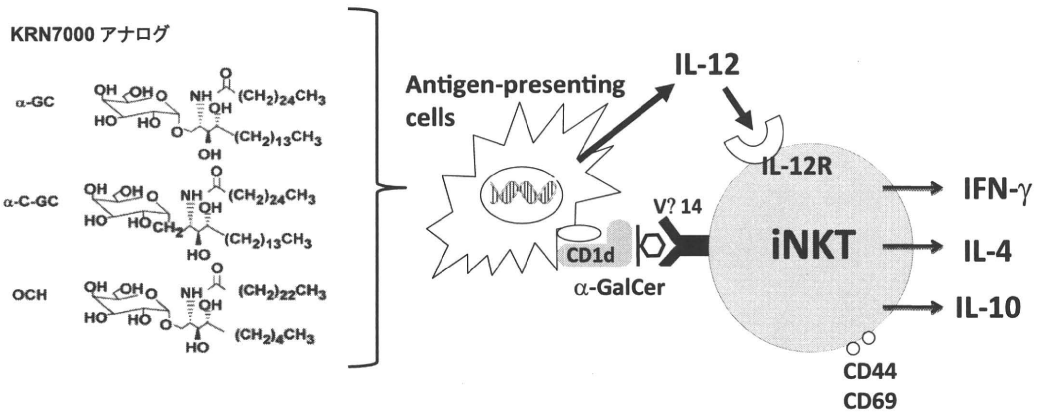
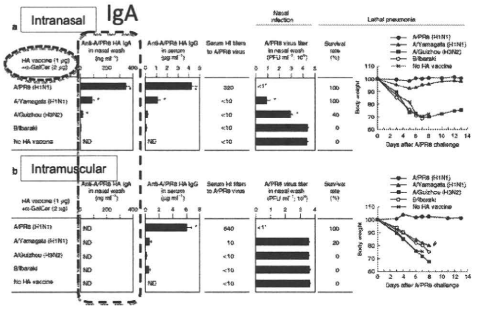
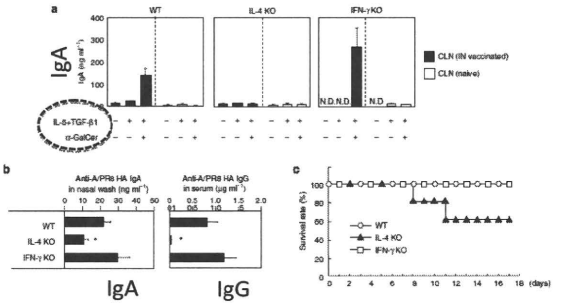
α-GalCerによるVα14 iNKT細胞の活性化



感染症に対する有効性

- マウスにα-GalCerと抗原を経鼻投与するとIgA産生が誘導される。
  - ➡ NKT細胞からのIL-4産生が重要

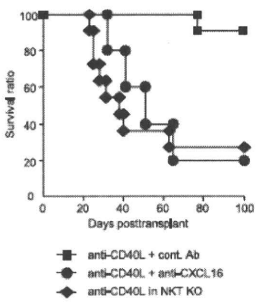




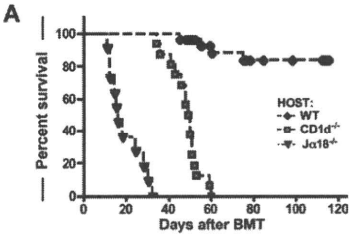
- 2005: Ko S-Y, Ko H-J, Chang W-S et al. "α-galactosylceramide can act as a nasal vaccine adjuvant inducing protective immune responses against viral infection and tumor. "*J Immunol*, 175:3309.
- 2008: Kamijuke H, Nagata T, Jiang X et al. "Mechanism of NKT cell activation by intranasal coadministration of α-galactosylceramide, which can induce cross-protection against influenza viruses." *Muc Immunity*, 1:208.
- 2010: Noda K, Kodama S, Umemoto S et al, "Nasal vaccination with P6 outer membrane protein and α-galactosylceramide induces nontypeable *Haemophilus influenzae*-specific protective immunity associated with NKT cells activation and dendritic cell expansion in nasophaynz." *Vaccine*, 28:5068.

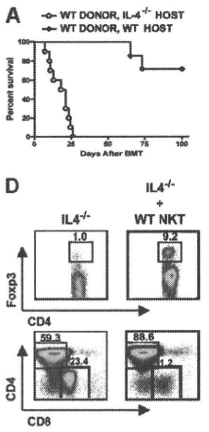
移植拒絶やGvHDをNKT細胞が保護

- NKT欠損マウスへの心臓移植モデルでは抗CD40L抗体による移植拒絶抑制が認められない。
  - ➡ NKT細胞が必須



- マウス骨髄移植モデルでは、レシピエントのNKT細胞がGvHD抑制に働く
  - ➡ NKT細胞のIL-4産生
  - ➡ Foxp3+CD4+Tregの増殖

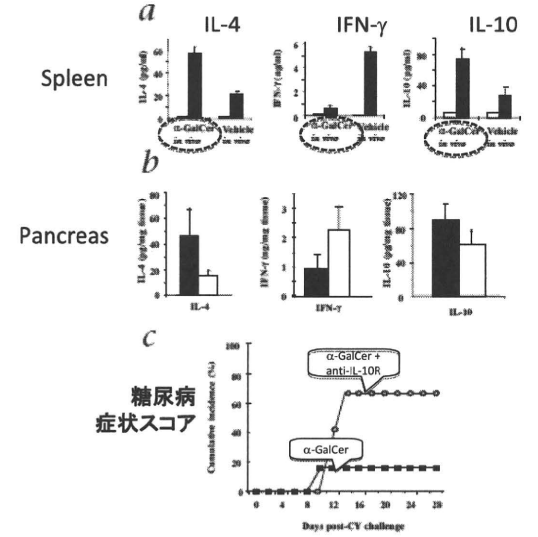


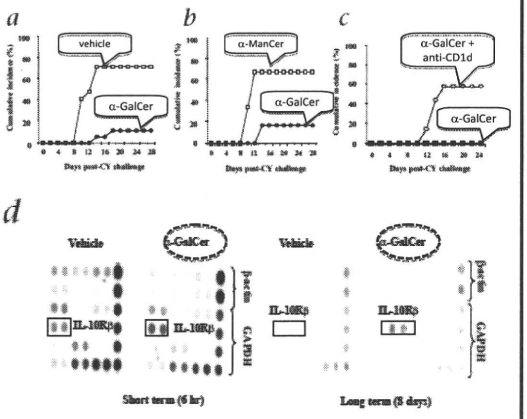


- 2000: Seino K-I, Fukao K, Muramoto K, et al. "requirement for natural killer T (NKT) cells in the induction of allograft tolerance." *Proc Natl Acad Sci USA*, 98:2577.
- 2005: Jiang X, Shimaoka T, Kojo S, et al. "Critical role of CXCL16/CXCR6 in NKT cell trafficking in allograft tolerance." *J Immunol*, 175:2051.
- 2007: Pillai AB, George TI, Dutt S, Strober S, "Host NKT cells can prevent graft-versus-host disease and permit graft antitumor activity after bone marrow transplantation." *J Immunol*, 178:6242.
- 2009: Pillai AB, George TI, Dutt S, Strober S, "Host natural killer T cells induce an interleukin-4-dependent expansion of おdonot CD4+CD25+Foxp3+ T regulatory cells that protects against graft-versus-host disease." *Blood* 113:4458.

I型糖尿病に対する有効性

- NODマウスにα-GalCerをi.p.連投すると、糖尿病症状が抑えられる。
  - NKT細胞のIL-4とIL-10産生が増強
  - IL-10R発現が増強





2001: Sharif S, Arrenza GA, Zucker P, et al. "Activation of natural killer T cells by α-galactosylceramide treatment prevents the onset and recurrence of autoimmune type 1 diabetes." *Nat Med,* 7:1057.

iNKT細胞の多彩な機能は α-GalCerを提示する APCsによって制御されている