京滋クリティカルケア・ヤングアカデミー：　特別講演「敗血症の病態メカニズム」資料

Nov 9, 2013. Teiji Sawa, Department of Anesthesiology, Kyoto Prefectural Univ. of Medicine

【実験ノート１】ラットに大腸菌エンドトキシン（0.1mg/g 体重）を静注して 4 時間観察

Physiologic Variables	Saline-infused	
	Control (n = 5)	Endotoxemia (n = 5)
MAP (mmHg)		
0 min	116 ± 4	111 ± 5
60 min	115 ± 6	100 ± 2
120 min	119 ± 4	101 ± 4
180 min	101 ± 6	94 ± 13
240 min	108 ± 7	90 ± 15
*p*H		
0 min	7.38 ± 0.03	7.37 ± 0.01
60 min	7.40 ± 0.02	7.33 ± 0.01
120 min	7.40 ± 0.03	7.32 ± 0.02*†
180 min	7.42 ± 0.01	7.35 ± 0.01
240 min	7.44 ± 0.01	7.35 ± 0.01
Pa_{O_2} (mmHg)		
0 min	75.2 ± 8.3	91.1 ± 5.5
60 min	98.8 ± 16.1	111.8 ± 5.5*
120 min	89.5 ± 12.5	115.1 ± 8.4*
180 min	89.9 ± 12.8	109.7 ± 4.6*
240 min	97.1 ± 14.2	127.2 ± 8.3*†
Pa_{CO_2} (mmHg)		
0 min	40.0 ± 5.9	37.0 ± 1.9
60 min	32.1 ± 2.8	32.5 ± 2.6
120 min	37.1 ± 2.8	27.4 ± 4.0*
180 min	34.6 ± 4.1	26.9 ± 2.3*
240 min	35.8 ± 3.5	26.1 ± 1.4*
Base excess (mEq/l)		
0 min	−1.7 ± 1.5	−0.9 ± 1.2
60 min	−2.9 ± 2.3	−5.2 ± 2.0*
120 min	−0.9 ± 2.2	−9.0 ± 1.5*†
180 min	−0.7 ± 2.3	−7.5 ± 0.4*
240 min	0.9 ± 2.0	−7.3 ± 1.4*†

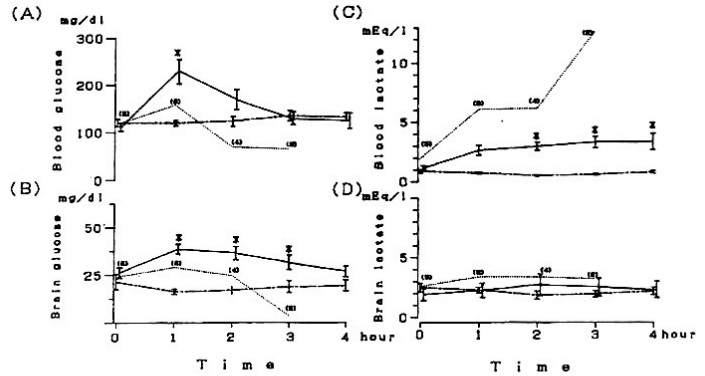


Figure 1. The changes of the concentrations in the blood glucose (A), brain glucose (B), blood lactate (C) and brain lactate (D). (———) The control group (n = 4). (——) The shock group that survived for 4 h (n = 6). (------) The shock group that died with 4 h. Numbers in the parentheses represent the number of rats surviving at that time. Values are reported as means ± SEM. *P < 0.05 compared with the control group.

- Sawa T, Okuda C, Mizobe T, Tanaka H, Lee E, Miyazaki M. Changes in glucose and lactate in the cerebral cortex of rats during endotoxin shock. Jpn Anaesth J Rev 4 : 63-65, 1990.
- Sawa T, Okuda C, Harada M, Murakami T. Effects of glucose infusion on cerebral cortical glucose and lactate concentrations during endotoxemia in rats. Anesthesiol 77 : 742-749, 1992.

【Keynote 1】Cachectin と Tumor Necrosis Factor (TNF)

Proc Natl Acad Sci U S A. 1975 Sep;72(9):3666-70.

An endotoxin-induced serum factor that causes necrosis of tumors.

Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B.

Nature. 1985 Aug 8-14;316(6028):552-4.

Identity of tumour necrosis factor and the macrophage-secreted factor cachectin.

Beutler B, Greenwald D, Hulmes JD, Chang M, Pan YC, Mathison J, Ulevitch R, Cerami A.

Science. 1985 Aug 30;229(4716):869-71.

Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin.

Beutler B, Milsark IW, Cerami AC.

【Keynote 2】Toll 様受容体（Toll-Like Receptors）の役割同定

Nature. 1998 Sep 17;395(6699):284-8.

Toll-like receptor-2 mediates lipopolysaccharide-induced cellular signalling.

Yang RB, Mark MR, Gray A, Huang A, Xie MH, Zhang M, Goddard A, Wood WI, Gurney AL, Godowski PJ.

Department of Molecular Biology, Genentech, South San Francisco, California 94080-4990, USA.

J Immunol. 1999 Apr 1;162(7):3749-52.

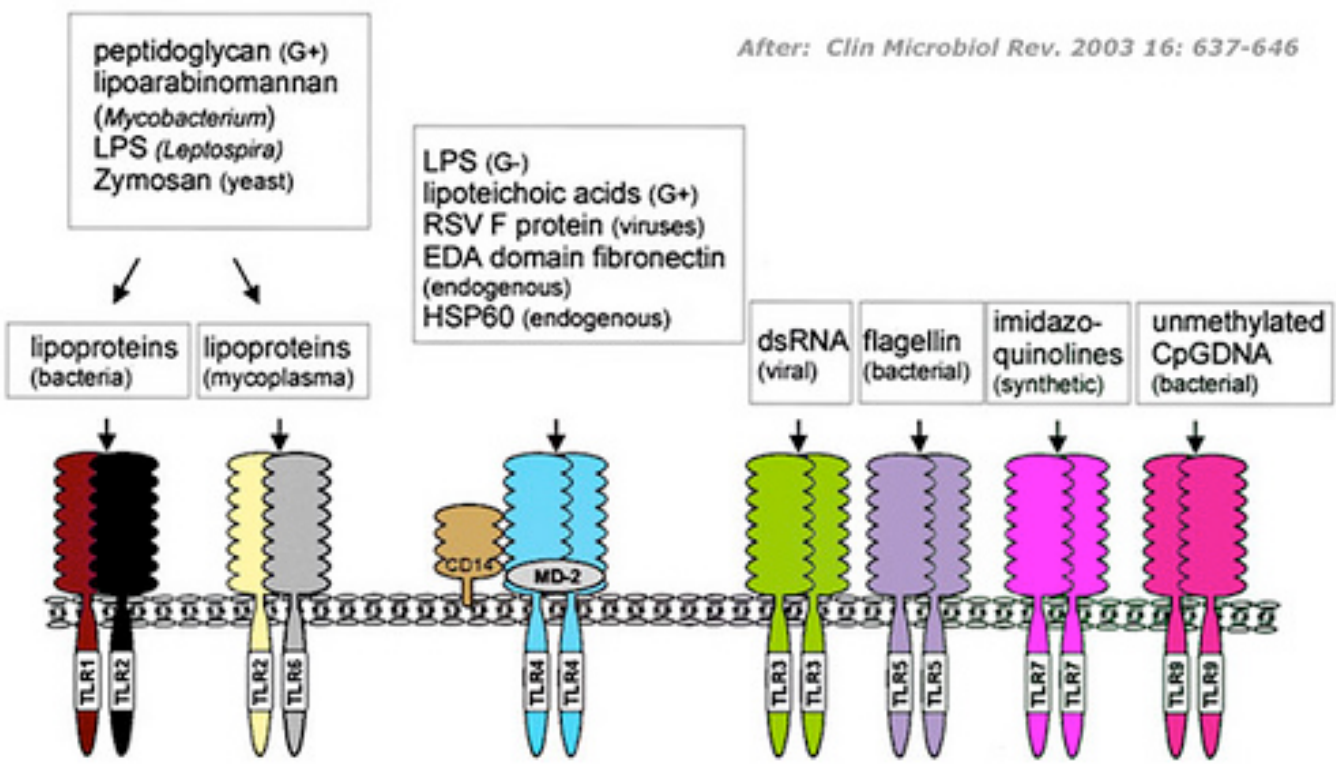
Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product.

Hoshino K, Takeuchi O, Kawai T, Sanjo H, Ogawa T, Takeda Y, Takeda K, Akira S.

Department of Biochemistry, Hyogo College of Medicine, Japan.

【ポイント１】

Toll-Like Receptors = パターン認識受容体 PRR



【Keynote 3】エンドトキシン抵抗性 C3H/HeJ マウスと TLR-4 の遺伝子変異の発見

Science. 1998 Dec 11;282(5396):2085-8.

Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene.

Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi-Castagnoli P, Layton B, Beutler B.

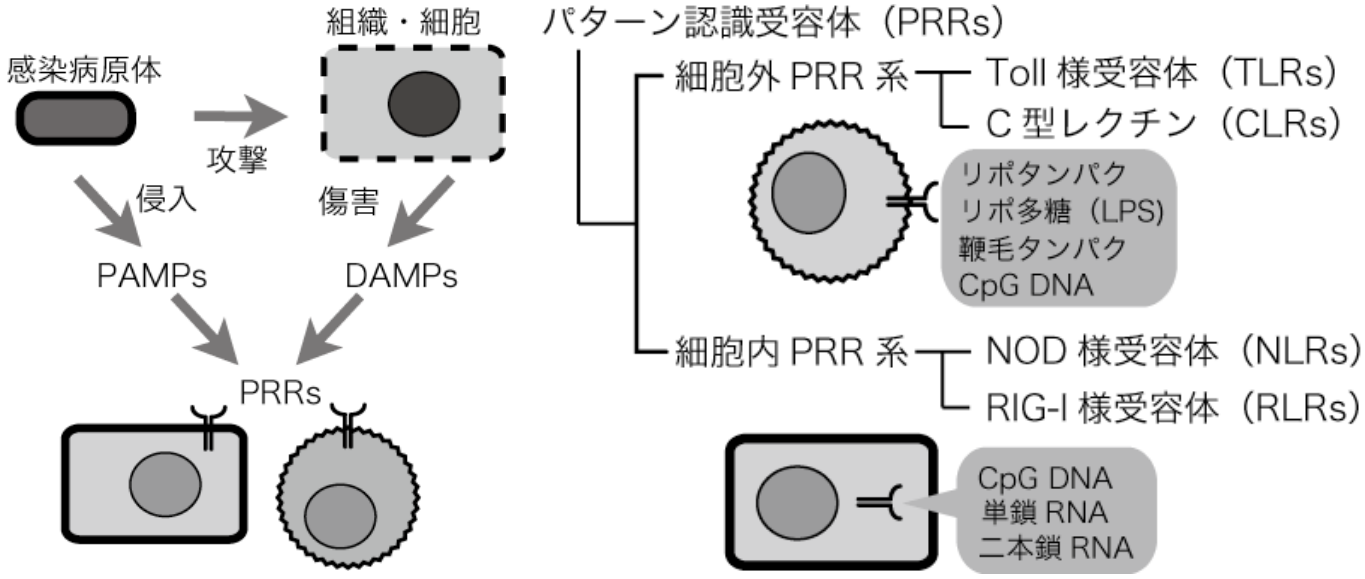
【Keynote 4】 High Mobility Group Box-1 (HMGB-1)が Late Mediator であるとした報告

Science. 1999 Jul 9;285(5425):248-51.

HMG-1 as a late mediator of endotoxin lethality in mice.

Wang H, Bloom O, Zhang M, Vishnubhakat JM, Ombrellino M, Che J, Frazier A, Yang H, Ivanova S, Borovikova L, Manogue KR, Faist E, Abraham E, Andersson J, Andersson U, Molina PE, Abumrad NN, Sama A, Tracey KJ.

【ポイント２】パターン認識受容体群　　Pattern Recognition Receptors (PRRs)



- 佐和貞治**. 敗血症と遺伝的素因. 特集 Sepsis. Intensivist 2 : 217-228, 2009.
- 佐和貞治**. 炎症の制御はどのようにおこなわれているか? 敗血症とヘルパーT 細胞. 特集 Sepsis. Intensivist 2 : 229-238, 2009.
- 佐和貞治. 手術侵襲と炎症・免疫反応. 臨床麻酔 35: 1483-1489, 2011.

【実験ノート 2】C3H/HeJ マウスの肺に緑膿菌を投与して色々と観察

図 1

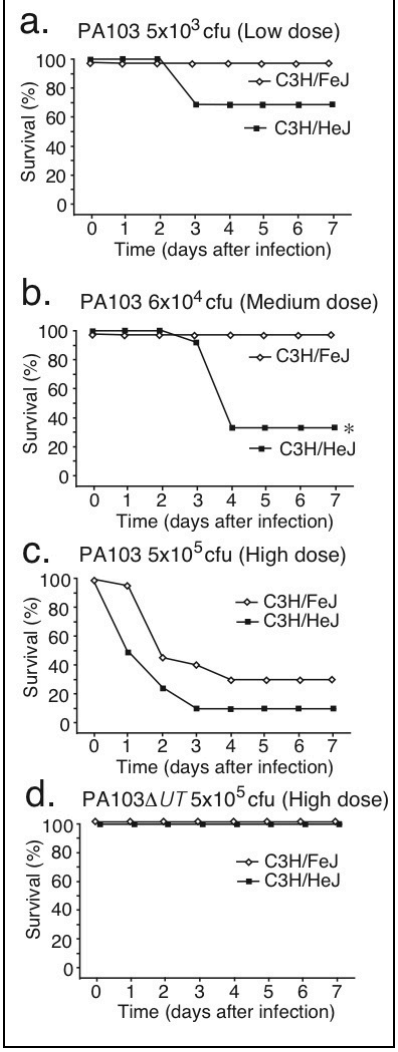


図 2

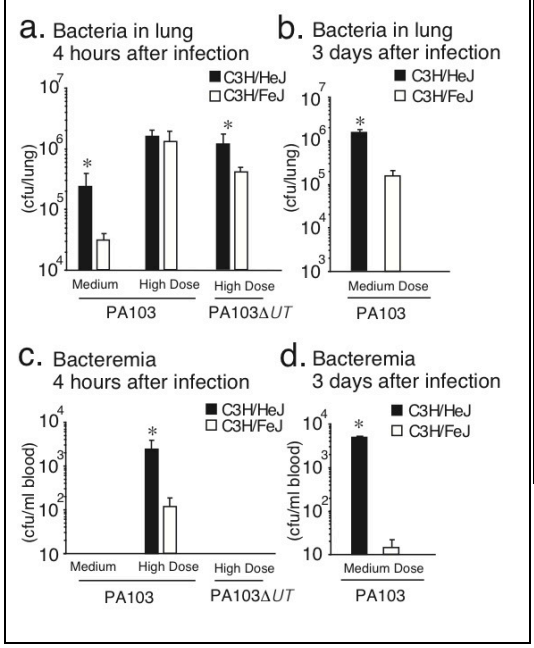


図 3

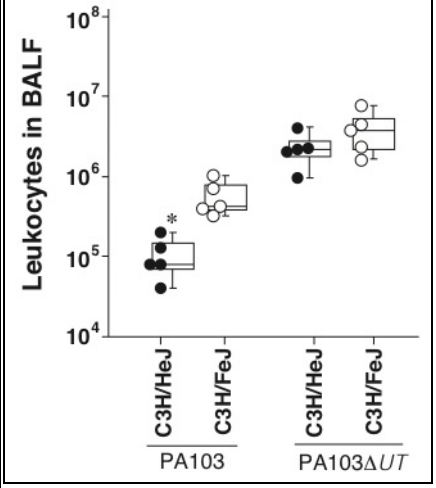
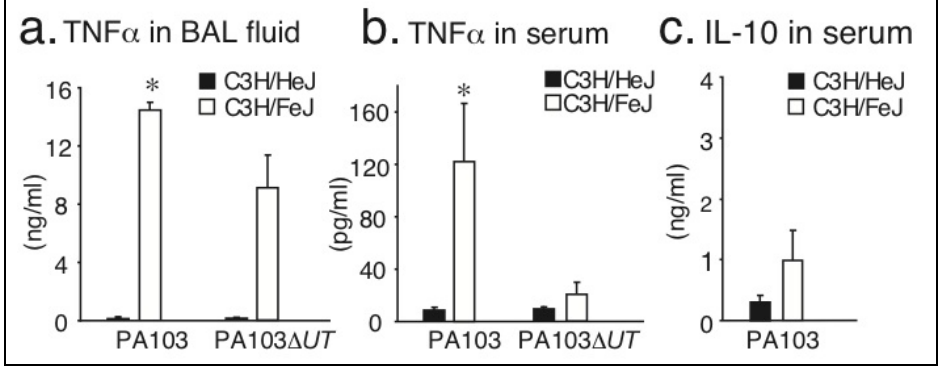


図 4



- Faure K, Sawa T**, Ajayi T, Fujimoto J, Moriyama K, Shime N, Wiener-Kronish JP. TLR4 signaling is essential for survival in acute lung injury induced by virulent *Pseudomonas aeruginosa* secreting type III secretory toxins. Respir Res 5: 1 (1-10), 2004.

ポイント：C3H/HeJ では緑膿菌感染で TNF が産生されず、菌血症が悪化し、有意に死亡率が高い。

【実験ノート 3】 緑膿菌 V 抗原の免疫効果発見：

Nat Med. 1999 Apr;5(4):392-8.

Active and passive immunization with the Pseudomonas V antigen protects against type III intoxication and lung injury.

Sawa T, Yahr TL, Ohara M, Kurahashi K, Gropper MA, Wiener-Kronish JP, Frank DW.

Department of Anesthesia and Perioperative Care, The University of California, San Francisco 94143-0542, USA.

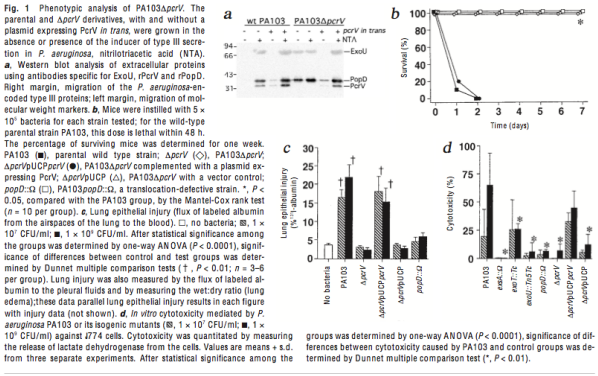


Fig. 1 Phenotypic analysis of PA103Δ*pcrV*. The parental and Δ*pcrV* derivatives, with and without a plasmid expressing PcrV *in trans*, were grown in the absence or presence of the inducer of type III secretion in *P. aeruginosa*, nitrilotriacetic acid (NTA). ***a***, Western blot analysis of extracellular proteins using antibodies specific for ExoU, rPcrV and rPopD. Right margin, migration of the *P. aeruginosa*-encoded type III proteins; left margin, migration of molecular weight markers. ***b***, Mice were instilled with 5×10^6 bacteria for each strain tested; for the wild-type parental strain PA103, this dose is lethal within 48 h. The percentage of surviving mice was determined for one week. PA103 (■), parental wild type strain; Δ*pcrV* (◇), PA103Δ*pcrV*; Δ*pcrV*pUCP*pcrV* (●), PA103Δ*pcrV* complemented with a plasmid expressing PcrV; Δ*pcrV*pUCP (△), PA103Δ*pcrV* with a vector control; *popD*::Ω (□), PA103*popD*::Ω, a translocation-defective strain. *, $P < 0.05$, compared with the PA103 group, by the Mantel-Cox rank test ($n = 10$ per group). ***c***, Lung epithelial injury (flux of labeled albumin from the airspaces of the lung to the blood). □, no bacteria; ▨, 1×10^7 CFU/ml; ■, 1×10^6 CFU/ml. After statistical significance among the groups was determined by one-way ANOVA ($P < 0.0001$), significance of differences between control and test groups was determined by Dunnet multiple comparison tests († , $P < 0.01$; $n = 3$–6 per group). Lung injury was also measured by the flux of labeled albumin to the pleural fluids and by measuring the wet:dry ratio (lung edema);these data parallel lung epithelial injury results in each figure with injury data (not shown). ***d***, *In vitro* cytotoxicity mediated by *P. aeruginosa* PA103 or its isogenic mutants (▨, 1×10^7 CFU/ml; ■, 1×10^6 CFU/ml) against J774 cells. Cytotoxicity was quantitated by measuring the release of lactate dehydrogenase from the cells. Values are means + s.d. from three separate experiments. After statistical significance among the groups was determined by one-way ANOVA ($P < 0.0001$), significance of differences between cytotoxicity caused by PA103 and control groups was determined by Dunnet multiple comparison test (*, $P < 0.01$).

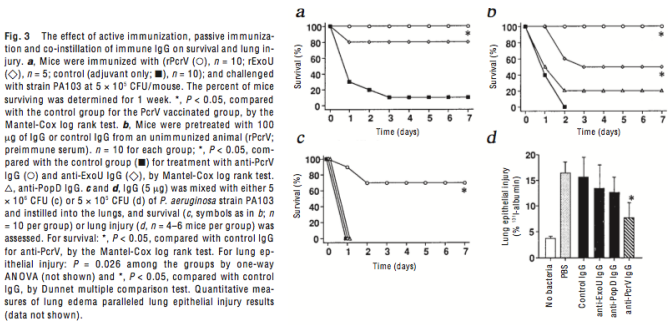
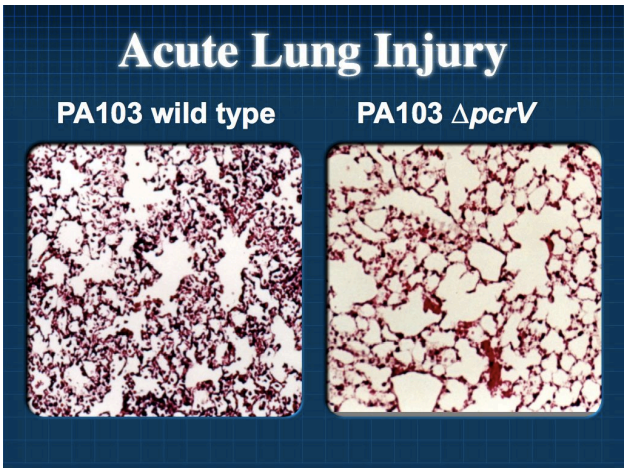
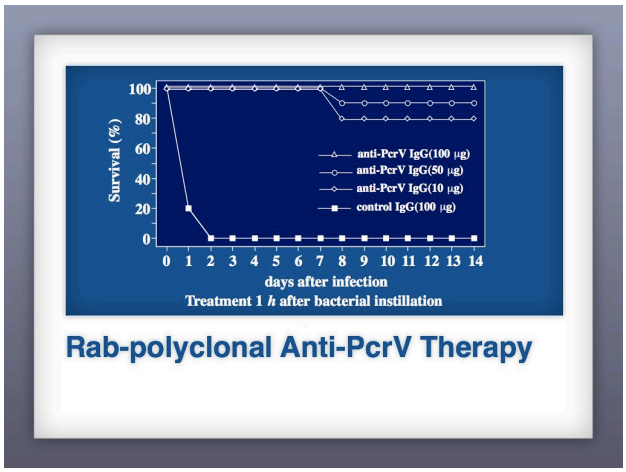


Fig. 3 The effect of active immunization, passive immunization and co-instillation of immune IgG on survival and lung injury. ***a***, Mice were immunized with (rPcrV (○), $n = 10$; rExoU (◇), $n = 5$; control (adjuvant only; ■), $n = 10$); and challenged with strain PA103 at 5×10^5 CFU/mouse. The percent of mice surviving was determined for 1 week. *, $P < 0.05$, compared with the control group for the PcrV vaccinated group, by the Mantel-Cox log rank test. ***b***, Mice were pretreated with 100 μg of IgG or control IgG from an unimmunized animal (rPcrV; preimmune serum). $n = 10$ for each group; *, $P < 0.05$, compared with the control group (■) for treatment with anti-PcrV IgG (○) and anti-ExoU IgG (◇), by Mantel-Cox log rank test. △, anti-PopD IgG. ***c*** and ***d***, IgG (5 μg) was mixed with either 5×10^5 CFU (c) or 5×10^7 CFU (d) of *P. aeruginosa* strain PA103 and instilled into the lungs, and survival (*c*, symbols as in *b*; $n = 10$ per group) or lung injury (*d*, $n = 4$–6 mice per group) was assessed. For survival: *, $P < 0.05$, compared with control IgG for anti-PcrV, by the Mantel-Cox log rank test. For lung epithelial injury: $P = 0.026$ among the groups by one-way ANOVA (not shown) and *, $P < 0.05$, compared with control IgG, by Dunnet multiple comparison test. Quantitative measures of lung edema paralleled lung epithelial injury results (data not shown).



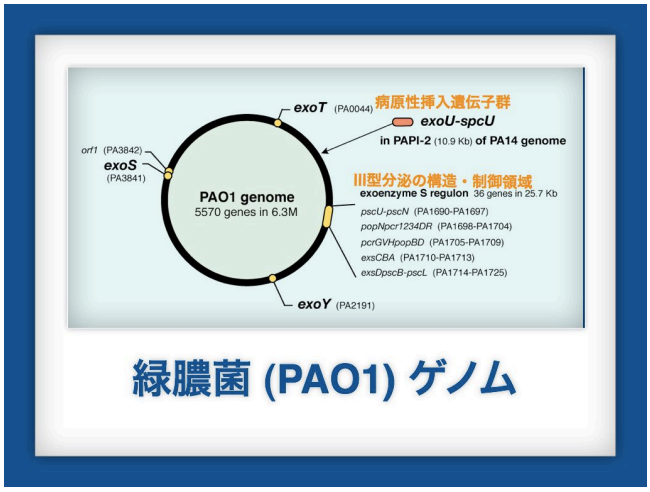


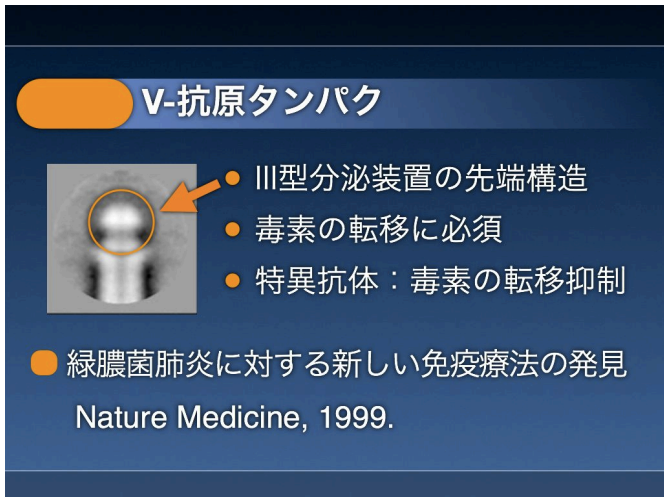
ポイント： 肺傷害 エンドトキシンではなくて、III 型分泌エクソトキシン

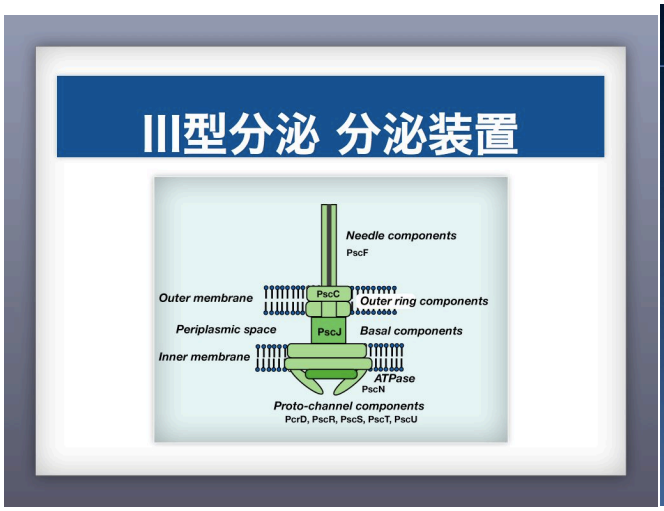
- Shime N, Sawa T, Fujimoto J, Faure K, Allmond LR, Karaca T, Swanson BL, Spack EG, Wiener-Kronish JP. Therapeutic administration of anti-PcrV F(ab')2 in sepsis associated with *Pseudomonas aeruginosa*. J Immunol 167 : 5880-5886, 2001.
- Roy-Burman A, Savel RH, Racine S, Swanson BL, Revadigar NS, Fujimoto J, Sawa T, Frank DW, Wiener-Kronish JP. Type III Protein Secretion is Associated with Death in Lower Respiratory and Systemic *Pseudomonas aeruginosa* infection. J Infect Dis 183 : 1767-1764, 2001.
- Frank DW, Vallis A, Wiener-Kronish JP, Roy-Burman A, Spack EG, Mullaney BP, Megdoud M, Marks JD, Fritz R, Sawa T**. Generation and characterization of a protective monoclonal antibody to *Pseudomonas aeruginosa* PcrV. J Infect Dis 186 : 64-73, 2002.
- Frank DW, Vallis A, Wiener-Kronish JP, Roy-Burman A, Spack EG, Mullaney BP, Megdoud M, Marks JD, Fritz R, Sawa T**. Generation and characterization of a protective monoclonal antibody to *Pseudomonas aeruginosa* PcrV. J Infect Dis 186 : 64-73, 2002.

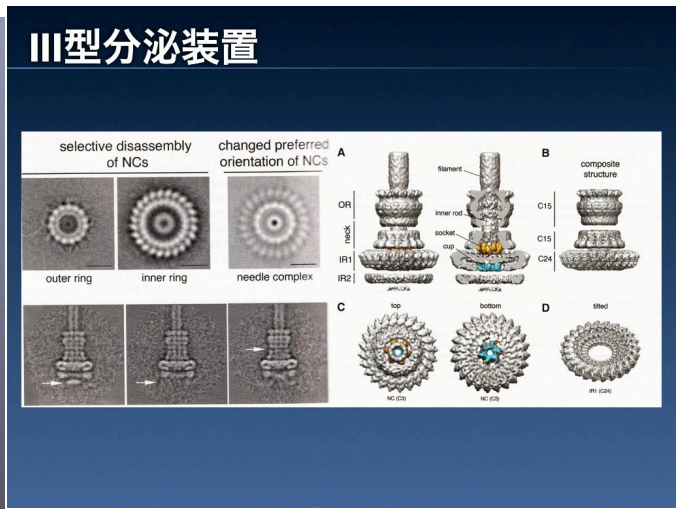
- Faure K, Shimabukuro DW, Ajayi T, Allmond LR, Sawa T, Wiener-Kronish JP. O-antigen serotypes and type III secretory toxins in clinical isolates of of *Pseudomonas aeruginosa*. J Clin Microbiol 41 : 2158-2160, 2003.
- Sato H, Frank DW, Cecilia J. Hillard, Pankhaniya R, Moriyama K, Finck-Barbancon V, Buchaklian A, Lei M, Long RM, Wiener-Kronish JP, Sawa T. The mechanism of action of the *Pseudomonas aeruginosa*-encoded type III cytotoxin ExoU. EMBO J 22 : 2959-2969, 2003.
- Ajayi T, Allmond LR, Sawa T, Wiener-Kronish JP. Single nucleotide polymorphism mapping of the *P. aeruginosa* type III secretion toxins for the development of a diagnostic multiplex PCR system. J Clin Microbiol 41 : 3526-3531, 2003.
- Faure K, Fujimoto J, Shimabukuro DW, Ajayi T, Shime N, Moriyama K, Spack EG, Wiener-Kronish JP, Sawa T**. Effects of monoclonal anti-PcrV antibody on *Pseudomonas aeruginosa*-induced acute lung injury in a rat model. J Immune Based Ther Vaccines 1: 2 (1-9), 2003.
- Tamura M, Ajayi T, Allmond LR, Moriyama K, Wiener-Kronish JP, Sawa T**. Lysophospholipase A activity of P*seudomonas aeruginosa* type III secretory toxin ExoU. Biochem Biophys Res Commun 316 : 323-31, 2004.
- Pankhaniya RR, Tamura M, Allmond LR, Moriyama K, Ajayi T, Wiener-Kronish JP, Sawa T**. *Pseudomonas aeruginosa* causes acute lung injury via the catalytic activity of the patatin-like phospholipase domain of ExoU. Crit Care Med 32 : 2293-2299, 2004.
- Neely AN, Holder IA, Wiener-Kronish JP, Sawa T. Passive anti-PcrV treatment protects burned mice against *Pseudomonas aeruginosa* challenge. Burns 31 : 153-158, 2005.
- Imamura Y, Yanagihara K, Fukuda Y, Kaneko Y, Seki M, Izumikawa K, Muyazaki Y, Hirakata Y, Sawa T, Wiener-Kronish JP, Kohno S. Effect of anti-PcrV antibody in a murine chronic airway *Pseudomonas aeruginosa* infection model. Eur Respir J 29 : 965-968, 2007.
- Baer M, Sawa T, Flynn P, Luehrsen K, Martinez D, Wiener-Kronish JP, Yarranton G, Bebbington C. An engineered human antibody Fab fragment specific for *Pseudomonas aeruginosa* PcrV antigen has potent anti-bacterial activity. Infect Immun 77 : 1083-1090, 2008.
- Moriyama K, Wiener-Kronish JP, Sawa T. Protective effects of affinity-purified antibody and truncated vaccines against *Pseudomonas aeruginosa* V-antigen in neutropenic mice. Microbiol Immunol 53: 587-594, 2009.

グラム陰性菌Ⅲ型分布システムとⅤ抗原









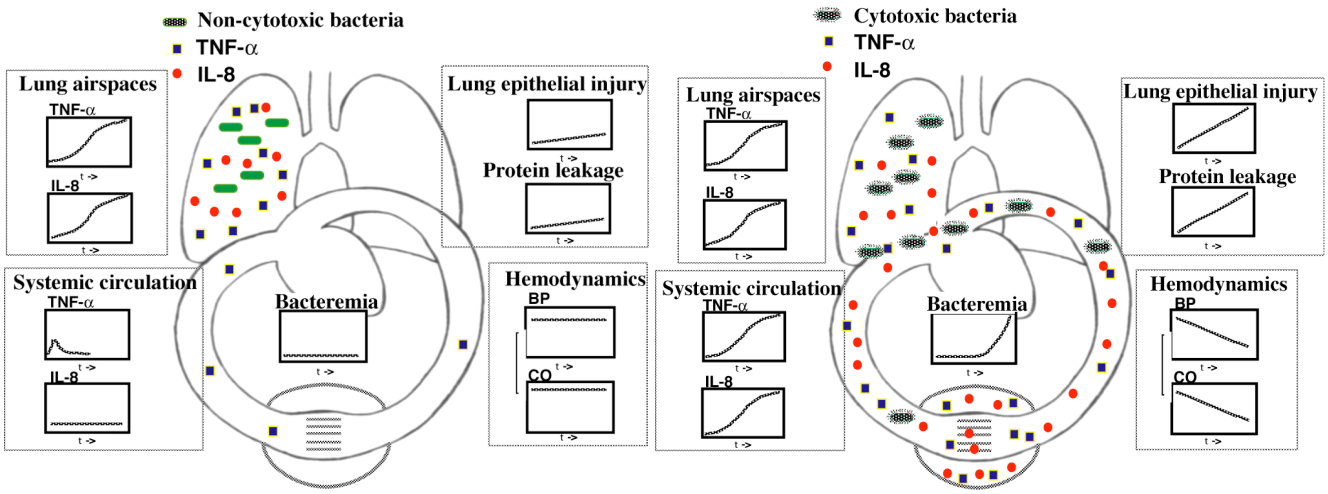
- 佐和ていじ. 緑膿菌性肺炎・敗血症と III 型分泌システム. 日本集中治療医学会誌 8 : 305-310, 2001.
- 森山潔、佐和貞治. グラム陰性菌の毒素について―緑膿菌 III 型分泌毒素を中心に- 臨麻 29 : 1279-1286, 2005.
- 佐和貞治、森山潔: 特集　細菌感染症への新たな治療戦略、緑膿菌 III 型分泌毒素に対する治療戦略、化療の領域 23 : 1265-1272, 2007.
- 佐和貞治. 慢性気道感染症-緑膿菌性肺炎における臨床分離株の比較検討-. 化療の領域 24 : 373-379, 2008.
- 佐和貞治.　緑膿菌ワクチンおよび抗緑膿菌抗体の開発. 緑膿菌感染症研究会講演記録 41 : 27-33, 2007.
- 佐和貞治, 加藤秀哉, 安本寛章. Topics グラム陰性菌の病原性と敗症. Anesthesia 21 Century 14(1-42): 2723-2329, 2012.
- 佐和貞治. 病原性グラム陰性菌のIII型分泌. 抗原ワクチン・抗体療法の開発. 京都府立医科大学雑誌 120: 659-671, 2011.

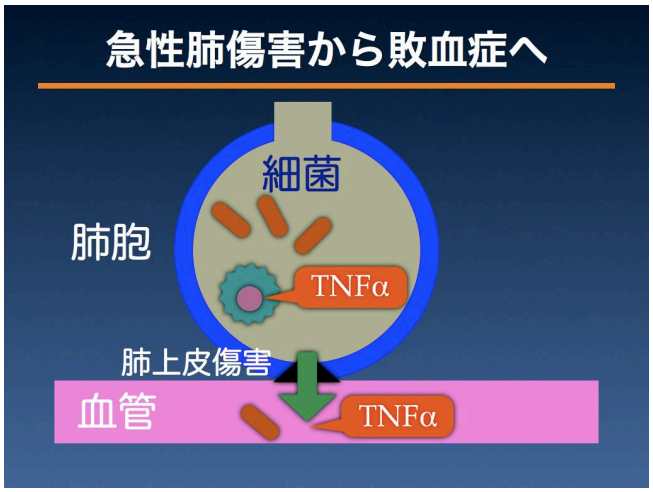
【実験ノート 4】　ウサギ緑膿菌肺炎モデル：肺傷害と敗血症

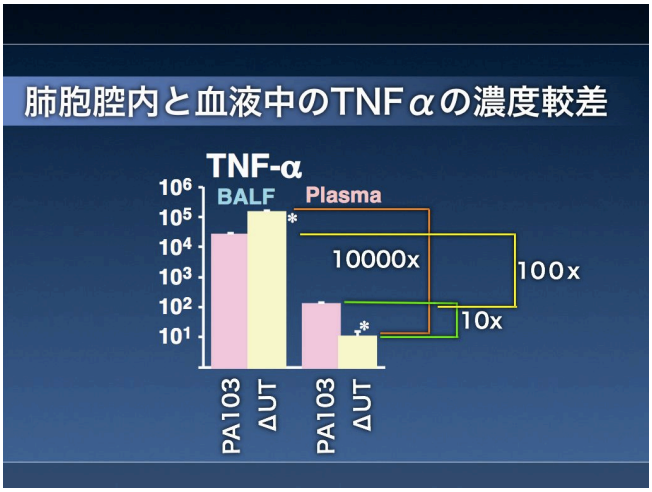
J Clin Invest. 1999 Sep;104(6):743-50.

Pathogenesis of septic shock in Pseudomonas aeruginosa pneumonia.

Kurahashi K, Kajikawa O, Sawa T, Ohara M, Gropper MA, Frank DW, Martin TR, Wiener-Kronish JP.
Department of Anesthesia and Perioperative Care, University of California-San Francisco, San Francisco, California 94143, USA.







ポイント：緑膿菌の静脈内投与ではショックにならない。肺胞内 TNF 濃度は、血液中の 10,000 倍！