interactions   to   investigate   vaccine's   molecular mechanism of action.
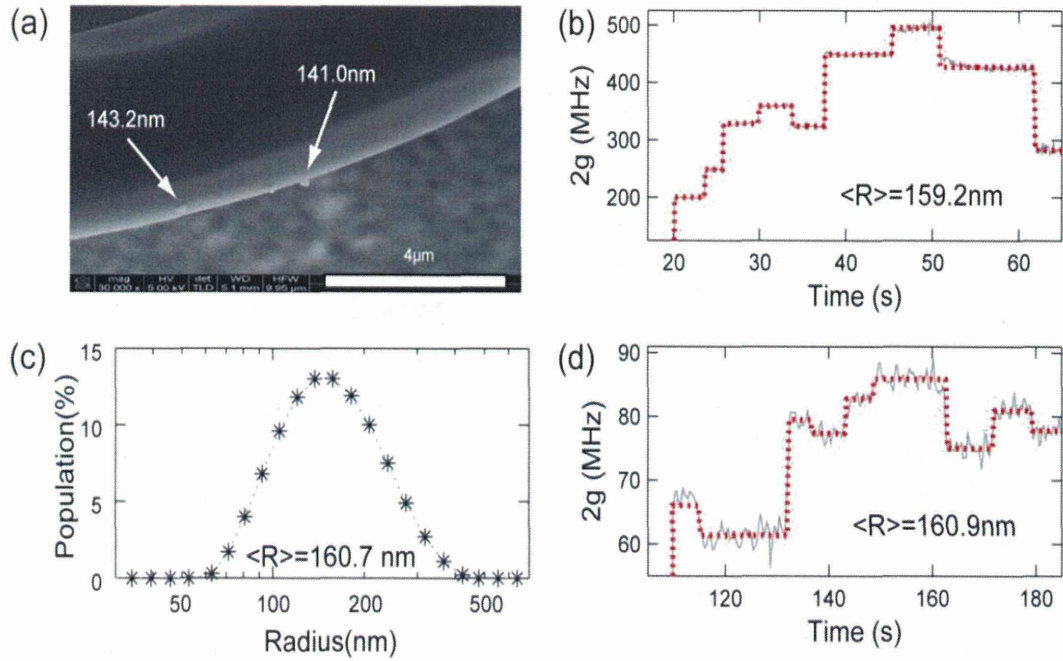

Figure 4. Detection and measurement of hemozoin crystals. (a) SEM image of hemozoin crystals deposited on a microtoroid resonator. (b) Amount of mode splitting induced by consecutively deposited hemozoin crystals on a microtoroid. Experiments were done in air. (c) Typical size distribution obtained from DLS measurements for the hemozoin crystals. (d) Result of mode splitting experiment performed in an aquatic environment for hemozoin crystals. Discrete jumps in (b) and (d) signals that a hemozoin is within the mode volume of the resonator. Figure was reproduced from *Kim et al., 2012, ref. #5.*

D. 考察

After the observation that synthetic hemozoin could be a cheap, easy and reliable adjuvant in dog allergy models (Coban et al., Cell Host Microbe, 2010), now, we've evaluated its effect in higher animals such as monkeys. We've found that synthetic hemozoin is as effective as potent CpG ODN adjuvant. We've deepen our investigation to understand its mechanism of action. Because there could be a clue that why some antigens/adjuvants work well in mouse but not in higher animals or humans. Our recent progresses in Alum research have also prompted us to study other particulate structures such as sHZ to make it possible to use as successful adjuvant at least in veterinary vaccines. It is known that alum exerts its Type 2 adjuvant properties MyD88-independently, but via TBK1. We currently investigate whether this is similar pathway for sHZ adjuvanticity. We're now working on the new detection systems such as WGM resonators or Raman microscopy to be able sense receptor-ligand interactions to understand how adjuvants interact with immune system.

These studies may lead us to optimize adjuvants for safer and potent usage in future.

E. 結論

Particulates could exert adjuvant properties, however, by which mechanism is not known. Therefore, understanding their mechanism of action may lead to find new and safer adjuvants.

G. 研究発表

-Publications

1- *Kuroda E, Coban C, Ishii* KJ. Particulate adjuvant and innate immunity: past achievements, present findings and future prospects. International Reviews of Immunology, 2013, 13; 32(2):209-20. doi: 10.3109/08830185.2013.773326.

2- *Hobro AJ, Konishi A, Coban C, Smith NI.* Raman spectroscopic analysis of malaria disease progression via blood and plasma samples. Analyst, 2013, Mar 26. [Epub ahead of print] PMID: 23529513.

3- *Tang CK, Aoshi T, Jounai N, Ito J, Ohata K, Kobiyama K, Dessailly BH, Kuroda E, Akira S, Mizuguchi K, Coban C, Ishii KJ.* The chemotherapeutic agent DMXAA as a unique IRF3-dependent type-2 vaccine adjuvant. PLOS ONE, 2013, 8(3): e60038. doi:10.1371/journal.pone.0060038.

4- *Tougan T, Aoshi T, Coban C, Katakai Y, Kai C, Yasutomi Y, Ishii KJ, Horii T.* TLR9 adjuvants enhance immunogenicity and protective efficacy of the SE36/AHG malaria vaccine in nonhuman primate models. Human Vaccines and Immunotherapeutics, 2013, Jan 4; 9(2). PMID: 23291928 [Epub ahead of print]

5- *Kim W, Ozdemir SK, Zhu J, Faraz M, Coban C, Yang L.* Detection and size measurement of individual hemozoin nanocrystals in aquatic environment using a whispering gallery mode resonator. Optics Express, 2012, Dec 31; 20(28):29426-46. doi: 10.1364/OE.20.029426.

6- *Hong Z, Konishi A, Fujita Y, Yagi M, Ohata K, Aoshi T, Itagaki S, Sato S, Narita H, Abdelgelil NH, Inoue M, Culleton R, Kaneko O, Nakagawa A, Horii T, Akira S, Ishii KJ, Coban C.* Lipocalin 2 bolsters innate and adaptive immune responses to blood-stage malaria infection by reinforcing host iron metabolism. Cell Host Microbe, 2012, 12(5):705-16. doi: 10.1016/j.chom.2012.10.010.PMID: 23159059.

7- *Tang CK, Coban C, Akira S, Ishii KJ.* Chapter 1: Route to discovering the immunogenic properties of DNA from TLR9 to cytosolic DNA sensing. Biological DNA Sensor: The Impact of Nucleic Acids on Diseases and Vaccinology (ELSEVIER), 2013, *in press.*

8- *Coban C, Tozuka M, Jounai N, Kobiyama K, Takeshita F, Tang CK, Ishii KJ.* Chapter 11: DNA vaccines: Common way of DNA sensing?. Biological DNA Sensor: The Impact of Nucleic Acids on Diseases and Vaccinology (ELSEVIER), 2013, *in press.*

–Presentations

1. *Coban C.* Lipocalin 2 and the iron metabolism during malaria infection.

Department of Tropical Medicine, Tulane University School of Public Health and Tropical Medicine, January 22nd, 2013, New Orleans, LA, USA.

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
Tang CK and Ishii KJ	The Impact of Nucleic Acids on Diseases and Vaccinology		Biological DNA Sensor:	Elsevier Inc	アメリカ	2013	In press
安田好文, 中西憲司	アレルギーとサイトカイン		医薬ジャーナル	医薬ジャーナル社	大阪	2013	683-687
安田好文, 中西憲司	蠕虫の排除と自然免疫・獲得免疫		臨床免疫・アレルギー科	科学評論社	東京	2012	307-15
中平雅清, 中西憲司	サイトカインのすべて, インターロイキン 18) IL-18.		臨床免疫・アレルギー科	科学評論社	東京	2012	125-36
安田好文, 中西憲司	自然免疫による好酸球性肺炎発症機構		医学のあゆみ	医歯薬出版株式会社	東京	2012	91-7
武藤太一朗, 安田好文, 中西憲司	寄生虫感染と肺におけるTh2型自然免疫応答		実験医学	羊土社	東京	2012	3056-61
中平雅清, 中西憲司	アレルギーに対するサイトカイン Ⅰ. IL-4.		アレルギー・免疫	医薬ジャーナル社	大阪	2012	12-21
Tang CK, **Coban C**, Akira S, Ishii KJ	Route to discovering the immunogenic properties of DNA from TLR9 to cytosolic DNA sensing	No	Biological DNA Sensor: The Impact of Nucleic Acids on Diseases and Vaccinology	ELSEVIER	Chapter 1	2013, in press	
Coban C, Tozuka M, Jounai N, Kobiyama K, Takeshita F, Tang CK, Ishii KJ.	DNA vaccines: Common way of DNA sensing?	No	Biological DNA Sensor: The Impact of Nucleic Acids on Diseases and Vaccinology	ELSEVIER	Chapter 11	2013, in press	

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Haenuki Y, Matsushita K, Futatsugi-Yumikura S, Ishii KJ, Kawagoe T, Imoto Y, Fujieda S, Yasuda M, Hisa Y, Akira S, Nakanishi K, Yoshimoto T.	A critical role of IL-33 in experimental allergic rhinitis.	J Allergy Clin Immunol.	130(1):	184-94. .e11	2012
Nakayama T, Kashiwagi Y, Kawashima H, Kumagai T, Ishii KJ, Ihara T.	Alum-adjuvanted H5N1 whole virion inactivated vaccine (WIV) enhanced inflammatory cytokine productions.	Vaccine.	6;30(26)	3885-90.	2012
Desmet CJ, Ishii KJ.	Nucleic acid sensing at the interface between innate and adaptive immunity in vaccination.	Nat Rev Immunol.	22;12(7)	479-91	2012
Shoji M, Tachibana M, Katayama K, Tomita K, Tsuzuki S, Sakurai F, Kawabata K, Ishii KJ, Akira S, Mizuguchi H.	Shoji M, Tachibana M, Katayama K, Tomita K, Tsuzuki S, Sakurai F, Kawabata K, Ishii KJ, Akira S, Mizuguchi H.	Biochem Biophys Res Commun.	17;425(1)	89-93	2012
Tetsutani K, Ishii KJ.	Adjuvants in influenza vaccines.	Vaccine			2012
Nakayama T, Kumagai T, Ishii KJ, Ihara T.	Alum-adjuvanted H5N1 whole virion inactivated vaccine (WIV) induced IgG1 and IgG4 antibody responses in young children.	Vaccine.			2012

Zhao H, Konishi A, Fujita Y, Yagi M, Ohata K, Aoshi T, Itagaki S, Sato S, Narita H, Abdelgelil NH, Inoue M, Culleton R, Kaneko O, Nakagawa A, Horii T, Akira S, Ishii KJ, Coban C.	Lipocalin 2 bolsters innate and adaptive immune responses to blood-stage malaria infection by reinforcing host iron metabolism.	Cell Host Microbe.	15;12(5)	705-16	2012
Jounai N, Kobiyama K, Takeshita F, Ishii KJ.	Recognition of damage-associated molecular patterns related to nucleic acids during inflammation and vaccination.	Front Cell Infect Microbiol.	2 (168)	1-13	2012
Shiraishi K, Hamano M, Ma H, Kawano K, Maitani Y, Aoshi T, Ishii KJ, Yokoyama M.	Hydrophobic blocks of PEG-conjugates play a significant role in the accelerated blood clearance (ABC) phenomenon.	J Control Release.	10;165(3)	183-90.	2013
Tougan T, Aoshi T, Coban C, Katakai Y, Kai C, Yasutomi Y, Ishii KJ, Horii T.	TLR9 adjuvants enhance immunogenicity and protective efficacy of the SE36/AHG malaria vaccine in non human primate models.	Hum Vaccin Immunother	4;9 (2)	1-8	2013
Kondo T, Kobayashi J, Saitoh T, Maruyama K, Ishii KJ, Barber GN, Komatsu K, Akira S, Kawai T.	DNA damage sensor MRE11 recognizes cytosolic double-stranded DNA and induces type I interferon by regulating STING trafficking.	Proc Natl Acad Sci U S A	110(8)	2969-74	2013

Kuroda E, Coban C, Ishii KJ	Particulate adjuvant and innate immunity: past achievements, present findings and future prospects.	Int. Rev. Immunol.	In press		2013
Tang CK, Aoshi T, Jounai N, Ito J, Ohata K, Kobiyama, K, Desailly BH, Kuroda E, Akira S, Mizuguchi K, Coban C and Ishii KJ	The chemotherapeutic agent DMXAA as a unique IRF3-dependent type-2 vaccine adjuvant	PLOS one	In press		2013
石井　健.	「宿主の生体バリア・腸管、肺、皮膚における新たな免疫細胞とその機能.」	実験医学増刊	vol.30 No.20	p134(3292)-137(3295).	2012
石井　健	「感染・共生・生体防御研究から生まれる新たな疾患予防、治療法ターゲット.」	実験医学増刊	vol.30 No.20	p172(3330)-175(3333)	2012
黒田悦史.	「粒子アジュバントのメカニズム.」	実験医学増刊	vol.30 No.20	p203(3361)-208(3366)	2012
城内　直、石井　健	「細胞外核酸の生物学的意義と臨床応用.」	実験医学増刊	vol.30 No.20	p209(3367)-216(3374).	2012
小檜山康司、石井　健	「自然免疫メカニズムを利用するワクチンアジュバント開発.」	THE LUNG	20(4)	54-61	2012
鉄谷耕平、石井　健.	「アジュバント開発研究の新展開：自然免疫から審査行政.」	ファームテクジャパン	28(4)	45-52	2012
鉄谷耕平、石井　健.	「ワクチンアジュバントの現状と展望.」	レギュラトリーサイエンス学会誌	2(2)	149-158	2012

石井　健.	「トップランナーに聞く　核酸による自然免疫および獲得免疫の制御機構の研究と核酸アジュバントのワクチンへの応用研究」	最新医学	68(2)	107-111	2013
大西　元康、石井健	「ワクチン（アジュバント）デザインの新展開」	医薬ジャーナル	49(2)	699-705	2013
城内　直、石井健.	「感染と免疫」	Medicina	50(3)	406-411	2013
Minowa Y., Kondo C., Uehara1 T., Morikawa Y., Okuno Y., Nakatsu N., Ono A., Maruyama T., Kato I., Yamate J., Yamada H., Ohno Y., Urushidani T.	Toxicogenomic multigene biomarker for predicting the future onset of proximal tubular injury in rats	Toxicology	297	47-56	2012
Yamada F., Sumida K., Uehara T., Morikawa Y., Yamada H., Urushidani T., Ohno Y.	Toxicogenomics discrimination of potential hepatocarcinogenicity of non-genotoxic compounds in rat liver	J. Appl. Toxicol.	Online publication		2012
Nakatsu N., Igarashi Y., Ono A., Yamada H., Ohno Y. and Urushidani T.	Evaluation of DNA microarray results in the Toxicogenomics Project (TGP) consortium in Japan	J. Toxicol. Sci.	37	791-801	2012
山田弘	総説：トキシコゲノミクスとバイオマーカー	日本薬理学雑誌	140	221-225	2012
笛木修,戸倉新樹,小野寺博志, 今井弘一, 細井一弘, 山田弘	光毒性試験代替法の第三者評価報告　評価対象:酵母光生育阻害試験と赤血球光溶血試験の組み合わせ	AATEX-JaCVAM	J1(1)	45-87,	2012
Uehara T., Kondo C., Morikawa Y., Hanafusa H., Ueda S., Minowa Y., Nakatsu N., Ono A., Maruyama T., Kato I., Yamate J., Yamada H., Ohno Y., Urushidani T.	Toxicogenomic Biomarkers for Renal Papillary Injury in Rats	Toxicology	303	1-8	2013

Matsumoto M, Sasaki Y, Yasuda K, Taki Y, Muramatsu M, Yoshimoto T, <u>N</u>akanishi K.	IgG and IgE collaboratively accelerate expulsion of Strongyloides 1 venezuelensis in a primary infection.	Infection and Immunity	In press		
Fukuoka A, Futatsugi-Yumikura S, Tkahashi S, Kazama H, Lyoda T, Yoshimoto T, Inaba K, <u>Nakanishi K</u>,Yonehara S	Identigication of a novel type 2 innate immunocyte with the ability to enhance IgE production.	Int Immunol	Epub		2013
Takahashi S, Futatsugi-Yumikura S, Fukuoka A, Yoshimoto T, <u>Nakanishi K</u>, Yonehara S.	Fas deficiency in mice with the Balb/c background induces blepharitis with allergic inflammation and hyper-IgE production in conjunction with severe autoimmune disease.	Int Immunol.	25(5)	287-293	2013
Yoshimoto T, <u>N</u>akanishi K.	Generation and characterization of mouse basophils from bone marrow and purification of basophils from spleen.	Curr Protec Immunol	98	3.24.1-3.24.16.	2012
Tsutsui H, <u>Nakanishi K.</u>	Immunotherapeutic applications of IL-18	Immunotherapy	4(12)	1883-94	2012
Yasuda K, Muto T, Kawagoe T, Matsumoto M, Sasaki Y, Matsushita K, Taki Y, Futatsugi-Yumikura S, Tsutsui H, Ishii K, Yoshimoto T, Akira S, <u>Nakaishi K</u>.	Contribution of IL-33-activated type II innate lymphoid cells to pulmonary eosinophilia in intestinal nematode-infected mice.	Proc Natl Acad Sci USA	109(9)	3451-6	2012
Haenuki Y, Matsushita K, Futatsugi-Yumikura S, Ishii K, Kawagoe T, Imoto Y, Fujieda S, Yasuda M, Hisa Y, Akira S, <u>Nakaishi K</u>, Yoshimoto T.	A critical role of IL-33 in experimental allergic rhinitis	J Allergy Clin Immunol	130(1)	184-94	2012

Enokizono, Y., H. K umeta,K.Funami,M. Horiuchi, J. Sarmie nto, K. Yamashita,D. Standley, T.Seya, M. Matsumoto,F.Inaga ki	Structures and interface ma pping of the Toll/Interleuki n-1 receptor-domain-contain ing adaptor molecules invol ved in interferon signaling.	*Proc Natl Aca d Sci USA.*	in press		2013
Oshiumi, H., M. Miyashita, M. Ma tsumoto, and T. S eya	Riplet ubiquitin ligase p lays a pivotal role in T RIM25-mediated RIG-I activation and is target ed by Hepatitis C Viru s.	*PLoS Pathog.*	in press		2013
Tatematsu, M., F. Nishikawa, T. Se ya, M. Matsumot o.	Toll-like receptor 3 reco gnizes single-stranded R NA with incomplete ste m structures.	*Nat Commun.*	in press		2013
Seya, T., M. Azu ma, and M. Mats umoto.	Targeting TLR3 with no RIG-I/MDA5 activation is effective in immunot herapy for cancer.	*Exp. Opn. Tar get. Therap.*	17(5)	533-544	2013
Oshiumi, H., K. F unami, H. H. Aly, M. Matsumoto, T. Seya.	Multi-step regulation of interferon induction by hepatitis C virus.	*Arch. Immuno l. Therap. Ex p.*	61	127-138	2013
Toscano, F., Y. Es tornes, F. Virard, A. Garcia-Cattan eo, A. Pierrot, B. Vanbervliet, K. F unami, T. Seya, M. Matsumoto, J. J. Pin, T. Renno, S. and Lebecque, K.	Cleavage of TLR3 by ca thepsins generates two fragments that remain associated to form a fu nctional receptor.	*J. Immunol.*	190	764-773	2013
Seya T, Shime H, Takaki H, Azum a M, Oshiumi H, Matsumoto M.	TLR3/TICAM-1 signalin g in tumor cell RIP3-de pendent necroptosis.	*Oncoimmunol.*	1	917-923	2012

Seya T, Shime H, Matsumoto M.	TAMable tumor-associated macrophages in response to innate RNA sensing.	*Oncoimmunol.*	1	1000-1001	2012
Oshiumi, H., M. Matsumoto, and T. Seya.	Ubiquitin-mediated modulation of the cytoplasmic viral RNA sensor RIG-I. (review)	*J. Biochem. (Tokyo).*	151	5-11	2012
Aly, H. H., K. Shimotohno, M. Hijikata, T. Seya.	In vitro models for analysis of the hepatitis C virus life cycle.	*Microbiol. Immunol.*	56(1)	1-9	2012
Yamazaki, S., A. Maruyama, K. Okada, M. Matsumoto, A. Morita, T. Seya.	Dendritic cells from oral cavity induce Foxp3+ regulatory T cells upon antigen stimulation.	*PLoS ONE*	7(12)	e51665	2012
Shime H, M. Matsumoto, H. Oshiumi, S. Tanaka, A. Nakane, Y. Iwakura, H. Tahara, N. Inoue, and T. Seya.	TLR3/TICAM-1 signaling converts tumor-supporting myeloid cells to tumoricidal effectors.	*Proc Natl Acad Sci USA.*	109	2066-2071	2012
Abe, Y., K. Fujii, N. Nagata, O. Takeuchi, S. Akira, H. Oshiumi, M. Matsumoto, T. Seya, and S. Koike.	Toll-like receptor 3-mediated antivirus response is important for protection against poliovirus infection in poliovirus receptor transgenic mice.	*J. Virol.*	86	185-194	2012
Azuma M., T. Ebihara, H. Oshiumi, M. Matsumoto, and T. Seya.	Cross-presentation and antitumor CTL induced by soluble Ag + polyI:C largely depend on the TICAM-1 pathway in mouse CD11c+/CD8a+ dendritic cells.	*OncoImmunol.*	1	581-592	2012

Hazeki, K., Y. Kametani, H. Murakami, M. Uehara, K. Nigorokawa, S. Takasuga, T. Sasaki, M. Matsumoto, T. Seya, and O. Hazeki.	Phosphoinositide 3-kinase<gamma> controls the intracellular localization of CpG to limit DNA-PKcs-dependent IL-10 production in macrophages.	*PLoS ONE.*	6(10)	e26836	2012
Fujiwara N, Porcelli SA, Naka T, Yano I, Maeda S, Kuwata H, Akira S, Uematsu S, Takii T, Ogura H, Kobayashi K.	Bacterial sphingophospholipids containing non-hydroxy fatty acid activate murine macrophages via Toll-like receptor 4 and stimulate bacterial clearance.	*Biochim Biophys Acta.*	S1388-1981(13)	00066-8	2013
Takeuchi C, Matsumoto Y, Kohyama K, Uematsu S, Akira S, Yamagata K, Takemiya T.	Microsomal prostaglandin E synthase-1 aggravates inflammation and demyelination in a mouse model of multiple sclerosis.	*Neurochem Int.*	62(3)	271-80	2013
Flores-Langarica A, Marshall JL, Hitchcock J, Cook C, Jobanputra J, Bobat S, Ross EA, Coughlan RE, Henderson IR, Uematsu S, Akira S, Cunningham AF.	Systemic Flagellin Immunization Stimulates Mucosal CD103+ Dendritic Cells and Drives Foxp3+ Regulatory T Cell and IgA Responses in the Mesenteric Lymph Node.	*J Immunol.*	189(12)	5745-54	2012

Shibata T, Takemura N, Motoi Y, Goto Y, Karuppuchamy T, Izawa K, Li X, Akashi-Takamura S, Tanimura N, Kunisawa J, Kiyono H, Akira S, Kitamura T, Kitaura J, **Uematsu S**, Miyake K.	PRAT4A-dependent expression of cell surface TLR5 on neutrophils, classical monocytes and dendritic cells.	*Int Immunol.*	189(12)	5745-54	2012
Kreutz M, Giquel B, Hu Q, Abuknesha R, **Uematsu S**, Akira S, Nestle FO, Diebold SS.	Antibody-antigen-adjuvant conjugates enable co-delivery of antigen and adjuvant to dendritic cells in cis but only have partial targeting specificity.	*PLoS One.*	7(7)	e40208	2012
Kuroki Y, Sasaki Y, Kamei D, Akitake Y, Takahashi M, **Uematsu S**, Akira S, Nakatani Y, Kudo I, Hara S.	Deletion of microsomal prostaglandin E synthase-1 protects neuronal cells from cytotoxic effects of β-amyloid peptide fragment 31-35.	*Biochem Biophys Res Commun.*	424(3)	409-13	2012
Chucair-Elliott AJ, Elliott MH, Wang J, Moiseyev GP, Ma JX, Politi LE, Rotstein NP, Akira S, **Uematsu S**, Ash JD.	Leukemia Inhibitory Factor Coordinates the Down-regulation of the Visual Cycle in the Retina and Retinal-pigmented Epithelium.	*J Biol Chem.*	287(29)	24092-102	2012
Yoshioka W, Aida-Yasuoka K, Fujisawa N, Kawaguchi T, Ohsako S, Hara S, **Uematsu S**, Akira S, Tohyama C.	Critical role of microsomal prostaglandin E synthase-1 in the hydronephrosis caused by lactational exposure to dioxin in mice.	*Toxicol Sci.*	127(2)	547-54	2012

Sasaki Y, Kamei D, Ishikawa Y, Ishii T, **Uematsu S,** Akira S, Murakami M, Hara S.	Microsomal prostaglandin E synthase-1 is involved in multiple steps of colon carcinogenesis.	**Oncogene.**	31(24)	2943-52	2012
Yoshhida, T., Omatsu, T., Saito, A., Katakai, Y., Iwasaki, Y., Kurosawa, T., Hamano, M., Higashimo, A., Nakamura, S.., Takasaki, T., <u>Yasutomi, Y.</u>, Kurane, I. and Akari, H.	Dynamics of cellular immune responses in the acute phase of dengue virus infection.	Archiv. Virol.			印刷中
Tougan,T., Aoshi, T., Coban,C., Katakai,Y., Kai,C., <u>Yasutomi,Y.</u>, Ishii, KJ. and Horii,T.	TLR9 adjuvants enhance immunogenicity and protective efficacy of the SE36/AHG malaria vaccine in nonhuman primate models.	Hum.Vac.Immunother			印刷中
Karamatsu,K., Matsuo,K., Inada,H., Tsujimura,Y., Shiogama,Y., Matsubara,A., Kawano, M. and <u>Yasutomi, Y.</u>	Single systemic administration of Ag85B of mycobacteria DNA inhibits allergic airway inflammation in a mouse model of asthma.	J Asthma Allergy	5	71-79	2012
Nomaguchi,M., Yokoyama,M., Kono, K., Nakayama,E. E., Shioda T., Saito,A., Akari,H., <u>Yasutomi,Y.</u>, Matano,T., Sato,H. and Adachi,A.	Gag-CA Q110D mutation elicits TRIM5-independent enhancement of HIV-1mt replication in macaque cells.	Microve. Infect			印刷中

Yoshida,T., Omatsu,T., Saito,A., Katakai,Y., Iwasaki, Y., Iijima,S., Kurosawa,T., Hamano, M., Nakamura,S., Takasaki,T., Yasutomi,Y., Kurane., I Akari,H.	CD16 positive natural killer cells play a limited role against primary dengue virus infection in tamarins	Archives Virol	15	363-368	2012
Tajiri,K., Imanaka-Yoshida,K., Matsubara,A., Tsujimura,Y., Hiroe,M., Naka,T.,Shimojo, N., Sakai,S., Aonuma,K. and Yasutomi,Y.	Suppressor of cytokine signaling 1 (SOCS1) DNA administration inhibits inflammatory and pathogenic responses in autoimmune myocarditis.	J.Immunol	189	2043-2053	2012
Uchdida,A., Sasaguri,H., Kimura,N., Tajiri,M., Ohkubo,T., Ono,F., Sakaue,F., Kanai,K., Hirai,T., Sano,T., Shibuya,K., Kobayashi,M., Yamamoto,M., Yokota,S.., Kuboddera,T., Tomori,M., Sakaki, K., Enomoto,M., Hirai,Y., Kumagai,J., Yasutomi,Y., Mochizuki,H., Kuwabara,S., Uchihara,T., Mizusawa, H. and Yokakota, T.	Non-human primate model of ALS with cytoplasmic mislocalization of TDP-43.	Brain	135	833-846	2012

Saito,A., Kono,K., Nomaguchi,M., Y asutomi,Y., Adach i,A., Shioda,T., Ak ari,H. and Nakay ama,E. E.	Geographical genetic an d functional diversity of antiretroviral host fact or TRIMCyp in cynomol gus macaque (Macaca f ascicularis).	J.Gen.Virol.	93	594-602	2012
Higashino,A., Sak ate,R., Kameoka, Y., Takahashi,I., Hirata,M., Tanum a,R., Masui,T., Ya sutomi,Y. and Os ada,N.	Whole-genome sequenci ng and analysis of the Malaysian cynomolgus macaque (Macaca fascic ularis) genome.	Genome Biol.	Epub		2012
Tachibana,S., Sull ivan,SA., Kawai, S., Nakamura,S., Goto,N., Arisue, N., Palacpac,NM Q., Honma,H., Ya gi,M., Tougan,T., Katakai,Y., Kanek o,O., Mita,T., Kit a,K., Yasutomi,Y., Kim,HR., Sutton, PL., Shakhbatya n,R., Horii,T., Yas unaga,T., Bamwel l,JW., Escalante,A A., Carlton,JM. A nd Tanabe,K.	Plasmodium cynomolgi genome sequences provi de insight into Plasmod ium vivax and the mon key malaria clade.	Nature Geneti cs	44	1051-1055	2012
Daron M. Standley et al.	Systems biology approaches to toll-like receptor signaling	WIREs SystBi ol Med	4	497 - 507	2012
Kuroda E, Coban C, Ishii KJ.	Particulate adjuvant an d innate immunity: past achievements, present findings and future pros pects.	International Reviews of Im munology	13; 32(2)	209-220.	2013

Hobro AJ, Konishi A, Coban C, Smith NI	Raman spectroscopic analysis of malaria disease progression via blood and plasma samples.	Analyst	March 26	Ahead of print PMID: 23529513	2013
Tang CK, Aoshi T, Jounai N, Ito J, Ohata K, Kobiyama K, Dessailly BH, Kuroda E, Akira S, Mizuguchi K, Coban C, Ishii KJ.	The chemotherapeutic agent DMXAA as a unique IRF3-dependent type-2 vaccine adjuvant.	PLOS ONE	8(3): e60038.	doi:10.1371/journal.pone.0060038.	2013
Tougan T, Aoshi T, Coban C, Katakai Y, Kai C, Yasutomi Y, Ishii KJ, Horii T.	TLR9 adjuvants enhance immunogenicity and protective efficacy of the SE36/AHG malaria vaccine in nonhuman primate models.	Human Vaccines and Immunotherapeutics	Jan 4; 9(2)	Ahead of print PMID: 23291928	2013
Kim W, Ozdemir SK, Zhu J, Faraz M, Coban C, Yang L.	Detection and size measurement of individual hemozoin nanocrystals in aquatic environment using a whispering gallery mode resonator.	Optics Express	Dec 31; 20(28)	29426-29446.	2012
Hong Z, Konishi A, Fujita Y, Yagi M, Ohata K, Aoshi T, Itagaki S, Sato S, Narita H, Abdelgelil NH, Inoue M, Culleton R, Kaneko O, Nakagawa A, Horii T, Akira S, Ishii KJ, Coban C.	Lipocalin 2 bolsters innate and adaptive immune responses to blood-stage malaria infection by reinforcing host iron metabolism.	Cell Host Microbe	12(5)	705-716	2012

Review

Adjuvants in influenza vaccines

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ARTICLE INFO	ABSTRACT
	The effectiveness of influenza vaccines is still controversial, and the role of adjuvants in such vaccines is briefly reviewed in this paper. Inactivated whole virus vaccines may include components that function as adjuvants, meaning that additive adjuvants are often not required. MF59 and AS03 showed higher adjuvanticity than aluminum salts in several clinical studies. Recent research has suggested that immune cell recruitment is the main mechanism underlying adjuvant actions in general, and that aluminum salts induce this recruitment via inflammation at the injected site. The aspect of how oil-based adjuvants, such as MF59 and AS03, recruit immune cells remains to be clarified. © 2012 Elsevier Ltd. All rights reserved.

Contents

1. Introduction

Influenza vaccines have been proven to induce high immunity in various trials. However, the coverage of seasonal influenza vaccine remains around half in Europe, America, and Asia [1], that may partially because its social usefulness is not yet fully shared in the population.

Vaccine effectiveness consists of vaccine immunogenicity, safety, and cost, and these aspects should be reviewed for assessment of influenza vaccines. In particular, vaccine adjuvants, vaccine administration routes, and/or immunization schedules may be the keys to improve vaccine efficacy and safety.

An adjuvant is used to enhance vaccine immunogenicity per se. The adjuvant effect, or adjuvanticity, would be measured by the ratio of immunogenicity (increase in geometric mean of antibody titer, percent responders, or seroconversion rate) of vaccine-with-adjuvant to vaccine-without-adjuvant in either non-clinical or clinical conditions. Recent clinical studies have suggested that AS03 or MF59 shows good adjuvanticity in influenza vaccines, but

these adjuvants also increase local and systemic adverse reactions, although they are not severe.

Recently developed alternative vaccination routes such as nasal, skin patch or oral route vaccines often show better efficiency than classical administration. Several nasal vaccines (influenza [3], measles [4]), microneedle skin patch vaccines [5,6], oral vaccines (rotavirus vaccine [7]) are well studied.

Boosting immunization is promising for improving protection. Even when the priming is not sufficiently immunogenic, sequential immunization has been shown to provide enough protection.

In this review, adjuvants for influenza vaccines are briefly overviewed and the current knowledge of their functions based on molecular biology is reviewed.

2. Clinical experiences of influenza vaccines: effects of adjuvants

The World Health Organization's list of influenza vaccine developments [8] includes several studies analyzing the immunogenicity and safety profiles of adjuvanted vaccines versus non-adjuvanted vaccines (Table 1). Aluminum salts, the most world-wide and historically used adjuvants, were mostly used in the listed studies, followed by MF59® from Novartis and AS03 from GlaxoSmithKline.

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Table 1

Profiles of reviewed clinical studies that compared vaccines with and without adjuvants (numbers indicate references).

Vaccine type		Adjuvant			
		Aluminum	AS03	MF59	Others[a]
Pandemic	Whole virion	9, 21, 22	Nil	Nil	Nil
	Subunit/split	12–15	10, 11, 22	16–19	20, 24
	Recombinant	23	Nil	Nil	Nil
Seasonal	Subunit	Nil	Nil	25–27	Nil

[a] One study used Matrix M™ [20] and the other used Inulin [24].

Immunogenicity was reviewed by the increase in geometric mean of the antibody titer (GMT), vaccinee ratio of seropositivity, and ratio of seroconversion. The antibody titer was measured by either hemagglutinin inhibition assays or microneutralizing assays. The safety profile was reviewed as the frequency of vaccine-related adverse reactions, comprising local reactions of pain, induration, erythema, etc., and systemic reactions of fever, malaise, headache, etc. Since the trial designs differed, especially in doses, schedules, subject backgrounds, and details of the definitions of immunogenicity, inter-trial comparisons were not reasonable, but the authors gained the impression that adjuvanted vaccines caused more frequent adverse reactions, regardless of the adjuvant used. The severity of the adverse events was slight or moderate, and no serious adverse events were reported, indicating that these influenza vaccines adjuvanted with aluminum salts, MF59 or AS03 are tolerable.

Seven studies on aluminum adjuvanted vaccines included various types of whole virion vaccines [9,21,22], subunit/split vaccines [12–15] and recombinant vaccines [23]. They satisfied the European Medical Agency's criteria for assessment of influenza vaccine [28,29], no matter which type of vaccine were used. For example in the two doses whole-virus H5N1 vaccine study, GMT increase on 21days after the second administration was between 2.7 and 5.2 when Aluminum adjuvant was added, and was between 3.2 and 5.9 without adjuvant [9].

On the other hand, compared with studies on vaccine with other adjuvants (AS03 [10,11,22], MF59 [16–19,25–27] and others [20,24]) the trends for the adjuvant effects on the vaccine immunogenicity differed among the adjuvants, in that aluminum showed lower adjuvanticity than MF59, AS03, or other adjuvants, irrespective of the dose of aluminum (300–1000 μg/dose) or the form of aluminum (hydroxide or phosphate). One study with two doses split vaccine (7.5 μg HA per dose) adjuvanted with MF59 showed 406.9 of GMT on 21days after the second administration, while non-adjuvanted vaccine showed 156.6 [19]. Higher adjuvanticity of MF59 than aluminum salts has also been shown in a trial on hepatitis B virus vaccines [30], etc.

The protective efficacy of influenza vaccines is mostly assessed by the clinical occurrence of confirmed influenza or influenza-like illness. Direct comparisons between MF59 adjuvanted and non-adjuvanted trivalent subunit influenza vaccines showed that adjuvanted vaccines exhibited higher effectiveness in both young children in Canada [27] and elderly people in Italy [31]. In the former study where influenza illness was confirmed by means of real-time polymerase-chain-reaction in nasopharyngeal aspirates or swabs, the effectiveness of the adjuvanted vaccine was shown by decreased influenza occurrence by 75%; 13 cases among 1937 adjuvanted vaccine group presented influenza illness whereas 50 cases of 1772 non-adjuvanted vaccine group showed influenza illness [27]. In the latter study in elderly people, the protective efficacy of the adjuvanted vaccine appeared to be less, since the odds ratio for developing influenza-like illness with the non-adjuvanted vaccine (versus adjuvanted vaccine) was 1.52, while the odds ratio for non-vaccinated people (versus vaccinated) was 2.16 [31].

From these experiences, it can be said that adjuvants in subunit influenza vaccines enhance the immunogenicity except for aluminum salts, but their adjuvanticity may need more improvement to prevent clinical influenza illness sufficiently.

3. Whole virion vaccines: vaccines with "unintended adjuvant"?

While subunit/split vaccines contain virus surface proteins as the vaccine antigens, whole virion vaccines are made of whole influenza virus particles that have been inactivated, typically by formaldehyde treatment. Therefore, these vaccines are composed of not only surface proteins, such as neuraminidase and hemagglutinin (for type A and type B, as the most commonly used vaccine antigens) or hemagglutinin esterase (for type C), but also matrix proteins and genomic RNA.

A review of three whole virion vaccines suggested that they were effective even though they were without aluminum adjuvants, and one of them was more effective than the aluminum-adjuvanted whole virion vaccine [9]. Superior immunogenicity of a whole virion influenza vaccine has been demonstrated in several Toll-like receptor (TLR) 7-knockout mouse experiments, which suggested it was dependent on TLR7 signaling [32,33]. Sialo-suger chains of host bind to influenza viruses but TLR7 specifically recognizes RNA of pathogens. These studies suggest that remaining RNA of influenza virus in the whole virion vaccine might unintentionally function as an adjuvant through TLR7 signaling. It is an interesting concept that a whole virion vaccine product might contain a "built-in adjuvant" when we call aluminum salts, MF59, or AS03 are artificially added as adjuvants. However, its generalization to other single-stranded RNA virus vaccines is controversial, since TLR7 and TLR8 polymorphisms did not affect the measles vaccine antibody response [34] and a transcriptional analysis of human blood cells found similar results for a vaccine against yellow fever and poly ICLC, the specific ligand of TLR3 [35].

4. Mechanisms of influenza vaccine adjuvants

The differences in the mechanisms of aluminum and other adjuvants are not yet fully understood, but they are commonly known to induce mild inflammation with immune cell recruitment at the injection site and not to induce Th1 cellular immunity.

Aluminum salts are generally thought to catch antigens and keep them at the local injection site for periods of days to weeks, such that the antigen is slowly presented and processed by the immune system. This "depot effect" was shown historically in diphtheria toxin experiments, in which immunity was impaired when the injection site was removed, while animals with transplantation of the injection site showed transferred immunity in parallel [2]. In addition, inflammation and cell damage caused by aluminum salts were recently shown to be a critical step in their Th2-biased adjuvanticity.

MF59 is still known to be effective when it is administered in advance of a vaccine antigen. However, when MF59 is administered

at 24 h after an antigen, it is not sufficiently immunogenic. These observations show MF59 does not act via a "depot effect", but instead is supposed to condition the immune system to respond effectively. At 2 days after injection, MF59 is found in lymph node mature macrophages and the gene profile of the "adjuvant core response genes" found in microarray analyses of the injected muscle of mice suggests that the mechanism of action of MF59 involves strong recruitment of antigen-presenting cells to the injection site as early as 12 h after injection [36].

A recent comparison study between aluminum salts and MF59 in mice [37] has suggested that the degree of cell recruitment may represent the current description of adjuvanticity. Specifically, in the first 24 h, MF59 recruited significantly more neutrophils, monocytes, eosinophils, macrophages, and dendritic cells than aluminum salts.

MF59 is composed of 0.5% Tween-80 as a water-soluble surfactant, 0.5% Span85 as an oil-soluble surfactant, 4.3% squalene oil, and water. It is an oil-in-water preparation and its emulsion droplet size is approximately 130 nm. Experience with nanoparticle adjuvants suggests that the particle size may be a key factor for adjuvanticity, since microspheres with diameters of <10 nm activate antigen-presenting cells, while those with diameters of 30–100 nm act via a "depot effect". A study comparing the sizes of silica particles showed that 30-nm-diameter particles induced the most inflammation and toxicity compared with 70-nm- or 300-nm-diameter particles [38]. If this situation is universal, the cell recruitment by MF59 may not depend on its size, but on its components. A recent study [39] compared several kinds of oil for particle size, emulsion stability, and adjuvanticity in a malaria vaccine candidate and an influenza vaccine, and found that the physical/chemical characters were similar among squalene, sesame oil, grape seed oil, and soybean oil, and that squalene oil showed the highest adjuvanticity in both vaccines.

5. Concluding remarks

Adjuvanticity of MF59 and AS03 has been shown in various studies, but their mechanisms of action still remain unclear. Regardless of how MF59 and AS03 act as vaccine adjuvants, there appears to be more to do to achieve social agreement on the importance of influenza vaccines. Vaccines that are "safer and more immunogenic" and "for the high-risk population" are the goals for vaccine development.

Conflicts of interest

None declared.

References

[1] OECD. Health at a glance 2011: OECD indicators; 2011. p. 125.
[2] Harrison WT. Some observations on the use of alum precipitated diphtheria toxoid. Am J Public Health Nations Health 1935;25:298–300.
[3] Carter NJ, Curran MP. Live attenuated influenza vaccine (FluMist®; Fluenz™): a review of its use in the prevention of seasonal influenza in children and adults. Drugs 2011;71:1591–622.
[4] Simon JK, Ramirez K, Cuberos L, Campbell JD, Viret JF, Muñoz A, et al. Mucosal IgA responses in healthy adult volunteers following intranasal spray delivery of a live attenuated measles vaccine. Clin Vaccine Immunol 2011;18:355–61.
[5] Laurent PE, Bourhy H, Fantino M. Safety and efficacy of novel dermal and epidermal microneedle delivery systems for rabies vaccination in healthy adults. Vaccine 2010;28:5850–6.
[6] Van Damme P, Oosterhuis-Kafeja F, Van der Wielen M, Almagor Y, Sharon O, Levin Y. Safety and efficacy of a novel microneedle device for dose sparing intradermal influenza vaccination in healthy adults. Vaccine 2009;27:454–9.
[7] Giaquinto C, Dominiak-Felden G, Van Damme P, Myint TT, Maldonado YA, Spoulou V, et al. Summary of effectiveness and impact of rotavirus vaccination with the oral pentavalent rotavirus vaccine: a systematic review of the experience in industrialized countries. Hum Vaccine 2011;7:734–48.
[8] World Health Organization. Clinical trials of influenza vaccine. http://www.who.int/vaccine_research/diseases/influenza/flu_trials_tables/en/
[9] Ehrlich HJ, Mueller M, Oh HM, Tambyah PA, Joukhadar C, Montomoli E, et al. A clinical trial of a whole-virus H5N1 vaccine derived from cell culture. N Engl J Med 2008;358:2573–84.
[10] Leroux-Roels I, Bernhard R, Gérard P, Dramé M, Hanon E, Leroux-Roels G. Broad clade 2 cross-reactive immunity induced by an adjuvanted clade 1 rH5N1 pandemic influenza vaccine. PLoS ONE 2008;3:e1665.
[11] Leroux-Roels I, Roman F, Forgus S, Maes C, De Boever F, Drame M, et al. Priming with AS03-adjuvanted H5N1 influenza vaccine improves the kinetics, magnitude and durability of the immune response after a heterologous booster vaccination: an open non-randomized extension of a double-blind randomized primary study. Vaccine 2010;28:849–57.
[12] Bresson JL, Perronne C, Launay O, Gerdil C, Saville M, Wood J, et al. Safety and immunogenicity of an inactivated split-virion influenza/Vietnam/1194/2004(H5N1) vaccine: phase I randomized trial. Lancet 2006;367:1657–64.
[13] Leroux-Roels I, Van der Wielen M, Kafeja F, Vandermeulen C, Lazarus R, Snape MD, et al. Humoral and cellular immune responses to split-virion H5N1 influenza vaccine in young and elderly adults. Vaccine 2009;27:6918–25.
[14] Brady RC, Treanor JJ, Atmar RL, Keitel WA, Edelman R, Chen WH, et al. Safety and immunogenicity of a subvirion inactivated influenza A/H5N1 vaccine with or without aluminum hydroxide among healthy elderly adults. Vaccine 2009;27:5091–5.
[15] Keitel WA, Campbell JD, Treanor JJ, Walter EB, Patel SM, He F, et al. Safety and immunogenicity of an inactivated influenza A/H5N1 vaccine given with or without aluminum hydroxide to healthy adults: results of a phase I–II randomized clinical trial. J Infect Dis 2008;198:1309–16.
[16] Stephenson I, Bugarini R, Nicholson KG, Podda A, Wood JM, Zambon MC, et al. Cross-reactivity to highly pathogenic avian influenza H5N1 viruses after vaccination with nonadjuvanted and MF59-adjuvanted influenza A/duck/Singapore97(H5N3) vaccine: a potential priming strategy. J Infect Dis 2005;191:1210–5.
[17] Galli G, Hancock K, Hoschler K, DeVos J, Praus M, Bardelli M, et al. Fast rise of broadly cross-reactive antibodies after boosting long-lived human memory B cells primed by an MF59 adjuvanted prepandemic vaccine. Proc Natl Acad Sci USA 2009;106:7962–7.
[18] Atmar RL, Keitel WA, Patel SM, Katz JM, She D, El Sahly H, et al. Safety and immunogenicity of nonadjuvanted and MF59-aduvanted influenza A/H9N2 vaccine preparations. Clin Infect Dis 2006;43:1135–42.
[19] Clark TW, Pareek M, Hoschler K, Dillon H, Nicholson KG, Groth N, et al. Trial of 2009 influenza A(H1N1) monovalent MF59-adjuvanted vaccine. N Engl J Med 2009;361:2424–35.
[20] Cox RJ, Pedersen G, Madhun AS, Svindland S, Sævik M, Breakwell L, et al. Evaluation of a virosomal H5N1 vaccine formulated with Matrix M adjuvant in a phase I clinical trial. Vaccine 2011;29:8049–59.
[21] Saenger R, Schussmann KM, Preusche A, Kuehl HO, Riemer N, Reiners B, et al. Immunogenicity and persistence of response to an alum-adjuvanted monovalente (H9N2) whole virus influenza vaccine in healthy adults aged 60 years and older. In: Presentation in International conference on influenza vaccines for the world-IVW2006. 2006.
[22] Hehme NW. GSK's pandemic vaccine development. In: Presentation in 3rd meeting on evaluation of pandemic influenza vaccines in clinical trials. 2007.
[23] Kanazashi S, Yagi Y, Komatsu F, Ninomiya Y. UMN-0501 Pandemic influenza recombinant HA vaccine phase I/II study in Japan. In: Presentation in WHO meeting. 2009.
[24] Cox M. Pandemic influenza vaccine clinical trial abstract minimum information.
[25] Vesikari T, Groth N, Karvonen A, Borkowski A, Pellegrini M. MF59-adjuvanted influenza vaccine (FLUAD) in children: safety and immunogenicity following a second year seasonal vaccination. Vaccine 2009;27:6291 5.
[26] Durando P, Fenoglio D, Boschini A, Ansaldi F, Icardi G, Sticchi L, et al. Safety and immunogenicity of two influenza virus subunit vaccines, with or without MF59 adjuvant, administered to human immunodeficiency virus type 1-seropositive and -seronegative adults. Clin Vaccine Immunol 2008;15:253–9.
[27] Vesikari T, Knuf M, Wutzler P, Karvonen A, Kieninger-Baum D, Schmitt HJ, et al. Oil-in-water emulsion adjuvant with influenza vaccine in young children. N Engl J Med 2011;365:1406–16.
[28] The European Agency for the Evaluation of Medical Products. Note for guidance on harmonization of requirements for influenza vaccines (CPMP/BWP/214/96); 1997.
[29] European Medicines Agency. Guideline on influenza vaccines prepared from viruses with the potential to cause a pandemic and intended for use outside of the core dossier context (CHMP/VWP/263499/2006); 2007.
[30] Heineman TC, Clements-Mann ML, Poland GA, Jacobson RM, Izu AE, Sakamoto D, et al. A randomized, controlled study in adults of the immunogenicity of a novel hepatitis B vaccine containing MF59 adjuvant. Vaccine 1999;17: 2769–78.
[31] Iob A, Brianti G, Zamparo E, Gallo T. Evidence of increased clinical protection of an MF59-adjuvant influenza vaccine compared to a non-adjuvant vaccine among elderly residents of long-term care facilities in Italy. Epidemiol Infect 2005;133:687–93.
[32] Geeraedts F, Goutagny N, Hornung V, Severa M, de Haan A, Pool J, et al. Superior immunogenicity of inactivated whole virus H5N1 influenza vaccine is primarily controlled by Toll-like receptor signaling. PLoS Pathog 2008;4:e1000138.
[33] Koyama S, Aoshi T, Tanimoto T, Kumagai Y, Kobiyama K, Tougan T, et al. Plasmacytoid dendritic cells delineate immunogenicity of influenza vaccine subtypes. Sci Transl Med 2010;2:25.