Adjuvants and their application: Update and the flagellin case

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Top public health achievement of modern medicine

Prophylactic vaccine era

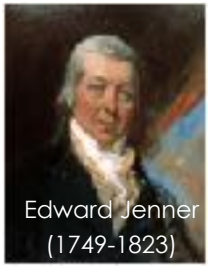
Therapeutic vaccine era

18th century
- Edward Jenner's Smallpox vaccine

19th century
- Cholera
- Rabies
- Tetanus
- Typhoid fever
- Bubonic plaque

20th century
- Diphtheria
- Pertussis
- Tuberculosis
- Yellow Fever
- Typhus
- Influenza
- Polio
- Japanese encephalitis
- Anthrax
- Adenovirus
- Measles
- Mumps
- Rubella
- Chicken pox
- Pneumonia
- meningitis
- Hepatitis A,B
- Haemophilus influenzae type b
- Lyme disease
- Rotavirus

21th century
- Human papillomavirus
- More cancer vaccines
- HIV and malaria, etc
- DNA vaccine
- Allergy
- Obesity, DM
- More and more (Zika, MERS, etc)


Edward Jenner (1749-1823)


Louis Pasteur (1822-1985)

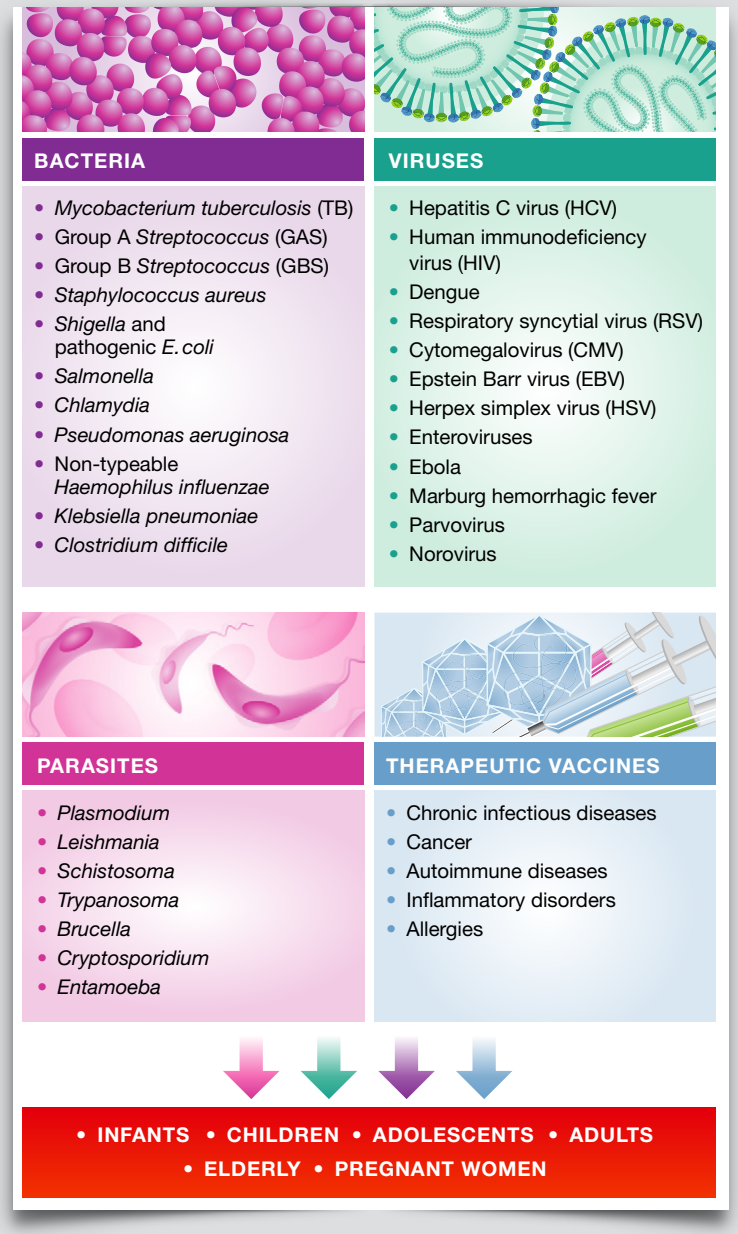

Robert Koch (1843-1910)


Gaston Ramon (1886-1963)


Maurice Ralph Hilleman (1919 – 2005)

Business insights Ltd

Target disease and population for 21st century vaccines



Delany I et al. EMBO Mol Med. doi:10.1002/emmm.201403876

The 21st century vaccinologists toolbox

Delany I et al. EMBO Mol Med. doi:10.1002/emmm.201403876

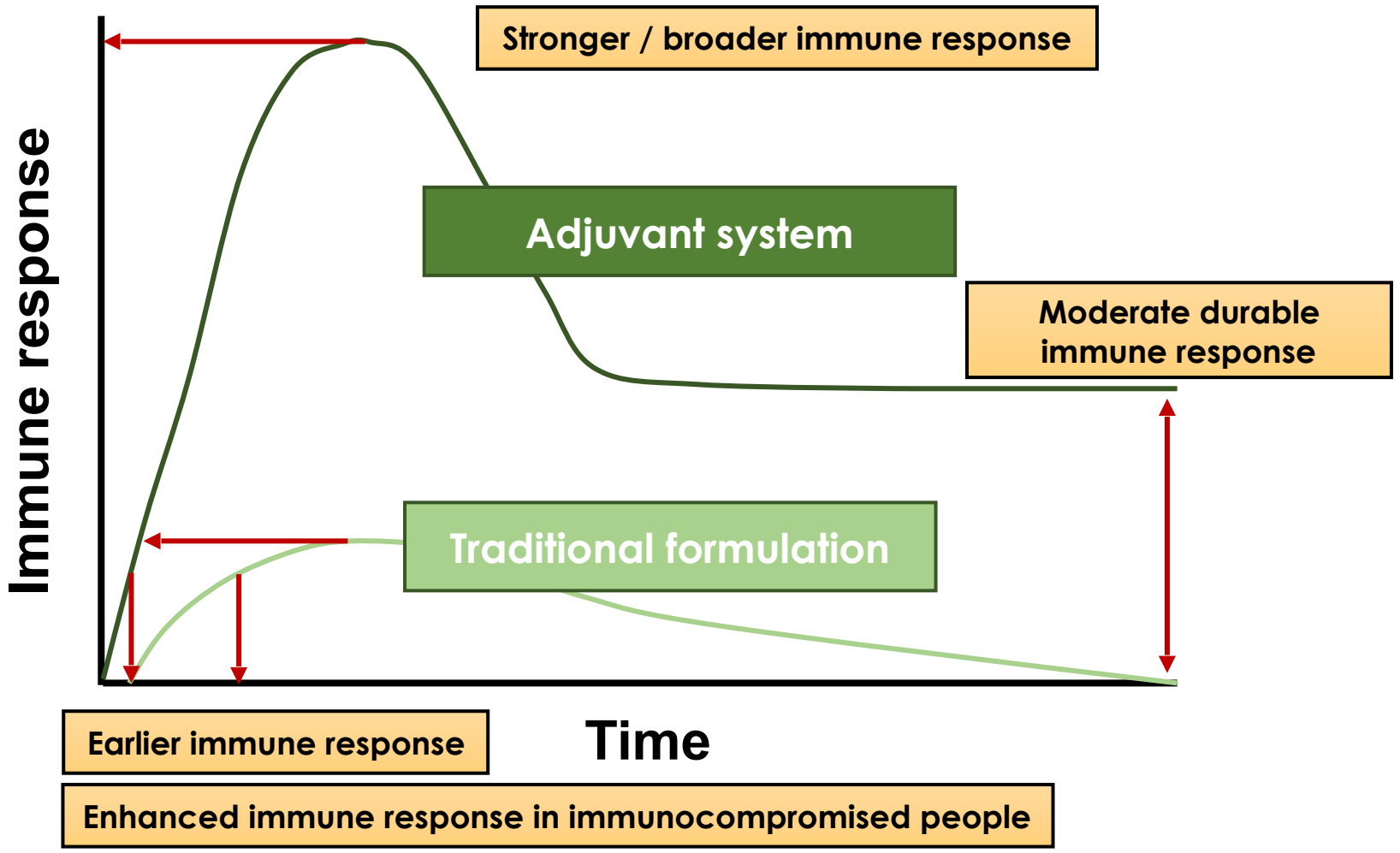
Adjuvant

* Adjuvare (in Latin) = help

* Ramon G (1926)

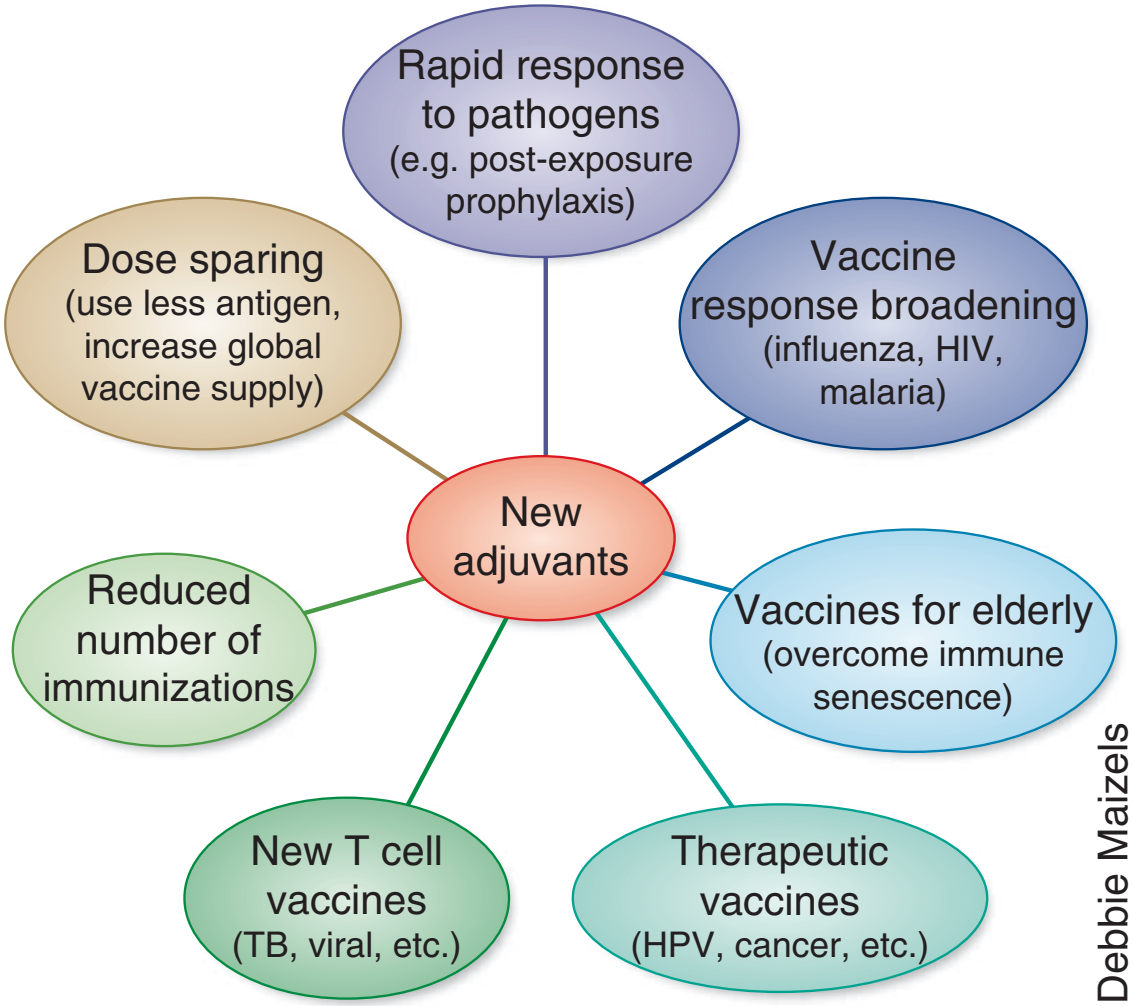
"substance used in combination with a specific antigen that produces a more robust immune response than the antigen alone"

Adjuvant paradigm



Tom-Heineman, GSK

Potential benefits of adjuvants



Why adjuvant?

- Most adjuvants in currently licensed vaccines were developed without a clear understanding of how they work.
- In contrast, modern vaccines are being developed based on rationally designed recombinant, highly purified antigens through structured based design, epitope focusing or genomic based screening
  - Inherent immunogenicity of these Ags are low in comparison to traditional vaccines
  - Increasing need for potent and safe vaccine adjuvants
  - Necessary to better understand how adjuvants work

Built in adjuvants - LPS, PG, DNA, RNA, CpG, etc

Target population for vaccines in the twenty-first century

a Age groups

Pre-birth
- Cytomegalovirus
- Group B streptococcus
- Hepatitis B virus
- Influenza virus
- Meningococcus serogroups A, B, C, Y and W135
- Pertussis
- Respiratory syncytial virus
- Tetanus

Infants and children
- Diphtheria
- Group A streptococcus
- *H. influenzae* type b
- *Helicobacter pylori*
- Hepatitis A virus
- Hepatitis B virus
- Inactivated poliovirus vaccine
- Influenza virus
- Measles
- Meningococcus serogroups A, B, C, Y and W135
- Mumps
- Pertussis
- Pneumococcus
- Respiratory syncytial virus
- Rotavirus
- Rubella
- Tetanus
- Varicella zoster virus

Adolescents
- Cytomegalovirus
- Diphtheria, tetanus acellular pertussis
- Epstein–Barr virus
- Herpes simplex virus
- Human papilloma virus
- Influenza virus
- Meningococcus serogroups A, B, C, Y and W135
- Parvovirus B19

Adults
- Diphtheria
- Hepatitis B virus
- Influenza virus
- Meningococcus serogroups A, B, C, Y and W135
- Pertussis
- Respiratory syncytial virus
- Tetanus

Elderly

Recurrent infections:
- Group B streptococcus
- Influenza virus
- Meningococcus serogroups A, B, C, Y and W135
- Pneumococcus
- Respiratory syncytial virus
- Varicella zoster virus

Antibiotic resistance:
- *Acinetobacter baumannii*
- *C. difficile*
- *Candida* spp.
- Enterotoxigenic *E. coli*
- *Klebsiella pneumoniae*
- *P. aeruginosa*
- *S. aureus*

Cancer:
- Breast cancer
- Colorectal cancer
- Prostate cancer

b Special target groups

Travellers
- Cholera
- Dengue
- Enterotoxigenic *E. coli*
- Hepatitis A virus
- Hepatitis B virus
- Influenza virus
- Japanese encephalitis virus
- Malaria
- Meningococcus serogroups A, B, C, Y, W135 and X
- Paratyphoid fever
- Rabies
- *Shigella* spp.
- Tick-borne encephalitis virus
- Tuberculosis
- Typhoid fever
- Yellow fever

Patients with chronic diseases
- Cytomegalovirus
- Fungal infections
- Influenza virus
- *P. aeruginosa*
- Parainfluenza
- Parvovirus B19
- Respiratory syncytial virus
- *S. aureus*
- Tuberculosis

Patients with HIV
- Influenza virus
- Pneumococcus
- Pneumocystosis
- Tuberculosis

Emerging infections
- AIDS
- Anthrax
- Avian influenza
- Cholera
- Dengue
- Diphtheria
- Ebola virus disease
- EV71
- Malaria
- Meningococcus serogroup X
- Plague
- SARS
- Smallpox
- Swine influenza
- Tuberculosis
- West Nile

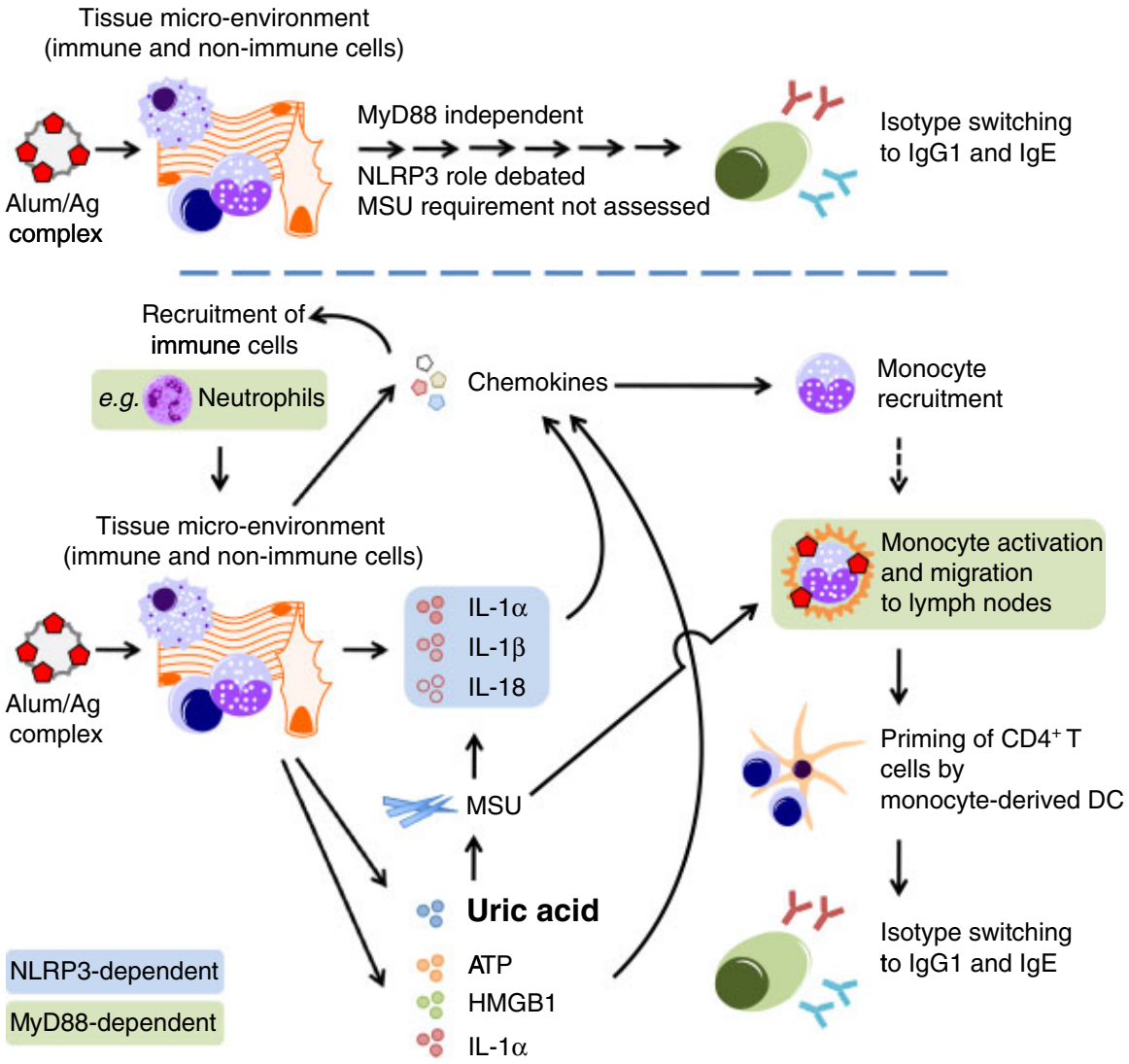
Poverty
- Cholera
- Dengue
- Enterotoxigenic *E. coli*
- Hepatitis A virus
- Hepatitis B virus
- Hepatitis E virus
- Influenza virus
- Japanese encephalitis virus
- Malaria
- Meningococcus serogroups A, B, C, Y, W135 and X
- Parasitic infections
- Paratyphoid fever
- Rabies
- Rotavirus
- *Salmonella* spp.
- *Shigella* spp.
- Tuberculosis
- Typhoid fever
- Yellow fever

Vaccine adjuvants

Adjuvant name	Class	Mechanism or receptor	Type of immune response	Clinical phase or licensed product name
dsRNA analogues (for example, poly(I:C))	IM	TLR3	Ab, T_H1, $CD8^+$ T cells	Phase 1
Lipid A analogues (for example, MPL, RC529, GLA, E6020)	IM	TLR4	Ab, T_H1	Cervarix, Supervax, Pollinex Quattro, Melacine
Flagellin	IM	TLR5	Ab, T_H1,T_H2	Phase 1
Imidazoquinolines (for example, Imiquimod, R848)	IM	TLR7 and TLR8	Ab, T_H1	Aldara
CpG ODN	IM	TLR9	Ab, T_H1, $CD8^+$ T cells	Phase 3
Saponins (for example, QS21)	IM	Unknown	Ab, T_H1,T_H2, $CD8^+$ T cells	Phase 3
C-type lectin ligands (for example, TDB)	IM	Mincle, Nalp3	Ab, T_H1, T_H17	Phase 1
CD1d ligands (for example, α- galactosylceramide)	IM	CD1d	Ab, T_H1, T_H2, $CD8^+$ NKT cells	Phase 1
Aluminum salts (for example, aluminum oxyhydroxide, aluminum phosphate)	PF	Nalp3, ITAM, Ag delivery	Ab, T_H2	Numerous licensed products
Emulsions (for example, MF59, AS03, AF03, SE)	PF	Immune cell recruitment, ASC, Ag uptake	Ab, T_H1, T_H2	Fluad, Pandemrix
Virosomes	PF	Ag delivery	Ab, T_H1,T_H2	Epaxal, Inflexal V
AS01 (MPL,QS21, liposomes)	C	TLR4	Ab, T_H1, $CD8^+$ T cells	Phase 3
AS02 (MPL,QS21, emulsion)	C	TLR4	Ab, T_H1	Phase 3
AS04 (MPL, aluminum salt)	C	TLR4	Ab, T_H1	Cervarix
AS15 (MPL, QS21, CpG, liposomes)	C	TLR4 and TLR9	Ab, T_H1, $CD8^+$ T cells	Phase 3
GLA-SE (GLA, emulsion)	C	TLR4	Ab, T_H1	Phase 1
IC31 (CpG, cationic peptide)	C	TLR9	Ab, T_H1, T_H2, $CD8^+$ T cells	Phase 1
CAF01 (TDB, cationic liposomes)	C	Mincle, Ag delivery	Ab, T_H1, $CD8^+$ T cells	Phase 1
ISCOMs (saponin, phospholipid)	C	Unknown	Ab, T_H1,T_H2, $CD8^+$ T cells	Phase 2

Ab, antibody; Ag, antigen; ASC, apoptosis-associated speck-like protein containing caspase recruitment domain; C, combination of immunomodulatory molecule and particulate formulation; dsRNA, double-stranded RNA; IM, immunomodulatory molecule; ITAM, immunoreceptor tyrosine-based activation motif; PF, particulate formulation; TDB, trehalose dibehenate. Some particulate formulations (such as aluminum salts and emulsions) also generate immunomodulatory activity.

Action Mechanism of Alum

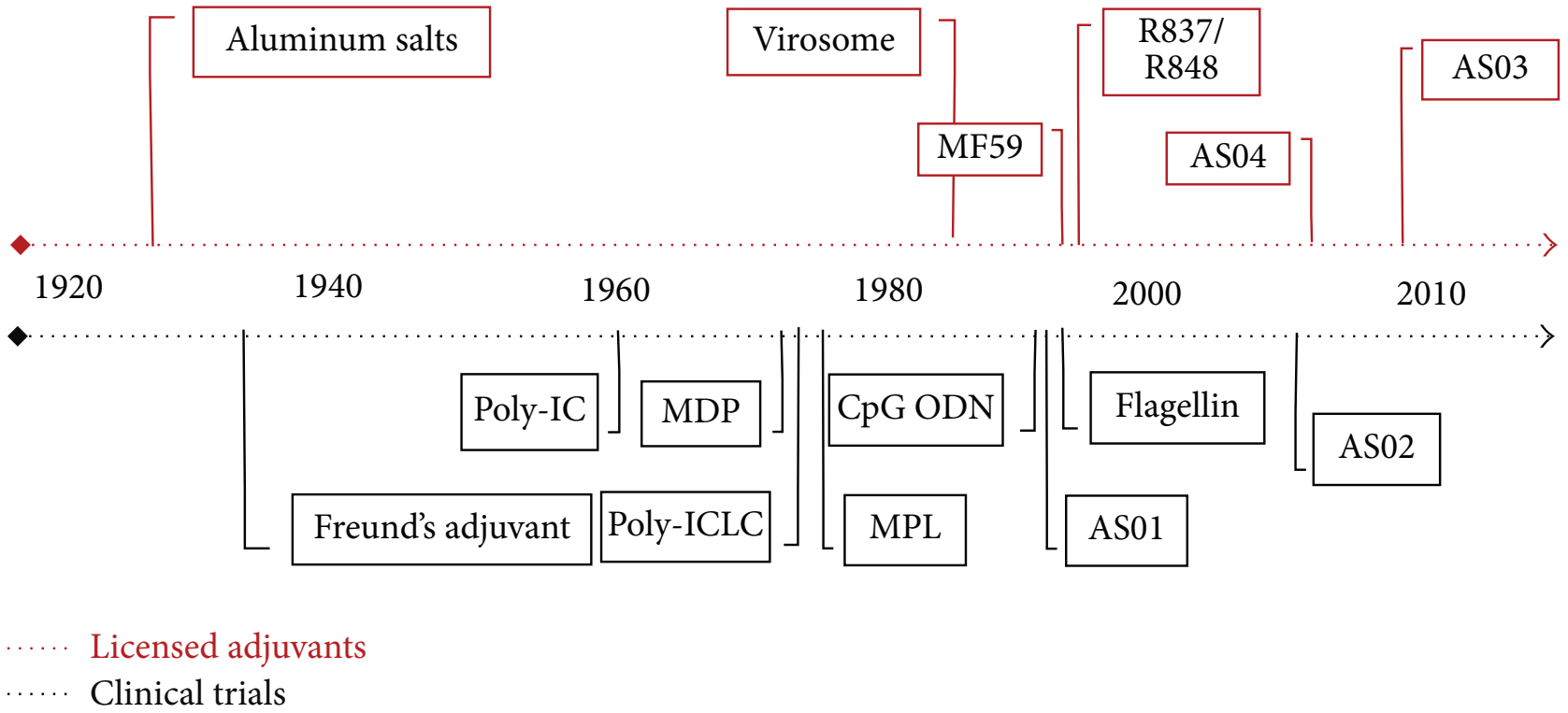


Eur. J. Immunol. 2010. 40: 595–653

Considerations for ideal adjuvants

Category	Subcategory	Considerations
Biological activity	Safety	Formulation must be safe and effective in all age groups; metabolizable components preferred; adjuvant activity should be localized and transient; adjuvant should not have direct effect on lymphocytes: no nonspecific B or T cell responses
	Immunization route	Each immunization route may have different formulation requirements
	Antigen dose sparing	Adjuvant should enable reduction in required antigen dose or number of immunizations
	Response broadening	Adjuvant should broaden protective responses against heterologous pathogen strains
	Antibody responses	Neutralizing antibody responses should be enhanced or prolonged by adjuvant
	Cell-mediated immunity	Adjuvant should induce and/or prolong pathogen-specific $CD4^+$ and/or $CD8^+$ T cell responses
	Immune response quality	Adjuvant should enable shaping of immune response (for example T_H1 versus T_H2 balance)
	Improve responses in weak immune systems	Immune responses should be enhanced in very young, elderly or immunocompromised populations
Physicochemical aspects	Raw materials	Synthetic adjuvants are preferable for purity, sustainability and safety; plant-based adjuvants may be acceptable if synthetic ones are too costly or have low yield; animal sources should be avoided for sustainability and disease concerns; multiple sources should be available at low cost; metabolizable or excretable components preferred
	Manufacturability	Equipment and process should be scalable, transferable and able to produce consistent batches
	Particle morphology	<200 nm particles can be terminally filtered, avoiding requirement for aseptic manufacturing, and may enter lymph node more easily than large particles; orientation and shape of nonspherical particles affects cell uptake; charge and chemical structure of surface groups are crucial factors in resulting bioactivity; targeting molecules such as mannose may enhance delivery to APCs; some concern regarding potential toxicity of cationic particles
	Antigen compatibility, association	Effects of adjuvant formulation on antigen structure should be characterized; generally it is thought that some level of association of the antigen to the formulation is preferred, although direct association is not required for biological activity
	Stability	Excipients and active pharmaceutical ingredients (APIs) should maintain chemical structure and particle size, shape, polydispersity and visual appearance, and API localization should be constant for several years; packaging under inert gas guards against oxidative degradation

Timeline of vaccine adjuvants discovery



Journal of Immunology Research
Volume 2016, Article ID 1459394, 16 pages

Adjuvant policy

Meetings of Public Health Authorities on Adjuvants

- FDA VRBPAC on Vaccine Adjuvants and Mode of Action ASO3 adjuvanted influenza vaccine (H5N1), Nov. 2012
- WHO Consultation on Nonclinical Evaluation of Adjuvanted Vaccines, Sept. 2011
- FDA/NIH Public Workshop: Adjuvants and Adjuvanted Preventative and Therapeutic Vaccines for Infectious Disease Indications, Dec. 2008
  - assess the scientific knowledge base regarding vaccine adjuvants
  - facilitate the development of a research agenda to improve the safety and efficacy assessments of adjuvanted vaccines for the treatment and prevention of disease.

Norman W. Baylor, PhD
Biologics Consulting Group, Inc.

Sleeping with your enemy ……

Topics: Clinical Trials | Influenza

Sanofi bird flu vax prompts immune response in PhII--but only with Novartis adjuvant

October 9, 2014 | By Carly Helfand

As it works to refine its vaccine strategy for the event of an avian flu pandemic, the NIH is trialing Sanofi's ($SNY) H7N9 vaccine at a range of doses and with a variety of adjuvant combinations. And now, it has one that looks promising.

NIAID Director Dr. Anthony Fauci

In a Phase II trial, the vaccine prompted an immune response in 59% of the 700 participating healthy adults--but only when mixed with Novartis' ($NVS) MF59 adjuvant. Without MF59, even those patients who received a higher-dosage vaccine had minimal immune responses, the NIH's National Institute of Allergy and Infectious Diseases (NIAID) said Wednesday.

H7N9 first hit the public health radar in March of last year, when the virus first surfaced in humans in China. Since then, the NIH has launched two trials to assess Sanofi's vaccine in conjunction with both the Novartis adjuvant and A503, an adjuvant from GlaxoSmithKline ($GSK).

POPULAR STORIES

MOST READ | MOST SHARED

UPDATED: Takeda appoints new vaccine development head as Novartis' Oswald heads for the door

GSK may give Ebola vaccine to West African healthcare workers in early 2015

Sanofi bird flu vax prompts immune response in PhII--but only with Novartis adjuvant

Pfizer's meningitis B vaccine is effective when given with HPV jab

Accidental poliovirus dump adds to GSK's production woes

Comparison of long-term immunogenicity and safety of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine and HPV-6/11/16/18 vaccine in healthy women aged 18-45 years: End-of-study analysis of a Phase III randomized trial

Mark H Einstein[1,*], Peter Takacs[2], Archana Chatterjee[3], Rhoda S Sperling[4], Nahida Chakhtoura[3], Mark M Blatter[5], Jacob Lazcano[6], Maria-Elena David[7], Lan Lin[8], Frank Struyf[9], and Gary Dubin[10], on behalf of the HPV-010 Study Group

The observer-blind, randomized, age-stratified, head-to-head study (NCT00423046) comparing immunogenicity and safety of HPV-16/18 and HPV-6/11/16/18 vaccines in healthy women aged 18-45 y was completed. Five y after vaccination, in subjects from the Month 60 according-to-protocol cohort (seronegative and DNA negative for HPV type analyzed at baseline), serum neutralizing antibody (nAb) responses induced by HPV-16/18 vaccine remained 7.8-fold (18-26-y stratum), 5.6-fold (27-35-y stratum) and 2.3-fold (36-45-y stratum) higher than those induced by HPV-6/11/16/18 vaccine for HPV-16. For HPV-18, the fold differences were 12.1, 13.0 and 7.8, respectively. At Month 60, all (100%) subjects in HPV-16/18 vaccine group and the majority (95.7%-97.5%) in HPV-6/11/16/18 vaccine group were seropositive for HPV-16. For HPV-18, the majority (98.1%-100%) of subjects in HPV-16/18 vaccine group were seropositive; however, seropositivity rates in HPV-6/11/16/18 vaccine group decreased considerably (61.1%-76.9%) across the 3 age strata. In the total vaccinated cohort (received ≥ 1 dose regardless of baseline HPV serostatus and DNA status), geometric mean titers for anti-HPV-16 and anti-HPV-18 nAb were higher in HPV-16/18 vaccine group than in HPV-6/11/16/18 vaccine group. Based on the 5-y data, piece-wise and modified power-law models predicted a longer durability of nAb response for HPV-16/18 vaccine compared to HPV-6/11/16/18 vaccine. Beyond the differences apparent between the vaccines in terms of immunogenicity and modeled persistence of antibody responses, comparative studies including clinical endpoints would be needed to determine whether differences exist in duration of vaccine-induced protection.

- **Cervarix (AS04, MPL+alum) >> Gardasil (alum)**

Vaccine Adjuvants

Benefits of Vaccine Adjuvants

Adjuvants have several important benefits, including the following:

Reducing the Amount of Antigen Required

Adding an adjuvant may reduce the amount of antigen, or pathogen component, require
protective immune response. The ability to increase the number of vaccine doses that c
may be especially important during an epidemic or pandemic. For example, NIAID-supp
formulating an experimental H9N2 influenza vaccine with MF59 adjuvant greatly reduce
needed to elicit a strong protective response. Currently, MF59 is licensed for use as a v
not in the United States.

Reducing the Number of Vaccine Doses Needed

A person may need fewer doses of a vaccine containing a certain adjuvant because the
more effective. For example, results from clinical trials indicate that two doses of an inve
vaccine containing a novel adjuvant given over one month elicit potent, long-lasting prot
B vaccine, which contains alum, requires three doses over six months. In addition, for a
current vaccine does not confer immunity against the hepatitis B virus. Research sugge
vaccine is effective for almost everyone.

Enhancing Vaccine Effectiveness in Immunocompromised People

People with compromised immune systems, such as the elderly or the very young, may
adjuvants because their immune systems may require an extra boost to provide protect
study showed that addition of MF59 adjuvant to a seasonal influenza vaccine boosted t
young children from 43 percent to 89 percent.

Boosting the Immune-Stimulating Effects of Vaccines

Adjuvants are especially effective at boosting the immune-stimulating effects of newer v
with purified antigens. By enhancing immune responses to pathogen antigens, adjuvant
vaccines against infectious diseases for which no effective vaccine currently exists, suc

Offering Broad Protection

Adjuvanted vaccines may offer broad protection against related strains (types) of pathog
human papillomavirus (HPV) vaccine Cervarix, which contains the AS04 adjuvant, is de
HPV 16 and 18, the two strains that cause approximately 70 percent of cervical cancers. Results from clinical trials
show that Cervarix protects against two additional cancer-causing strains, HPV 45 and 31.

Directing Specific Immune Responses

Adjuvants can direct specific immune responses to provide protection against the pathogen that the vaccine targets. Bacteria, viruses, and parasites use different infection strategies, and therefore, each is thwarted by different components of the immune system. Certain adjuvants may be more effective at stimulating responses to a particular vaccine antigen. Vaccine developers must tailor each antigen-adjuvant combination to maximize the safety and

National Institute of Allergy and Infectious Diseases
Leading research to understand, treat, and prevent infectious, immunologic, and allergic diseases.

Vaccine Adjuvants

Related Links

Immune System

Vaccines

View a list of links for more information about vaccine adjuvants.

Funded Research Programs

NIAID supports an array of adjuvant research, from basic studies on immune receptors to clinical testing of new adjuvanted vaccine candidates. NIAID-funded programs involving adjuvant research include the following:

Innate Immune Receptors and Adjuvant Discovery Program

Established in 2003, this program was renewed in 2009 and again in 2014 to continue support for the identification and optimization of promising adjuvants. NIAID-funded researchers are screening thousands of compounds for adjuvant activity and have identified several promising leads.

Adjuvant Development Program

NIAID initiated this program in 2008 to advance novel vaccine adjuvants toward licensure for human use. The program, which was renewed in 2013, supports the optimization of adjuvant candidates, vaccine formulation studies, and preclinical adjuvant pharmacology, toxicity, and efficacy studies.

Human Immunology Project Consortium

NIAID established the Human Immunology Project Consortium (HIPC) in 2010 to create a public resource that characterizes the diverse states of the human immune system. HIPC investigators use modern analytic tools to profile the immune system before and after infection, vaccination, or treatment with an adjuvant. The information gained from HIPC promises to improve understanding of the human immune system and its regulation. It also will help scientists evaluate the safety and effectiveness of different vaccine formulations and administration techniques.

Vaccine Treatment and Evaluation Units

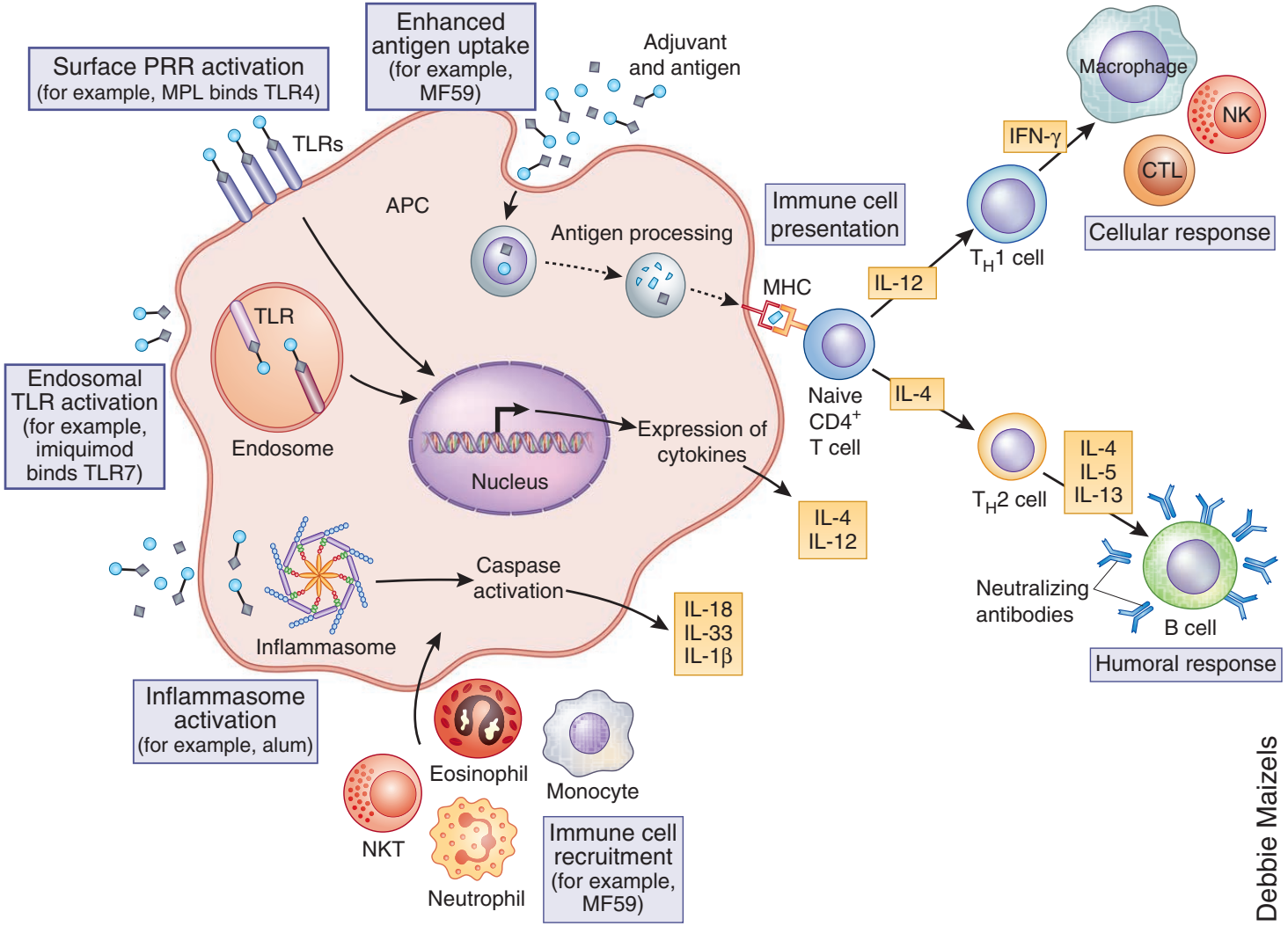
The NIAID-sponsored Vaccine and Treatment Evaluation Units (VTEUs), first established in 1962, have conducted hundreds of clinical trials, many of which have contributed to vaccine licensure. Researchers at the VTEUs, which are located at universities and health centers across the United States, test novel vaccines and vaccine delivery methods. This includes the evaluation and study of adjuvants.

Adjuvant may act in one or more of five ways

* Immune potentiation
* Presentation
* Induction of preferred (Th1 or Th2) immune response
* Targeting
* Depot generation

The best adjuvant will never correct the choice of the wrong epitope……

Vaccine adjuvant: putative mechanism of action



World Health Organization

FINAL
ENGLISH ONLY

Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines

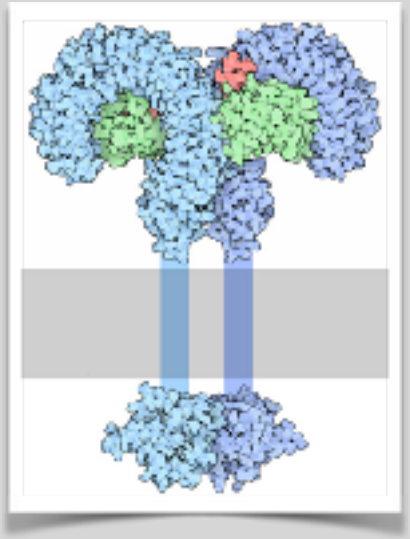
Adopted by the 64th meeting of the WHO Expert Committee on Biological Standardization, 21–25 October 2013. A definitive version of this document, which will differ from this version in editorial but not scientific details, will be published in the WHO Technical Report Series.

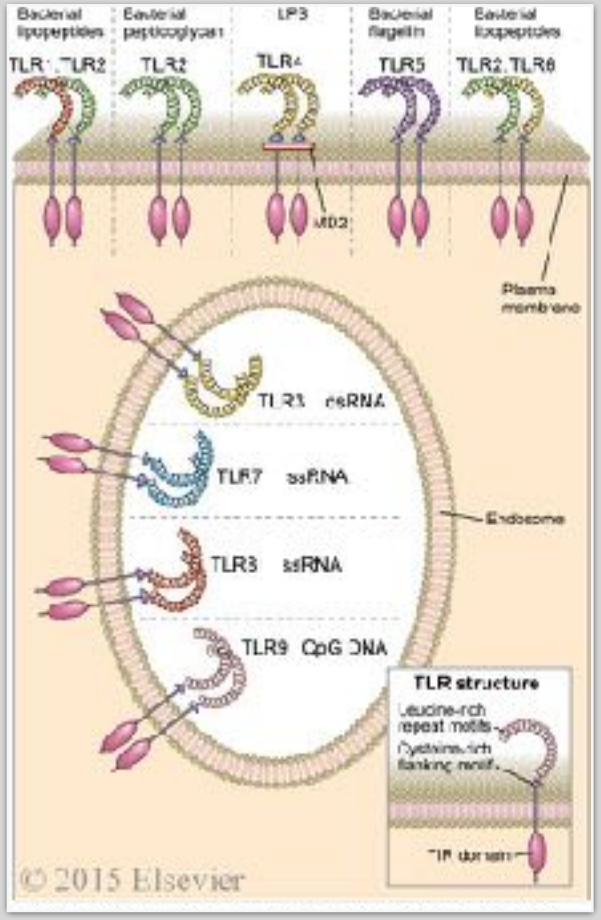
Classification of Adjuvants

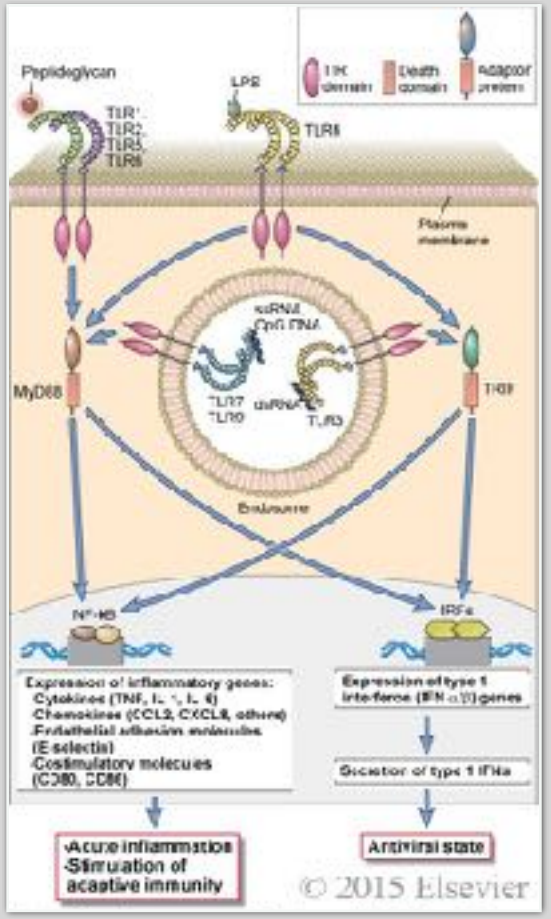
- ✔ **Mineral salts or gels** - aluminum salts or calcium phosphate
- ✔ **Oil-in-water and water-in-oil emulsions, amphiphilic molecules and surfactant-base formulations** - MF59, QS-21, AS03, and Montanide
- ✔ **Particulate adjuvants** – liposomes, virosomes, DC Chol, ISCOMS, Iscomatrix, biopolymers such as PLGA, etc
- ✔ **PAMPs (natural and synthetic)** – low-toxicity LPS or lipid A (MPL, MPLA, OM-174), CpG, flagellin, nontoxic bacterial toxins(mLT and CTB), Poly IC, Poly ICLC, imiquimod/resiquimod (R837/R848), etc
- ✔ **Endogenous human immunostimulators** – cytokines (hGM-CSF and hIL-12) administered as proteins or as plasmid preparations
- ✔ **Inert vehicles** – gold particles
- ✔ **Adjuvants derived from inulin** – delta inulin (Advax)
- ✔ **Combination adjuvants or adjuvant systems** - AS01, AS03, AS04, AS15, glucopyranosyl lipid adjuvant-stable emulsion (GLA-SE), CAF01, etc

WHO guideline, 2013

TLR signaling serves linker between innate and adaptive immunity







TLR ligands are considered as an attractive adjuvant candidate in vaccine development.

TLR Adjuvant Preference

TLR4 (LPS, MPL) > **TLR9** (CpG ODN) > **TLR3** (poly I:C) > **TLR5** (flagellin)

TLR2 (LTA, lipoproteins)

Flagellin:
TLR5-targeting Mucosal Adjuvant

Shee Eun Lee, Soo Young Kim, and Young Ran Kim

Flagellin

The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5

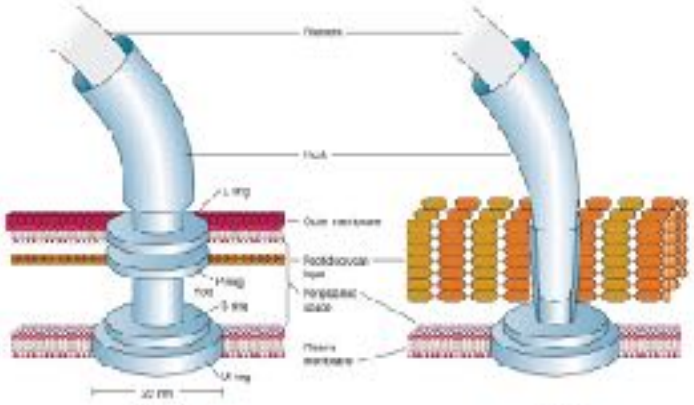
Fumitaka Hayashi*, Kelly D. Smith*‡, Adrian Ozinsky†, Thomas R. Hawn†§, Eugene C. Yi*, David R. Goodlett†, Jimmy K. Eng†, Shizuo Akira||, David M. Underhill† & Alan Aderem†

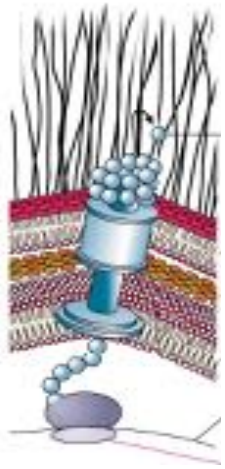
Nature. 2001 Apr 26;410(6832):1099-103.

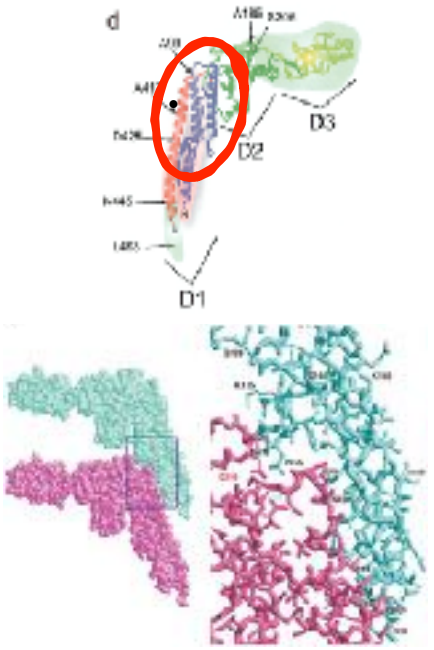
Structure of the bacterial flagellar protofilament and implications for a switch for supercoiling

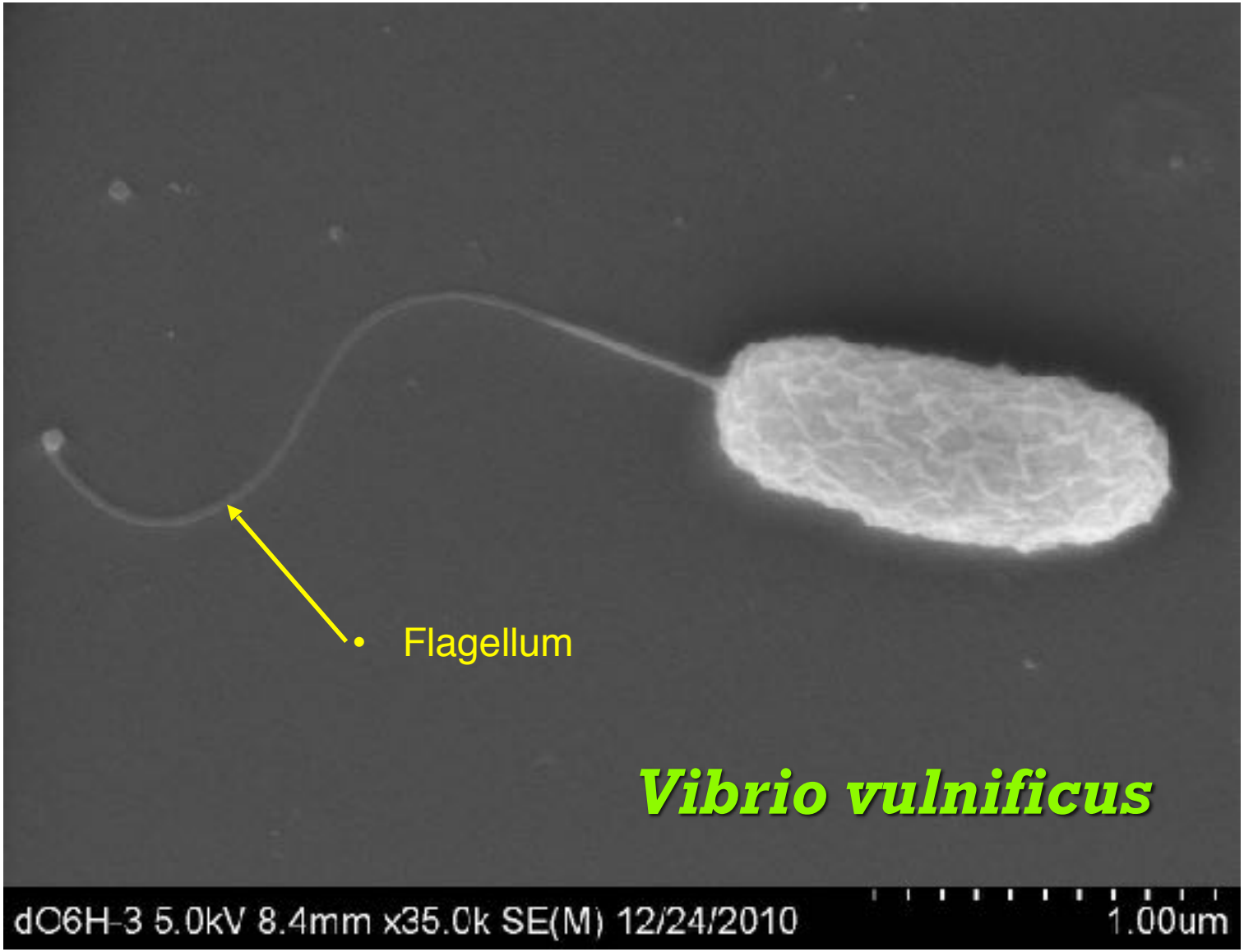
Fadel A. Samatey*, Katsumi Imada*, Shigehiro Nagashima*, Ferenc Vonderviszt*, Takashi Kumasaka†, Masaki Yamamoto† & Keiichi Namba*§

Nature. 2001 Mar 15:410(6826) 331-7.

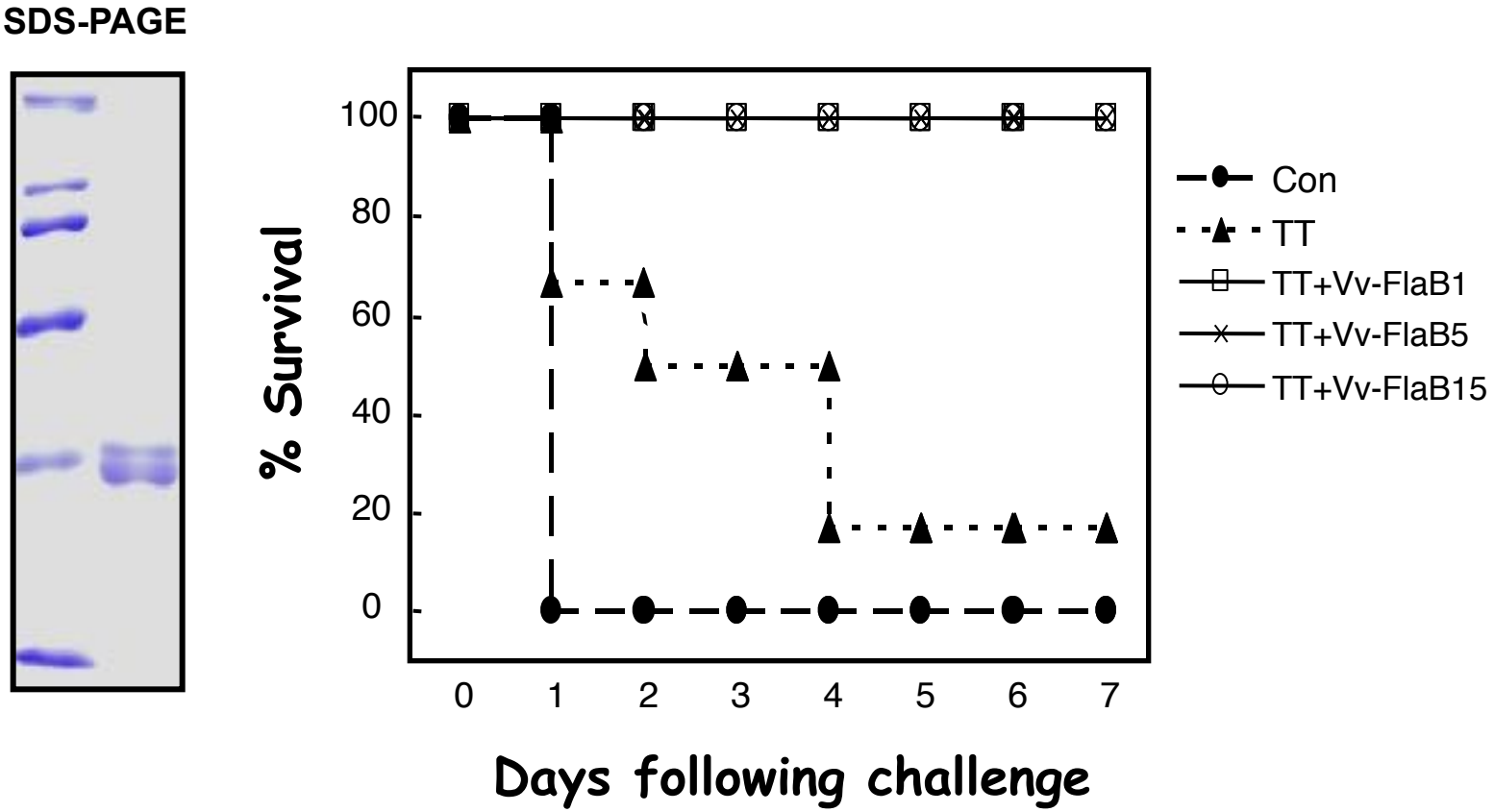







Flagellum
Vibrio vulnificus
dC6H-3 5.0kV 8.4mm x35.0k SE(M) 12/24/2010
1.00um

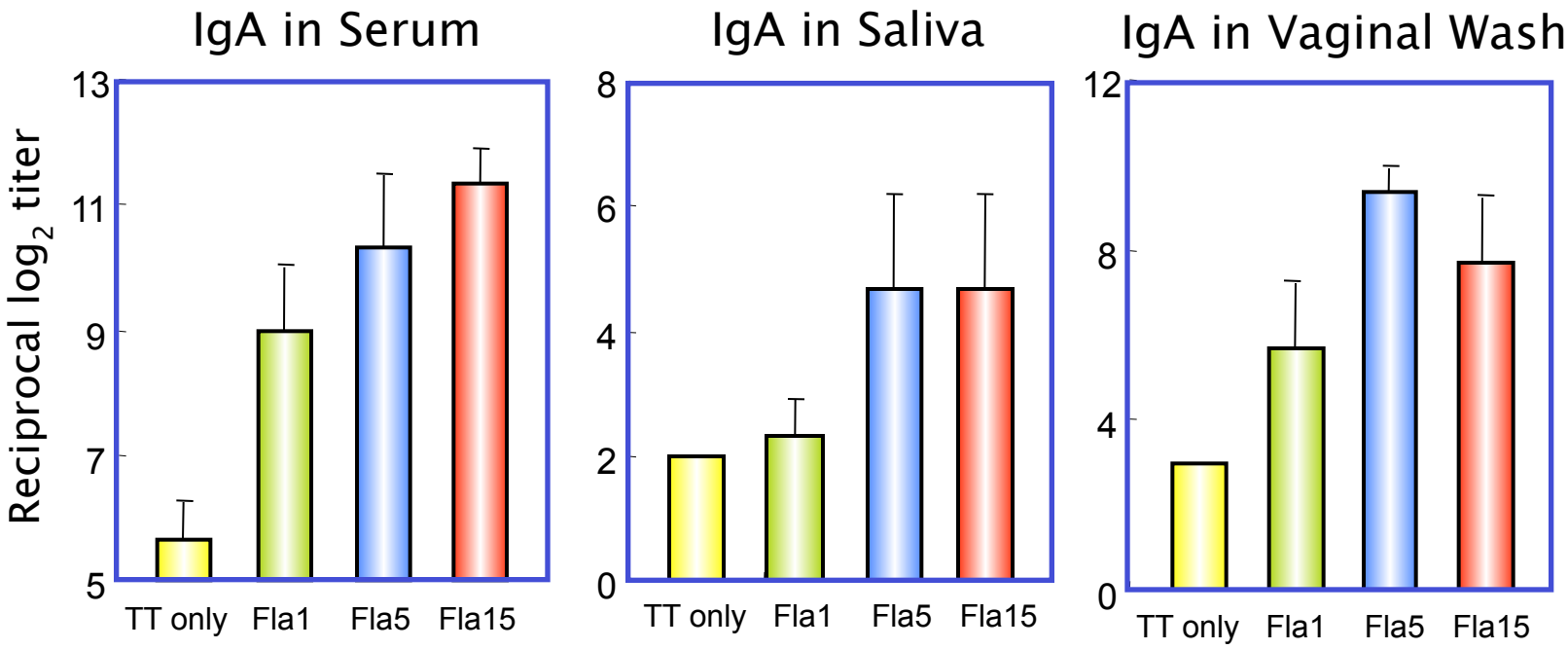
Nasal Vv-FlaB potentiates protective immune response



Nasal immunization with tetanus toxoid -> Tetanus toxin challenge

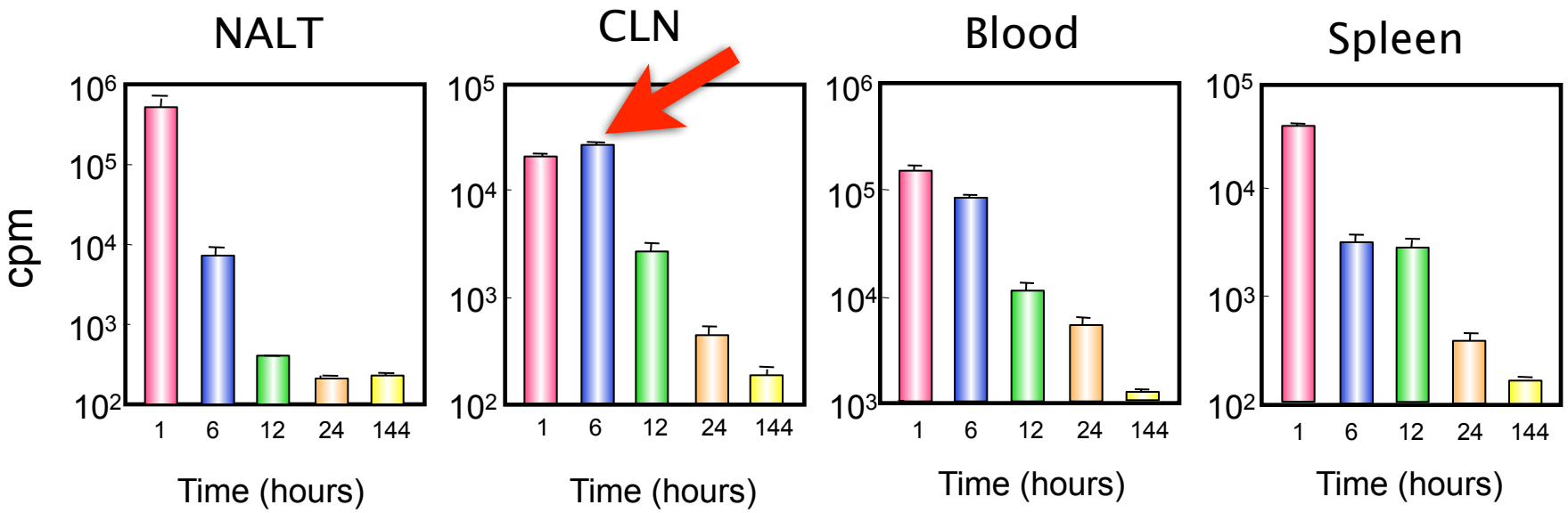
IAI 74:694-702, 2006

Antigen specific antibody production



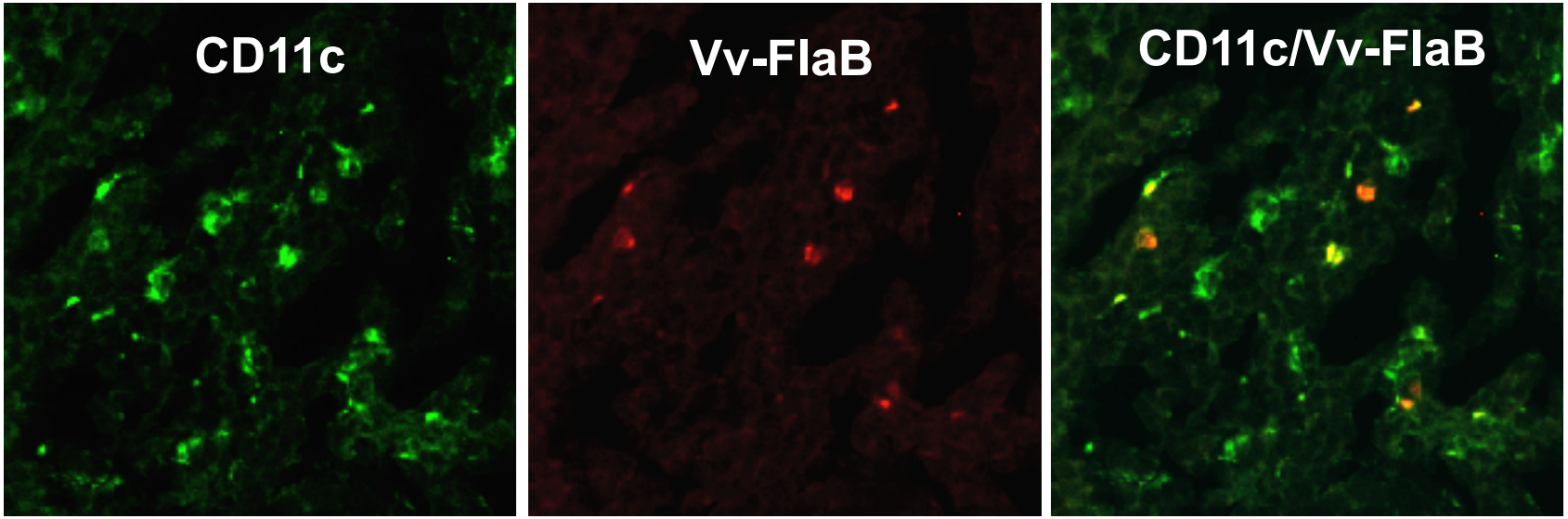
Intranasally administered Vv-FlaB enhanced antigen-specific systemic & mucosal IgA

Trafficking of ^{131}I-Vv-FlaB



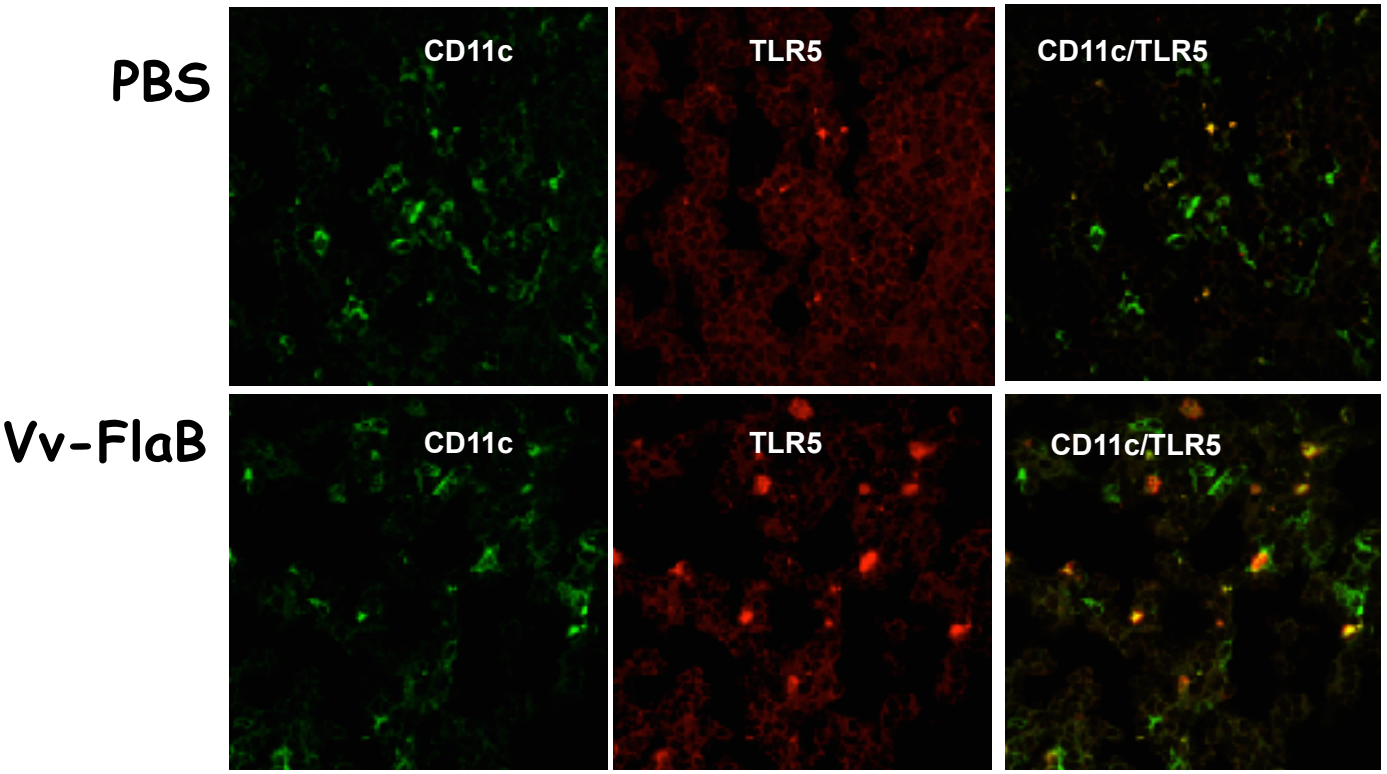
Intranasally administered ^{131}I-Vv-FlaB readily reached systemic circulation while the regional draining cervical lymph nodes retained the adjuvant protein relatively longer than spleen.

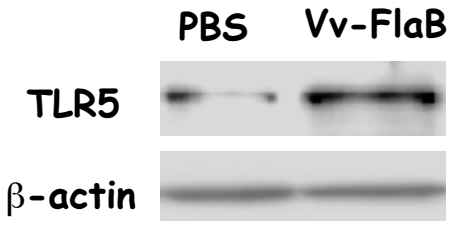
In vivo colocalization of Vv-FlaB with DCs in cervical lymph node

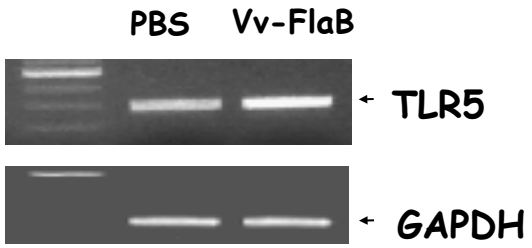


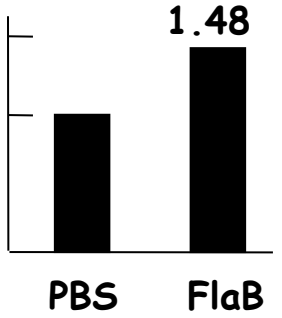
Intranasally administered Vv-FlaB colocalized with CD11c in the draining cervical lymph nodes.

Vv-FlaB treatment stimulates TLR5 expression in vivo





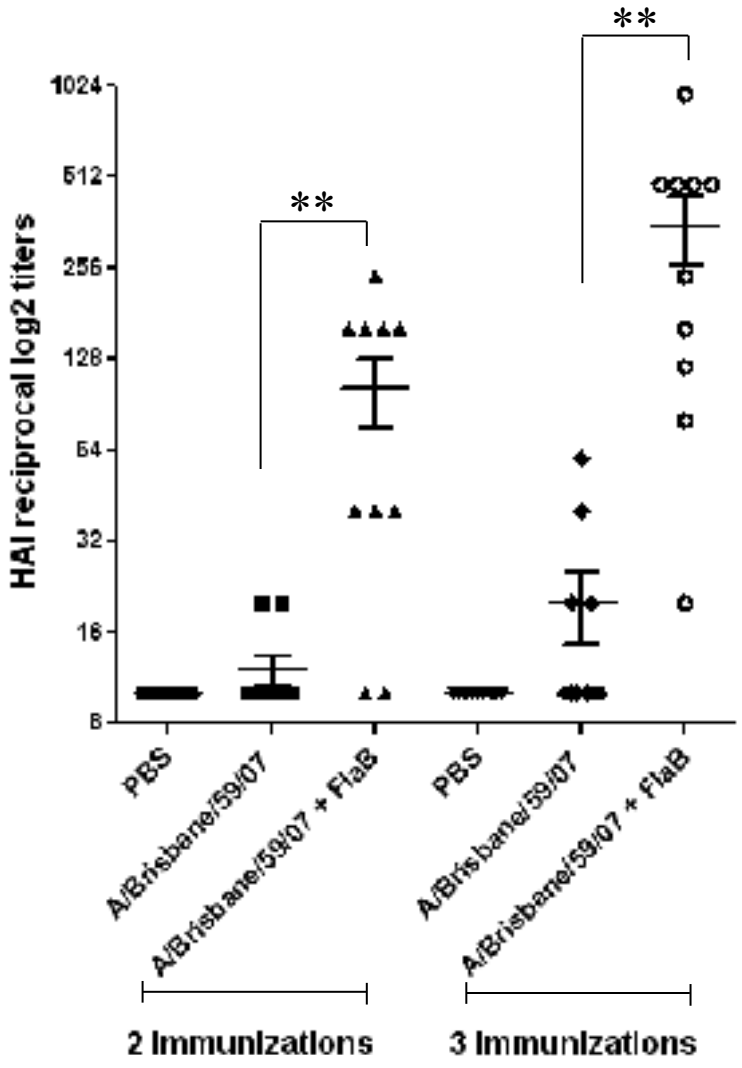


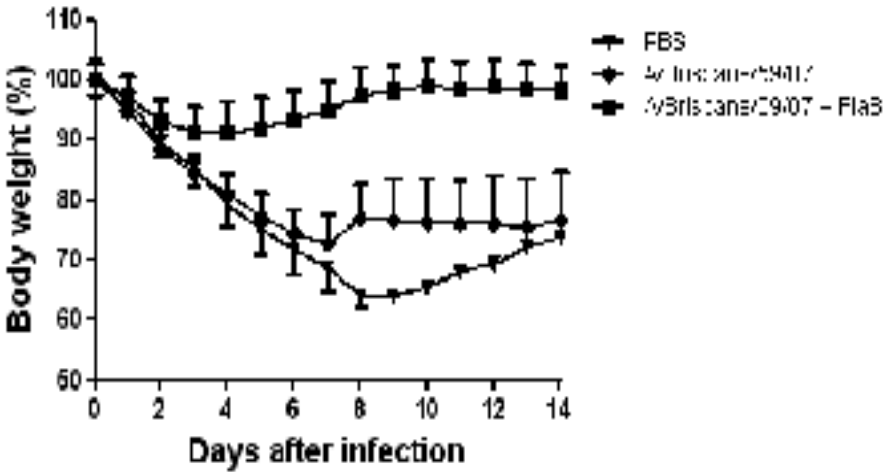


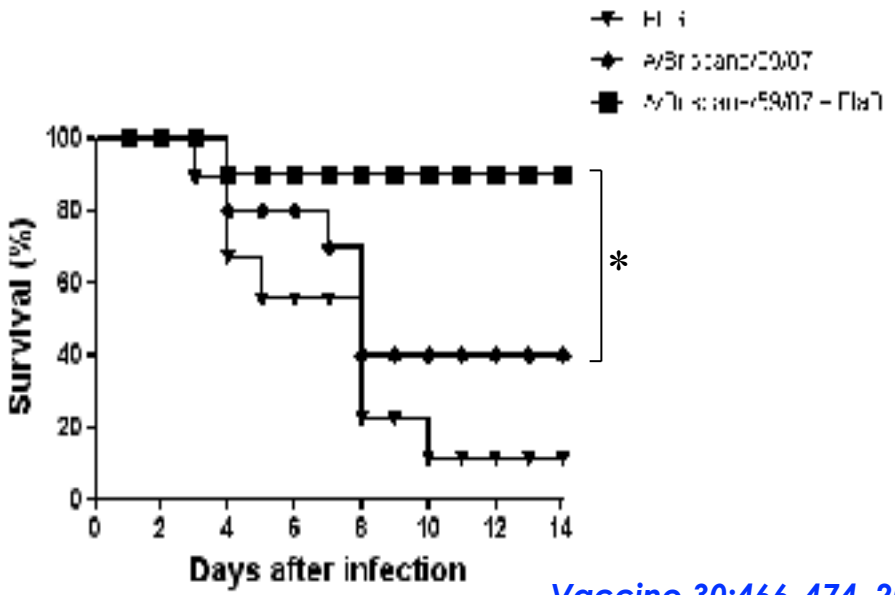
Then, can FlaB make the commercial killed influenza vaccine needle-free?

Shee Eun Lee, Seol Hee Hong, and Soo Young Kim

Intranasal co-administration of flagellin with inactivated influenza vaccine protects mice against lethal challenge with mouse-specific-pathogenic influenza virus

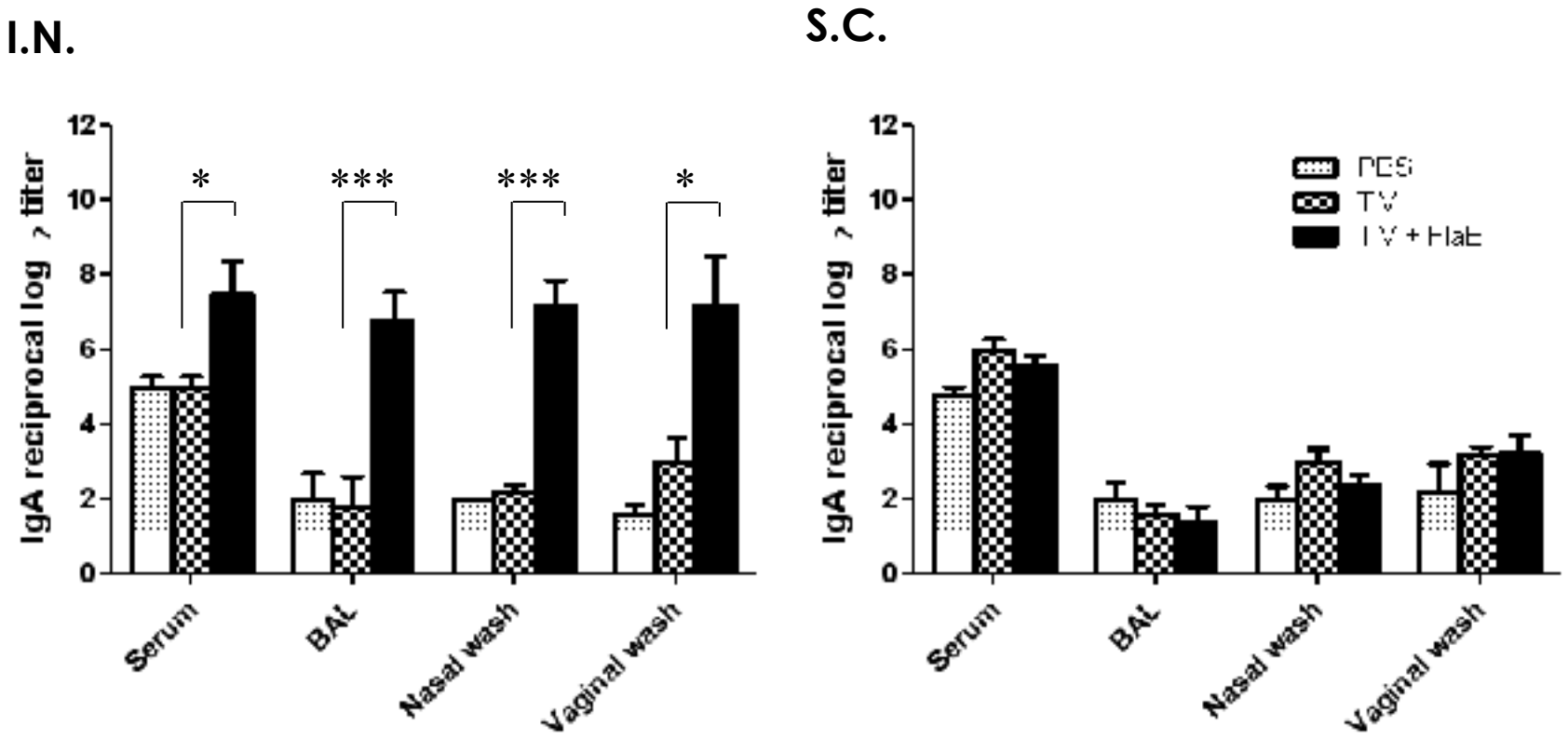






Vaccine 30:466-474, 2012

Intranasal administration of flagellin-adjuvanted inactivated influenza vaccine potents IgA production in serum and mucosal secretions

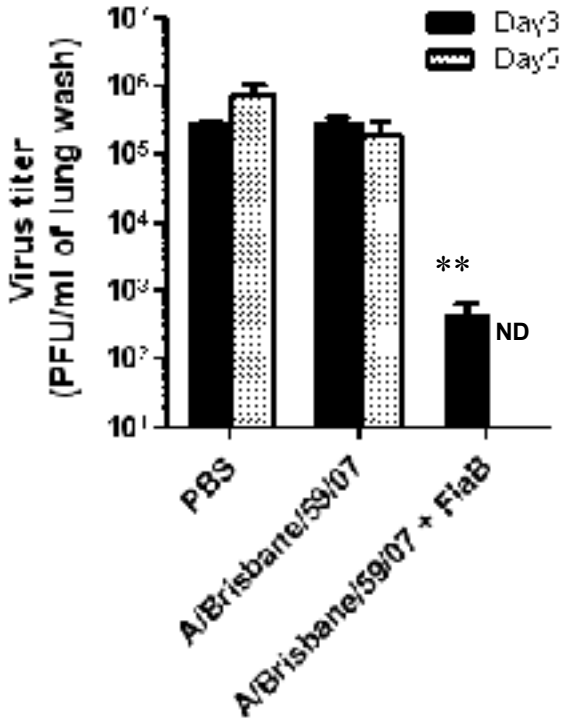


Vaccine 30:466-474, 2012

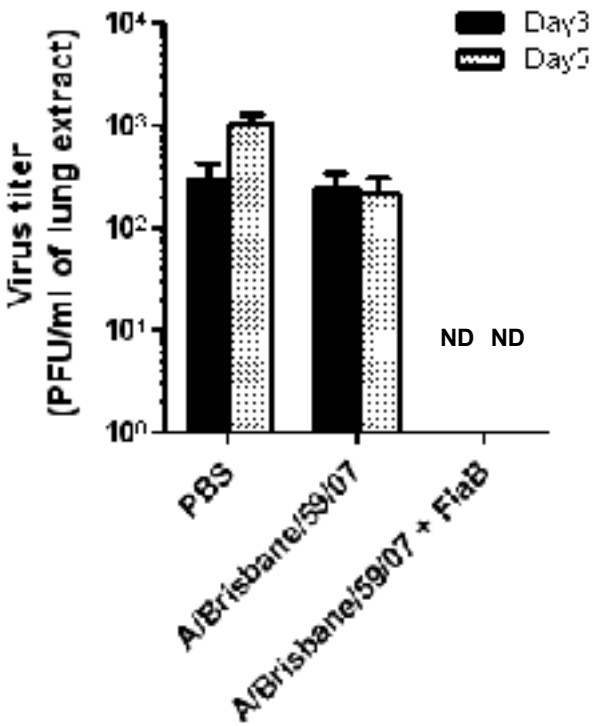
Protective Immunity

- Challenge with live virus: Viral titer -

Lung wash

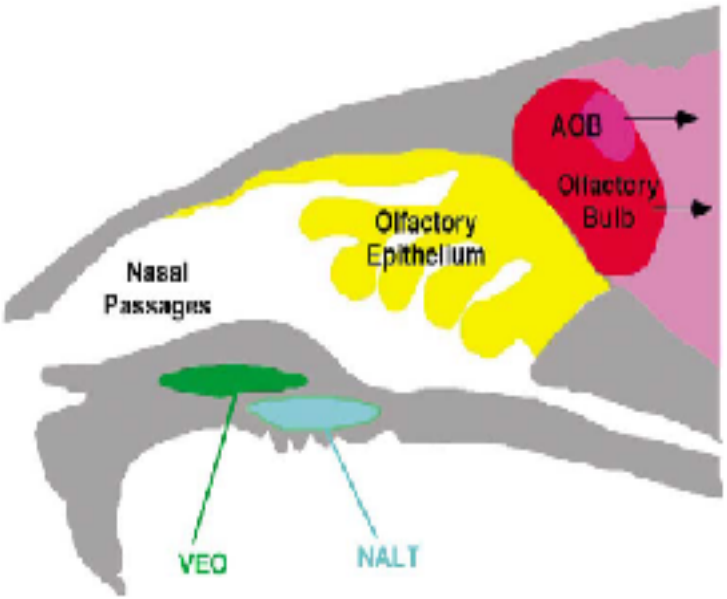


Lung extract



Vaccine 30:466-474, 2012

Vv-FlaB: Safety



LT/CT IN adjuvant failure history

Retrograde CNS uptake - GM1 ganglioside

Facial paralysis : Bell's palsy

Murine nasal tissues. Anatomical position of nasopharyngeal associated lymphoreticular tissues with olfactory tissues and the CNS

	Treatment		
Tissue	6.0 µg CT	5.0 µg Vv-FlaB	*p**
ON/E	1.26 ± 0.57	0.29 ± 0.05	<0.01
OB	1.28 ± 0.24	0.20 ± 0.02	<0.01

FlaB accumulation in the CNS - significantly lower than the CT

Vaccine 30:466-474, 2012

Conclusion

- ✓ FlaB adjuvant converted the killed influenza injection vaccine into a higher value mucosal vaccine
- ✓ Cleared GLP preclinical safety test in accordance with WHO guidelines
- ✓ IND submission process to KFDA

Would FlaB-*Ag* fusion work?

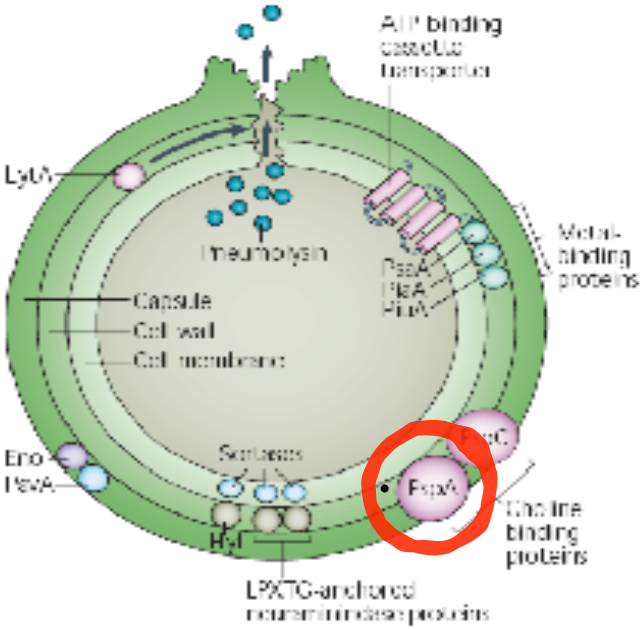
Mucosal vaccine & enhanced efficacy?

Chung Truong Nguyen, Soo Young KIm, and Shee Eun Lee

PspA

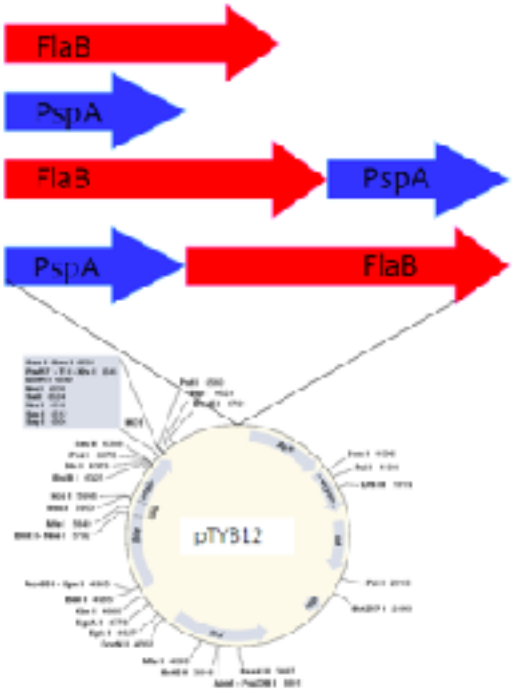
(pneumococcal surface protein A)

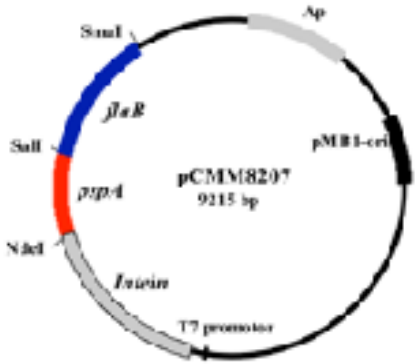
- Present on all strains of *S. pneumoniae*
  - expressed during invasion
  - expressed during colonization
- Virulence factor for
  - invasion
  - colonization
- Elicits protection against
  - bacteremia, sepsis, and pneumonia
  - colonization
- Inhibits
  - C3 activation
  - killing of pneumococci by cationic peptides
- Serologically diverse(2-3 PspAs are needed for a vaccine).

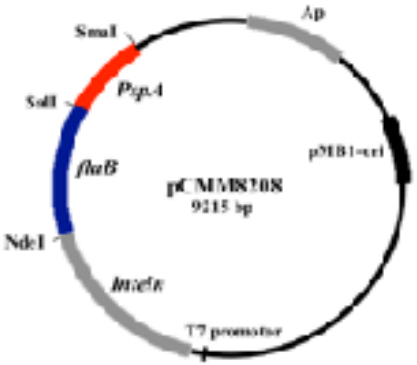


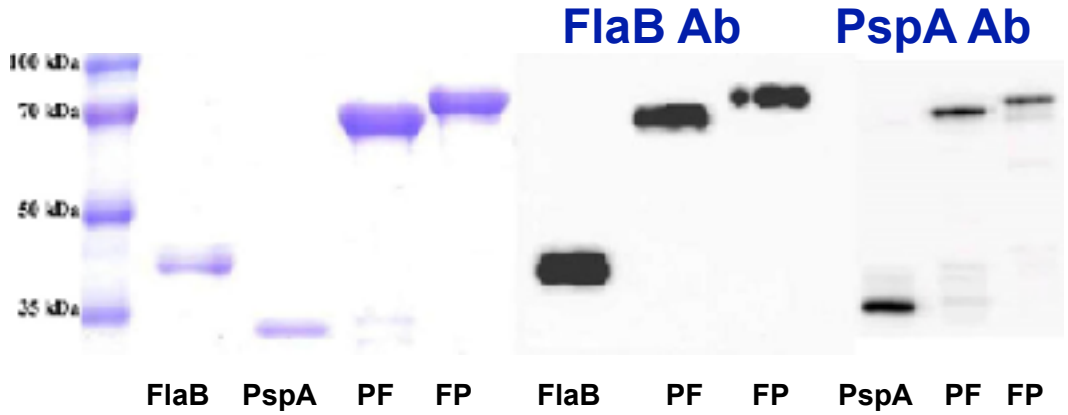
- **Aras Kadioglu, *et al.*** Nat Rev Micro 6(4): 288-301

Recombinant fusion proteins



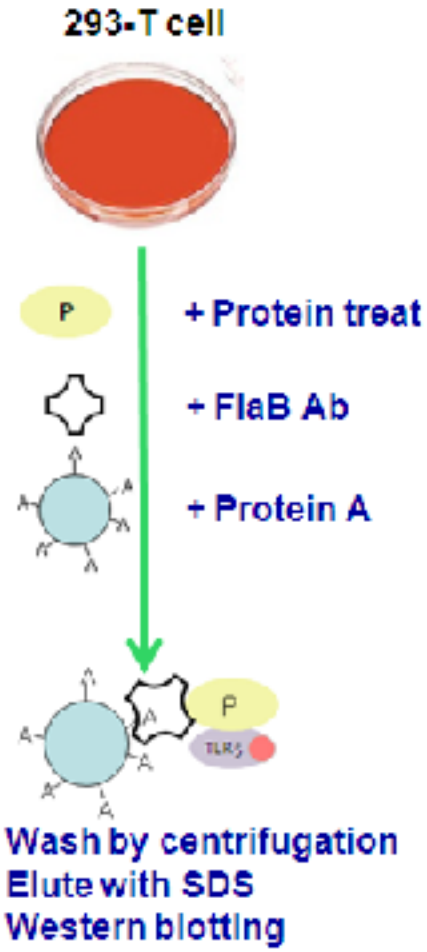


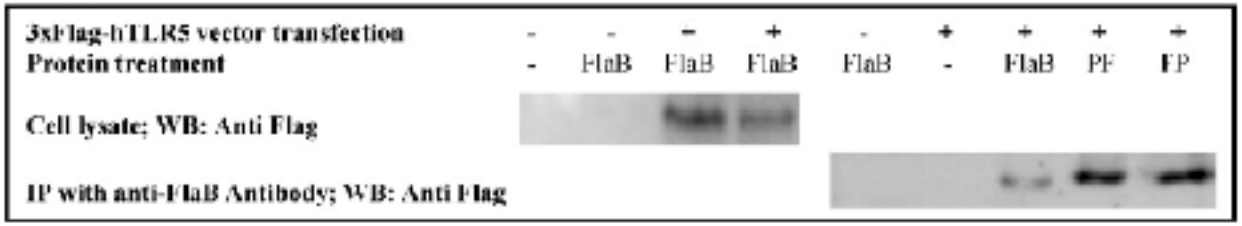




Vaccine 29:5731-9, 2011

Direct association of recombinant fusion protein with TLR5

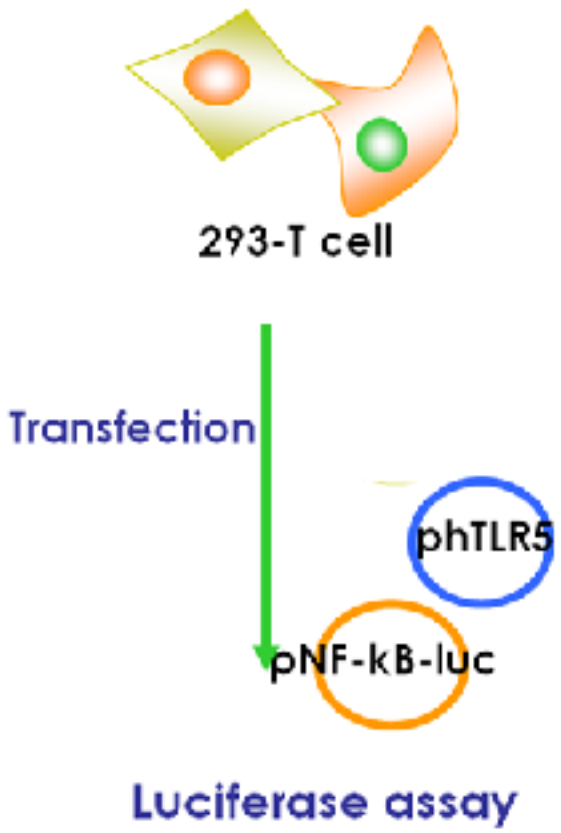


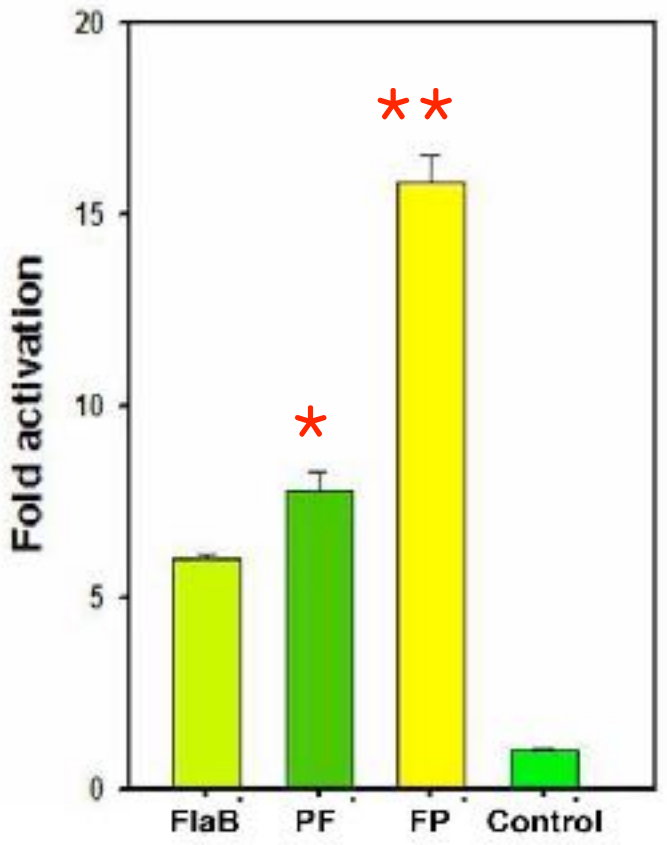


Recombinant fusion proteins directly interacted with TLR5 expressed in epithelial cells

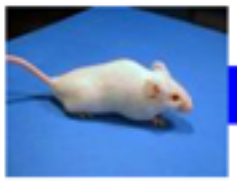
Vaccine 29:5731-9, 2011

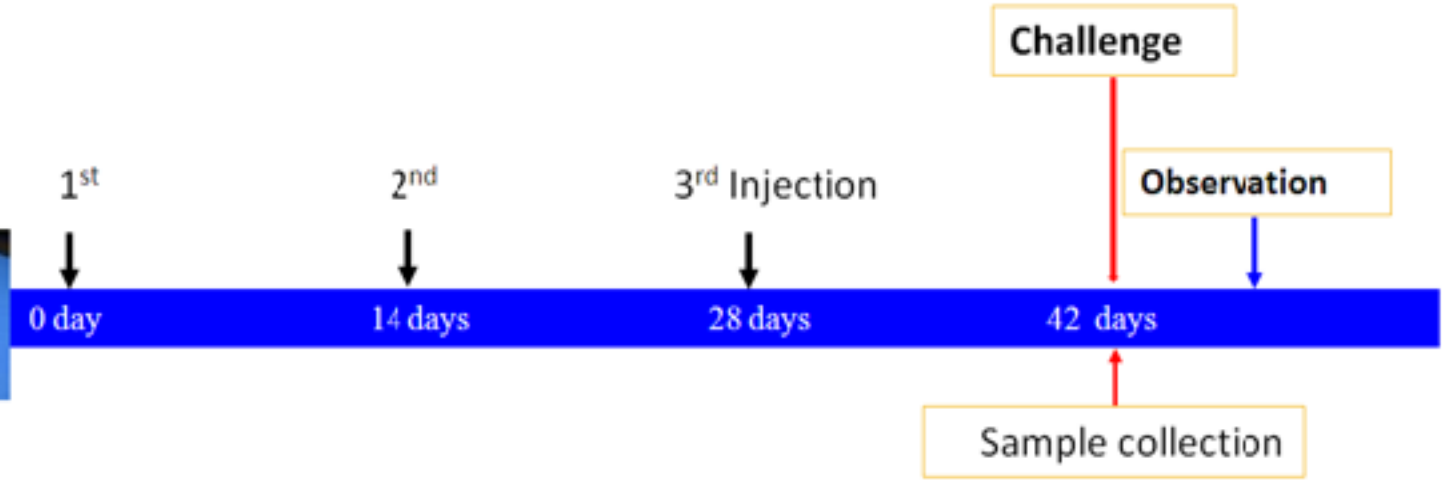
NF-κB activation through TLR-5





Immunization schedule





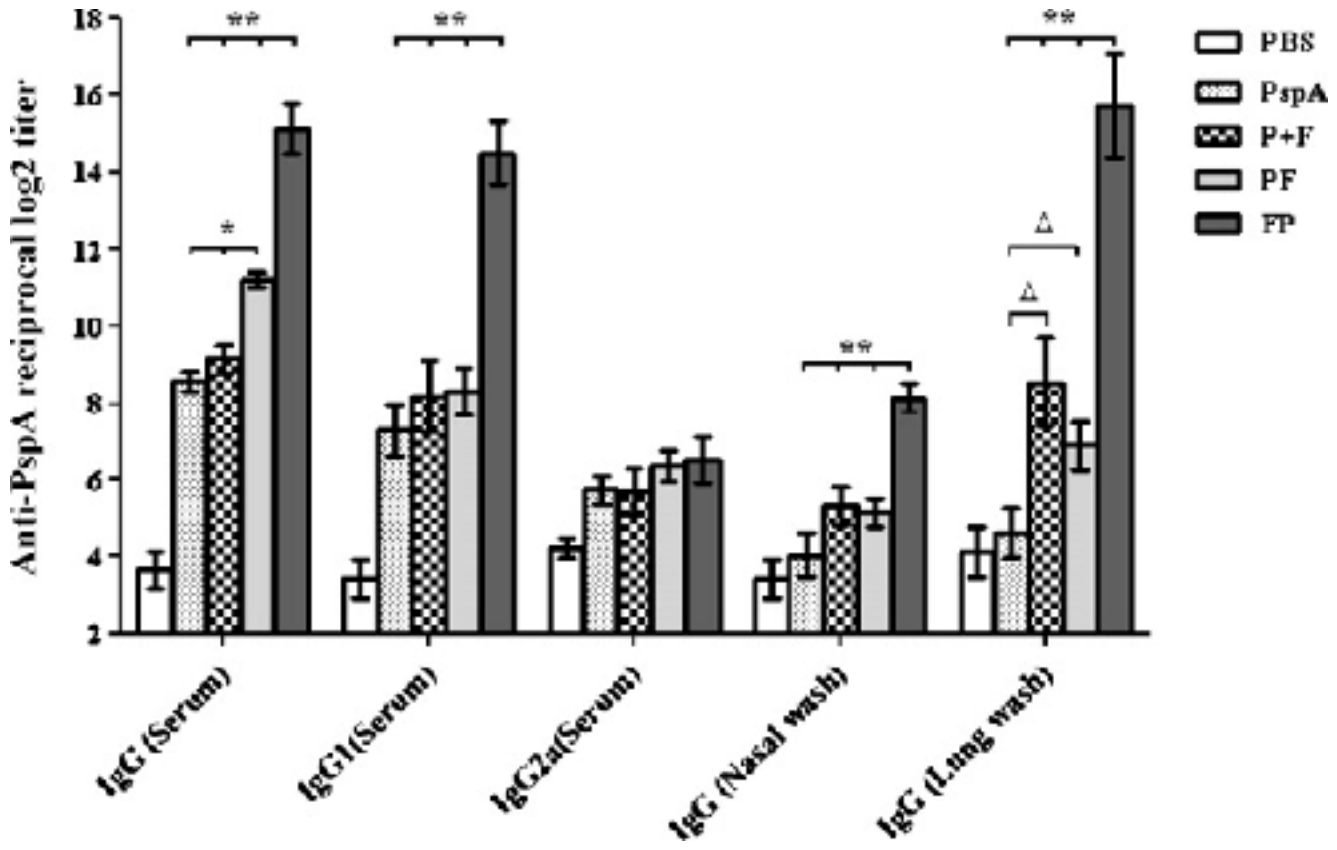
- Intranasal immunization: Balb/c mice

PBS :	20 µl
PspA only:	2.5 µ g/20 µl
P+F:	2.5+ 4 = 6.5 µg/20 µl
P-F or **F-P**:	6.5 µg/20 µl

- Challenge: *S. pneumoniae* in 20 µl PBS/ intranasal route
- Sampling: Blood, saliva, vaginal washes, lung washes, nasal washes, feces and spleen
- PspA-specific antibody response: ELISA
- PspA-specific cytokine production: ELISA

Vaccine 29:5731-9, 2011

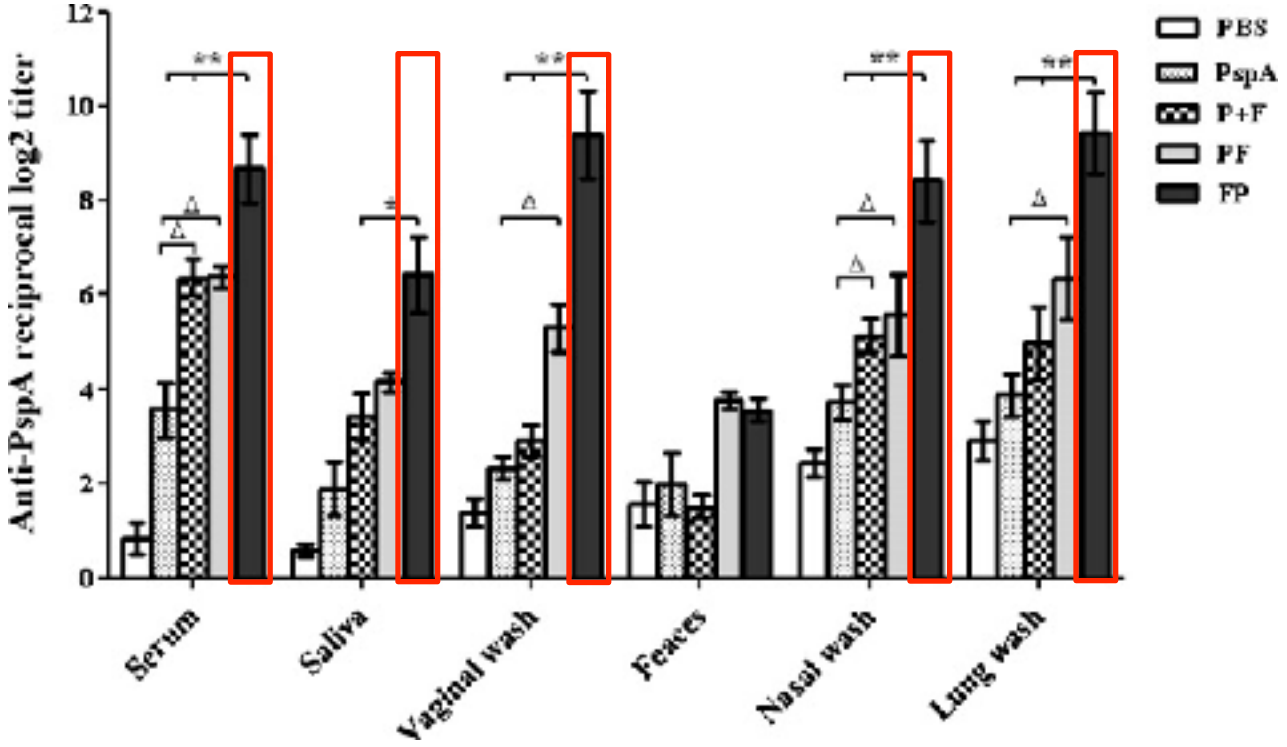
PspA-specific IgG Antibody



Enhanced Ab responses in both systemic and mucosal compartments

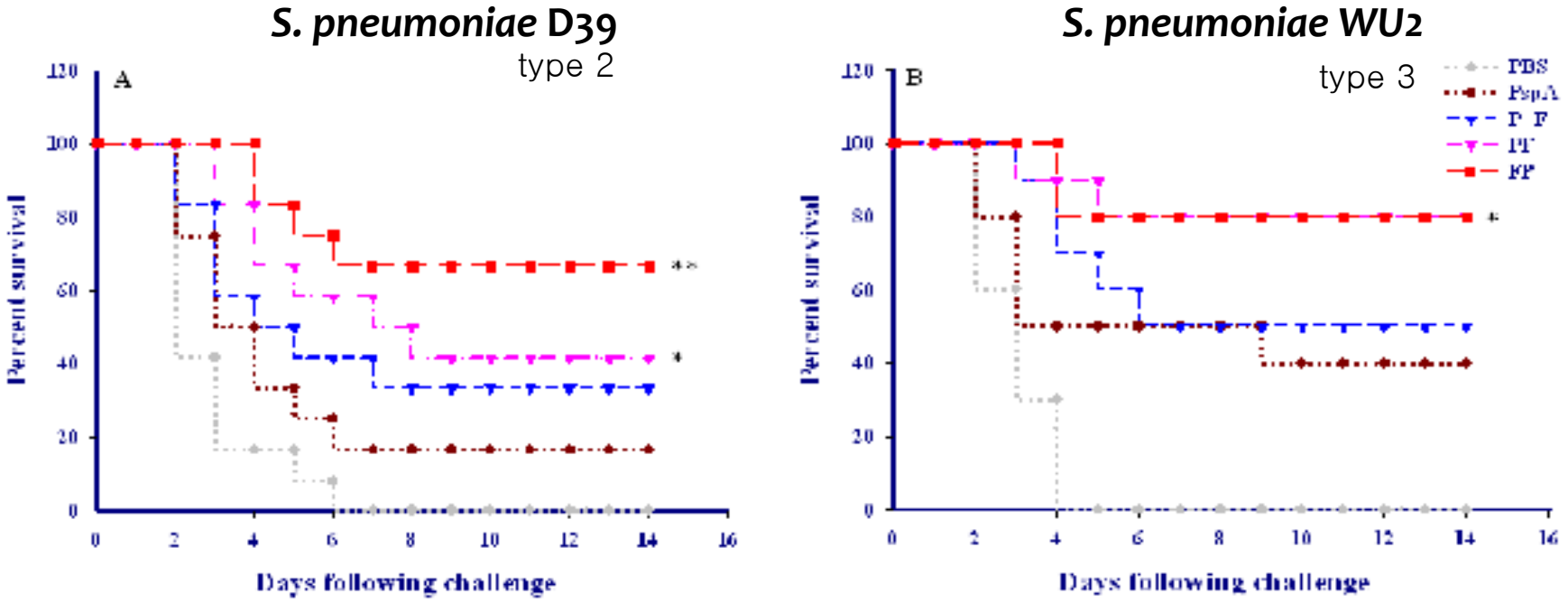
Vaccine 29:5731-9, 2011

PspA-specific IgA Antibody



Vaccine 29:5731-9, 2011

Protective immunity

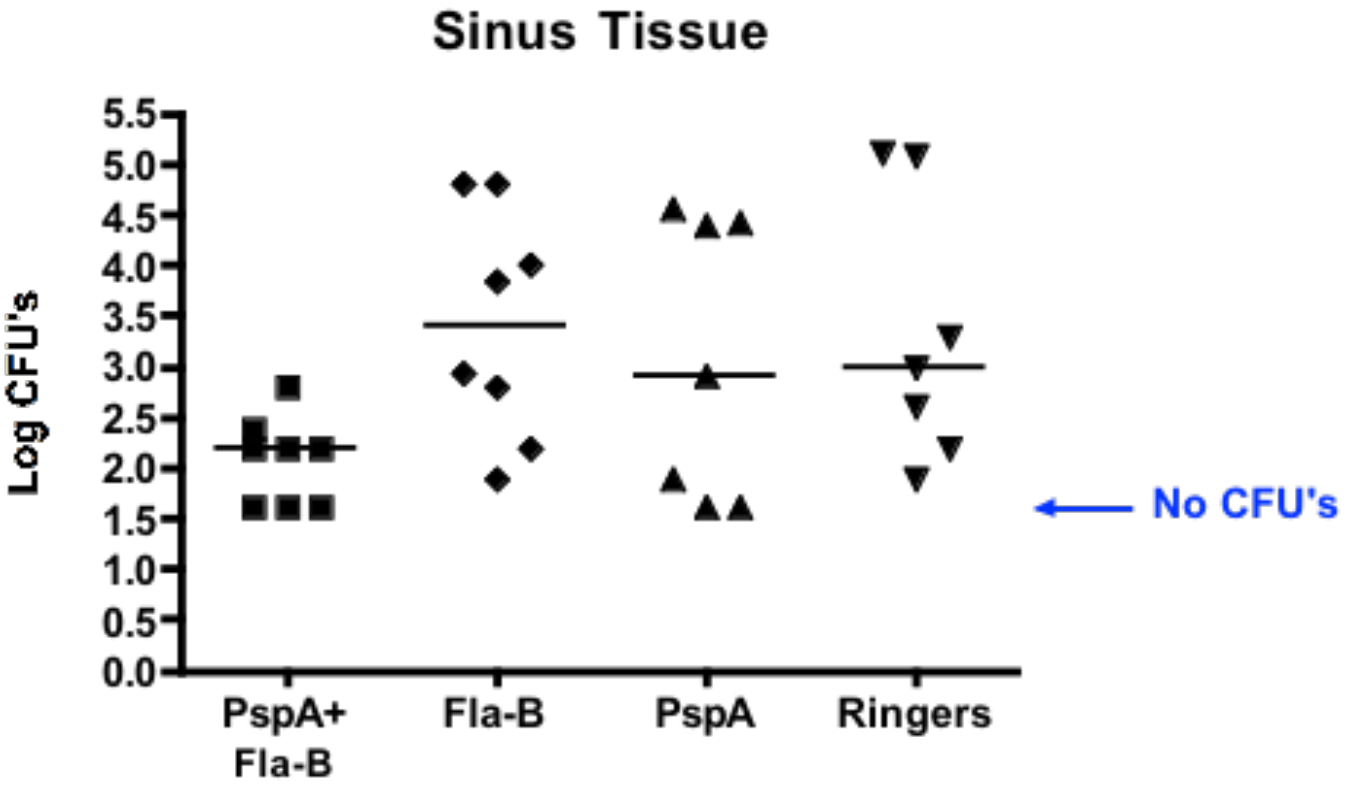


Survival of mice challenged with 200x the LD_{50} *S. pneumoniae* D39 (A) and 5 x 10^8 CFU *S. pneumoniae* WU2 (B).

➡ FlaB–PspA fusion proteins provided the best protection against intranasal challenge with pathogenic *S. pneumoniae*

Vaccine 29:5731-9, 2011

Effects on colonization?



By David E. Briles of UAB

Conclusion

- ✓ FlaB-PspA fusion protein - successful intranasal vaccine, heteroserotypic protection, could replace existing capsular polysaccharide-based vaccine?

- ✓ FlaB-PspA could be considered as an effective carrier for multivalent capsular polysaccharide conjugate vaccine for the development of new pneumococcal vaccine

Would sublingual route work?

Gastrointestinal immune responses?

Vivek Verma, Wenzhi Tan, Sao Puth, and Shee Eun Lee

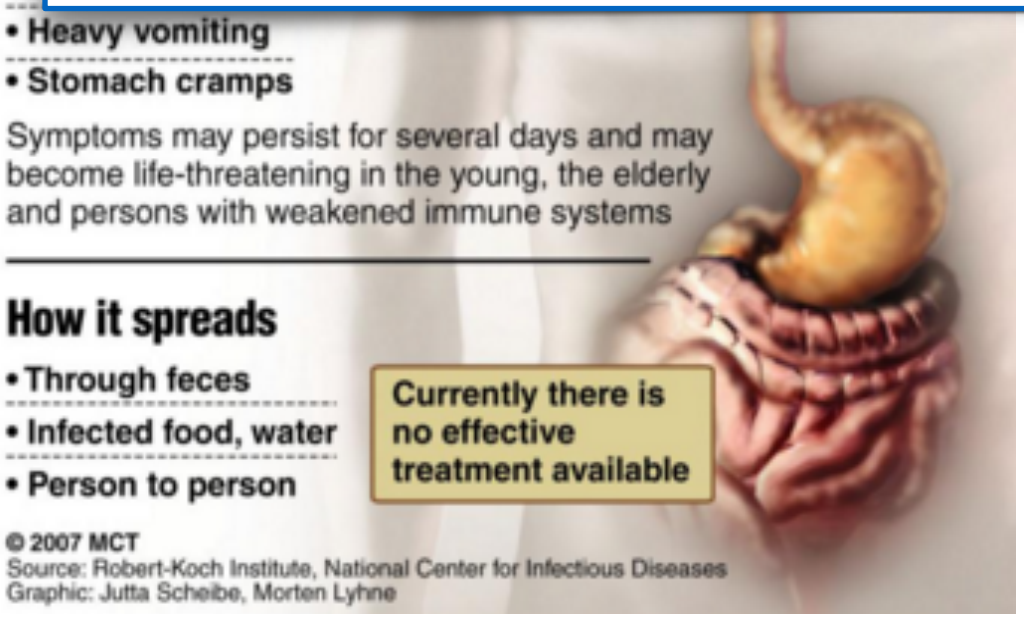
Verma *et al. J Transl Med (2016) 14:135*
DOI 10.1186/s12967-016-0899-4

Journal of Translational Medicine

RESEARCH Open Access

Norovirus (NoV) specific protective immune responses induced by recombinant P dimer vaccine are enhanced by the mucosal adjuvant FlaB

Vivek Verma[1,3,5], Wenzhi Tan[1], Sao Puth[1], Kyoung-Oh Cho[4], Shee Eun Lee[1,2] and Joon Haeng Rhee[1,3*]

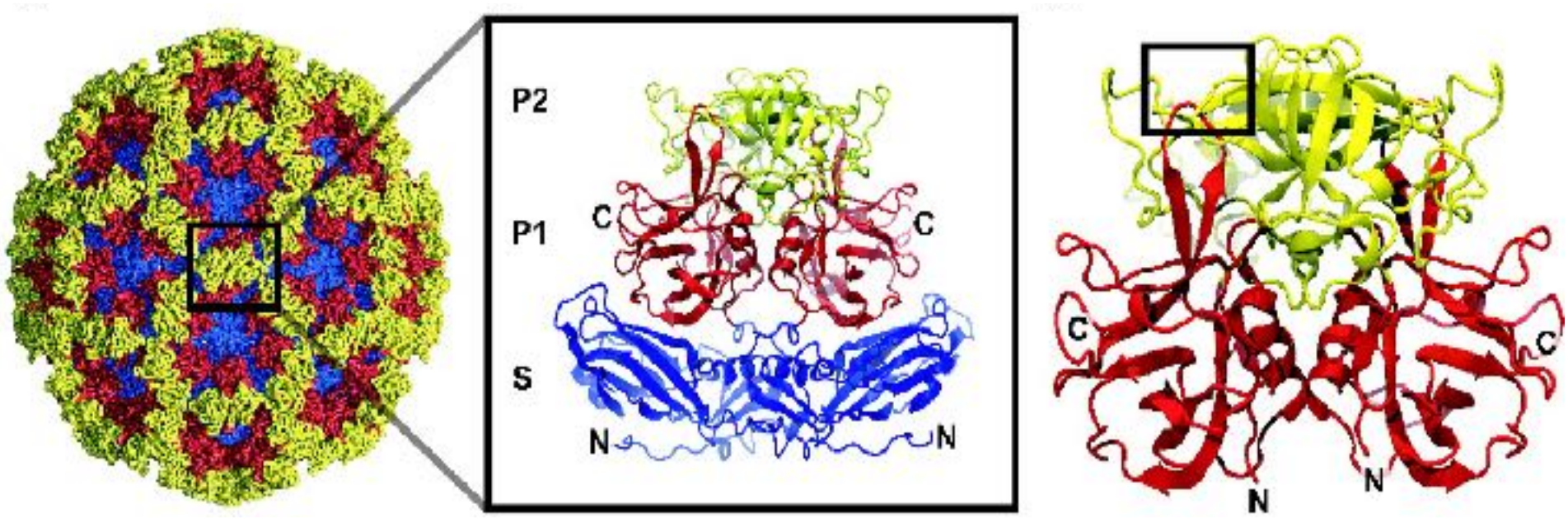


- **ORF3**: Minor capsid VP2
- **ORF4** an alternative reading frame overlapping the VP1

JTM, 14:135, 2016

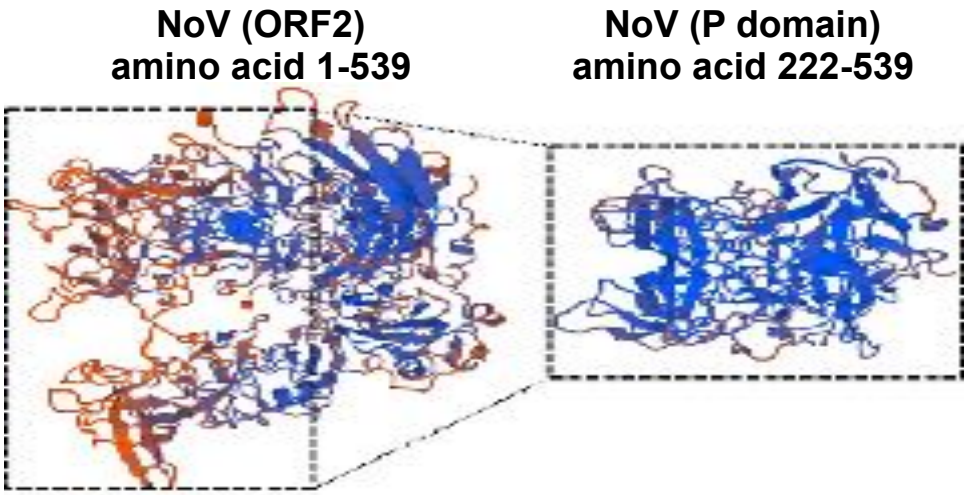
Norovirus P domain

P domain dimer has the same conformation as in the whole capsid structure

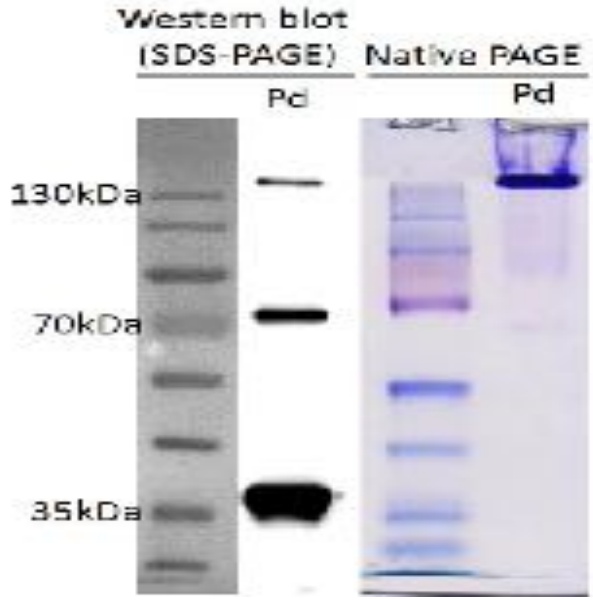


Atomic resolution structural characterization of recognition of histo-blood group antigens by Norwalk virus

Vaccine Target: NoV P domain dimer (Pd)



The three dimensional (3D) organization of Pd



Pd recombinant protein formed VLP like polymers:

» **Dimers and trimers by SDS-PAGE**

» **Trapped in the stacking gel by native PAGE**

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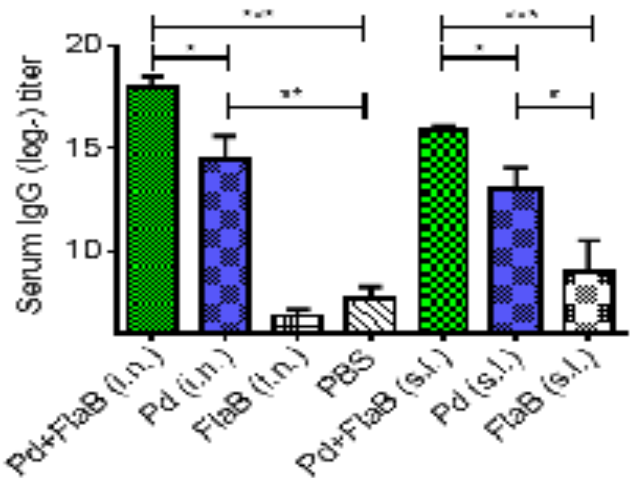
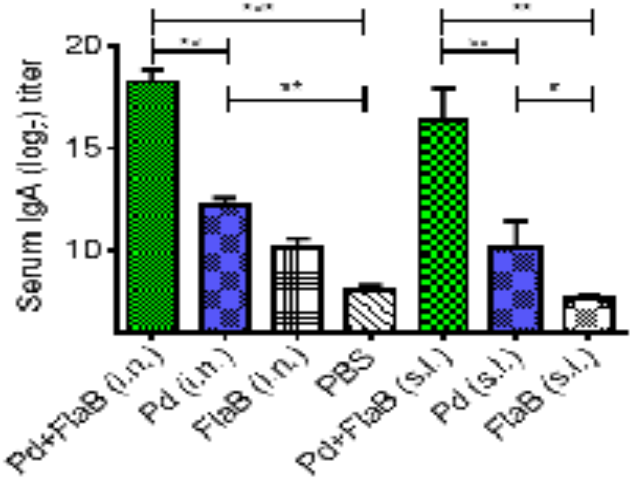
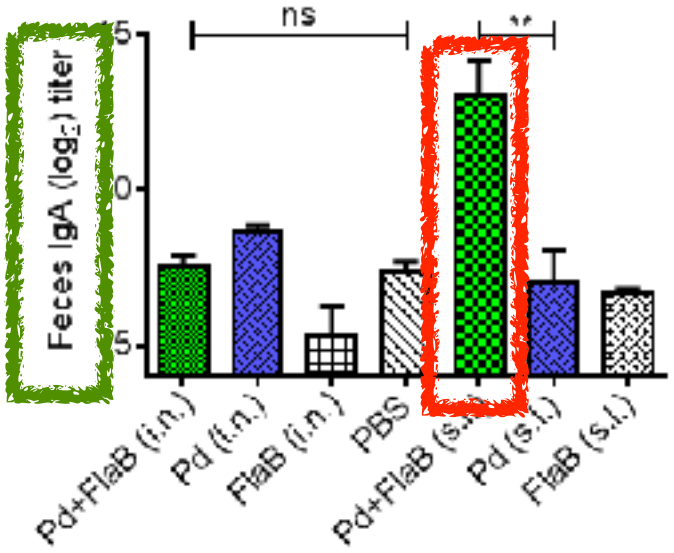
FlaB enhances Pd-specific Ab responses - Mucosal Vx







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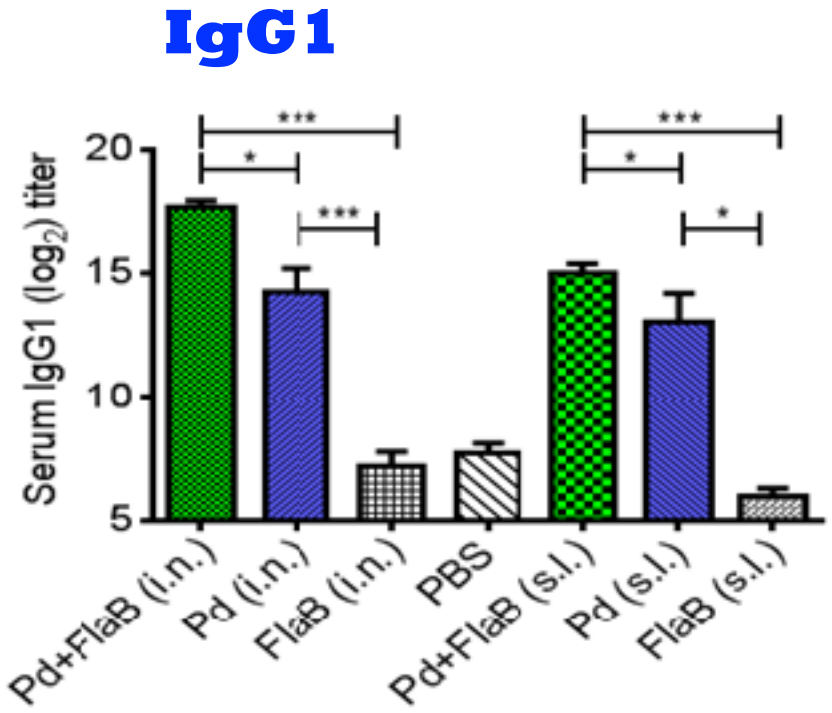
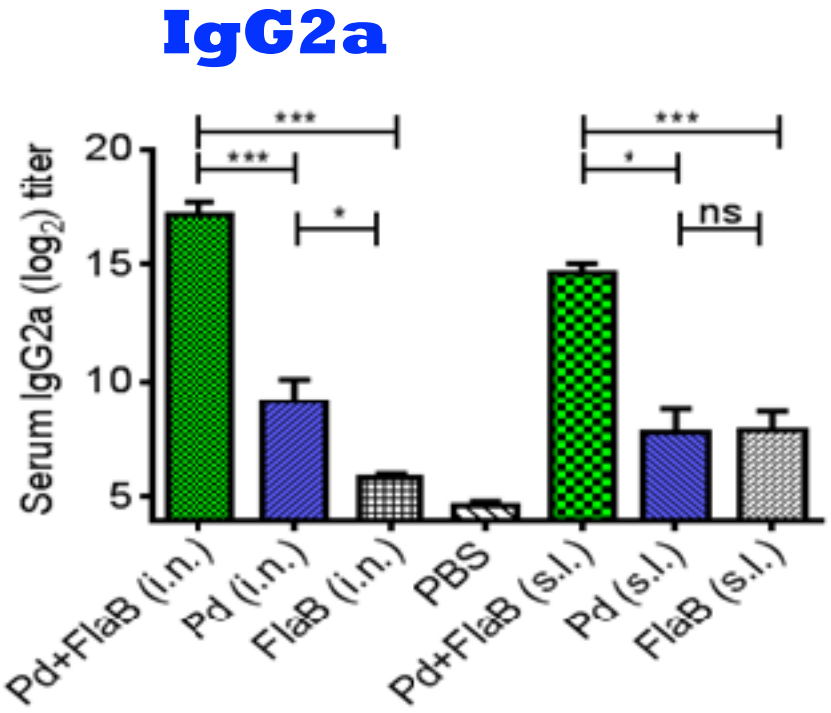
Balanced induction of Th1/Th2 immune responses





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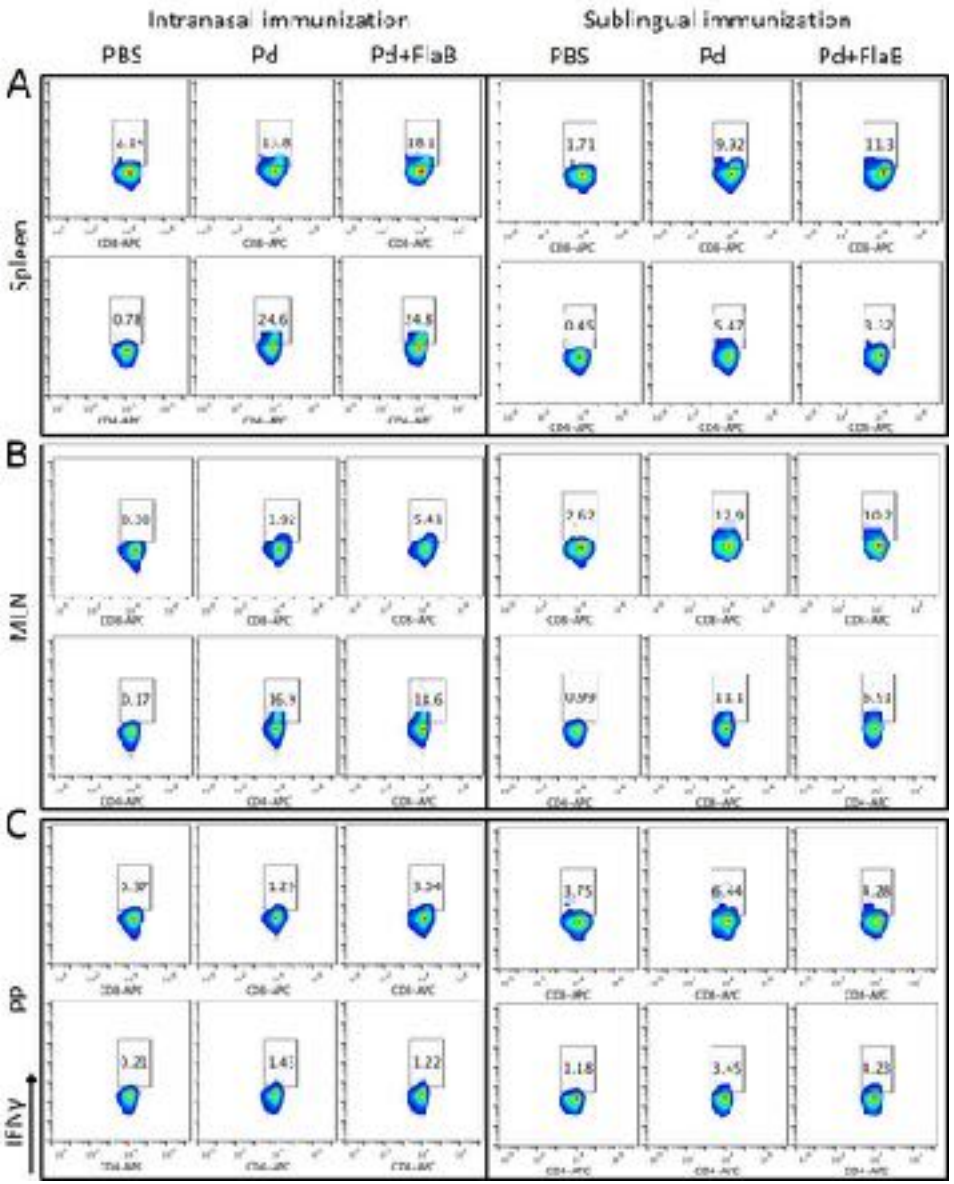
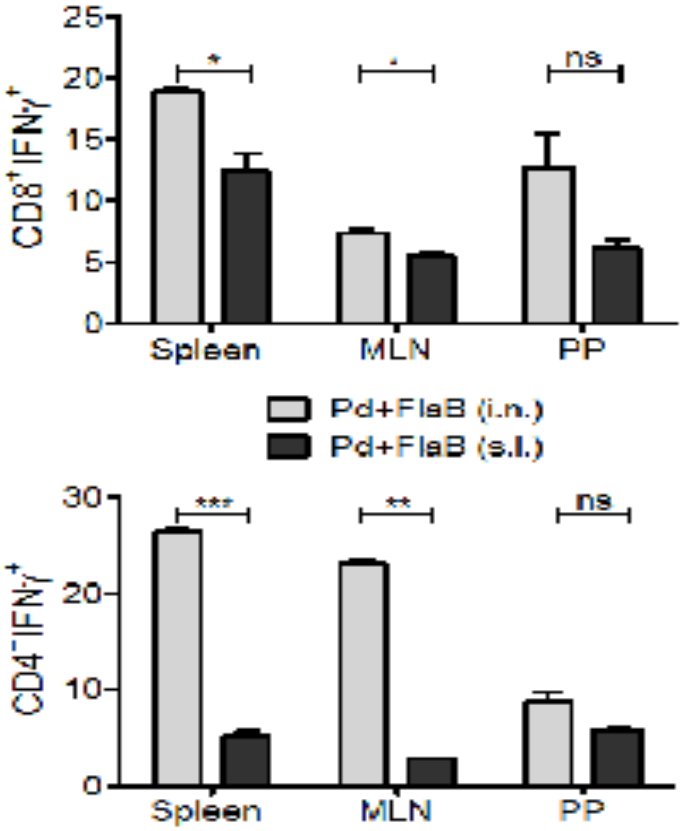
FlaB potentiates Pd-specific cell mediated immune responses in systemic and local immune compartments





The combination with FlaB stimulated a robust $CD4^+IFN\gamma^+$ and $CD8^+IFN\gamma^+$ T cell response in spleen as well as in mLNs.

JTM, 14:135, 2016

Final conclusion

Flagellin is a versatile mucosal adjuvant.

Thank you for your attention !

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6Th Vaccine and ISV Congress
(Shanghai Oct 14-16, 2012)

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부산대학교