Adjuvants: 21st century toolkit for vaccines and immunotherapeutics, with a special emphasis on mucosal routes

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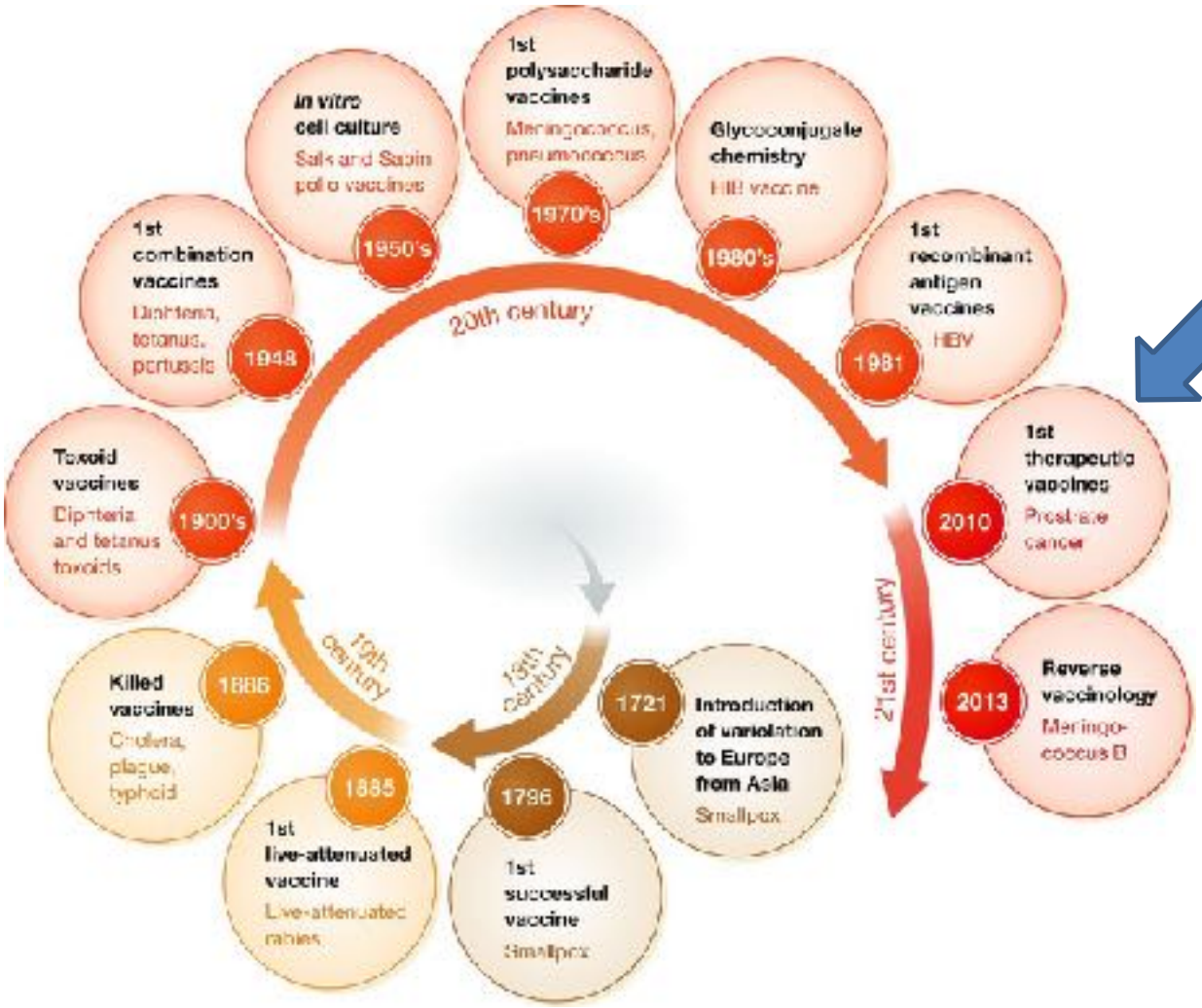
Major milestones in vaccinology

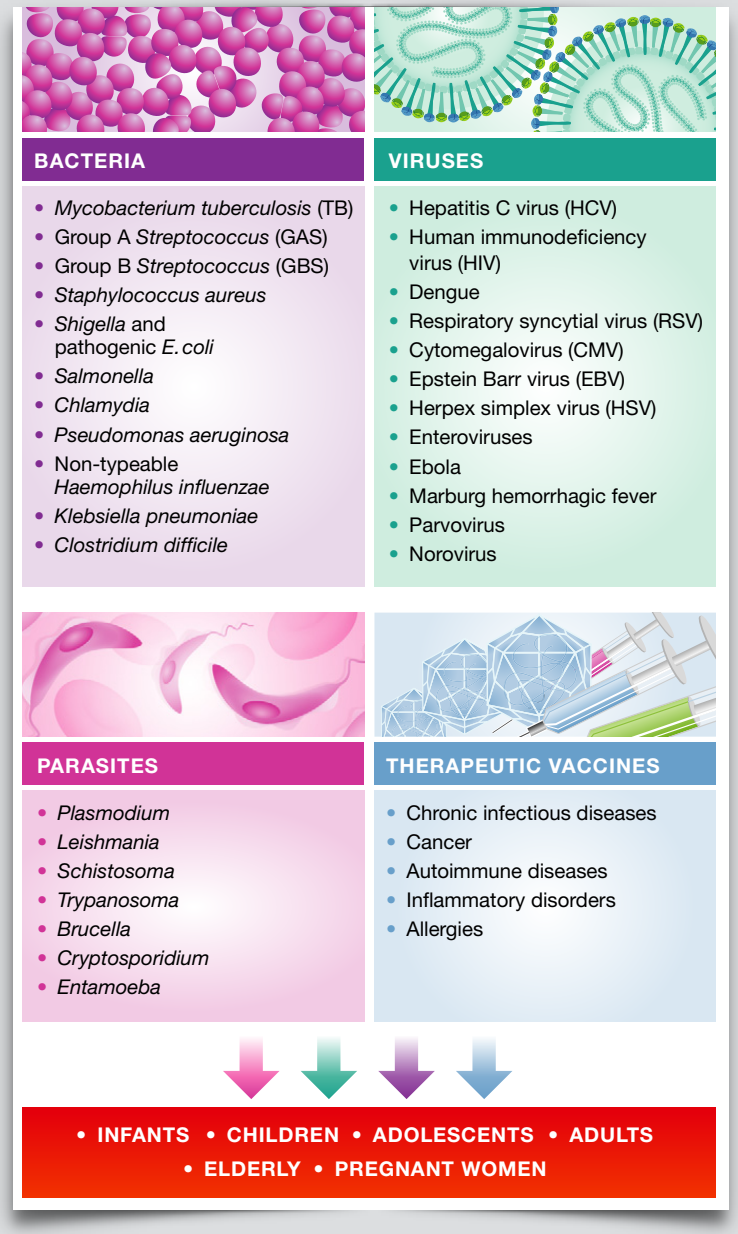


Sipuleucel-T
The first therapeutic vaccine for prostate cancer

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

EMBO Molecular Medicine

Target disease and population for 21st century vaccines



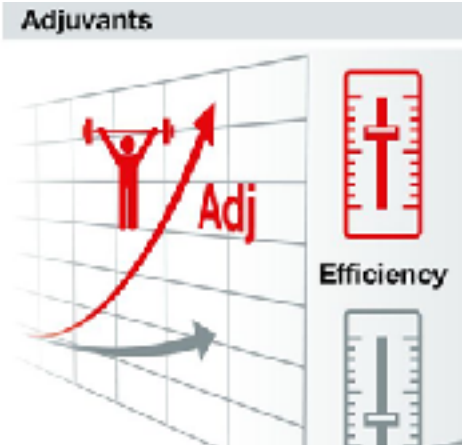
Delany I et al. EMBO Mol Med. doi:10.1002/emmm.201403876

The 21st century vaccinologists toolbox

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

21st century vaccine: Adjuvant

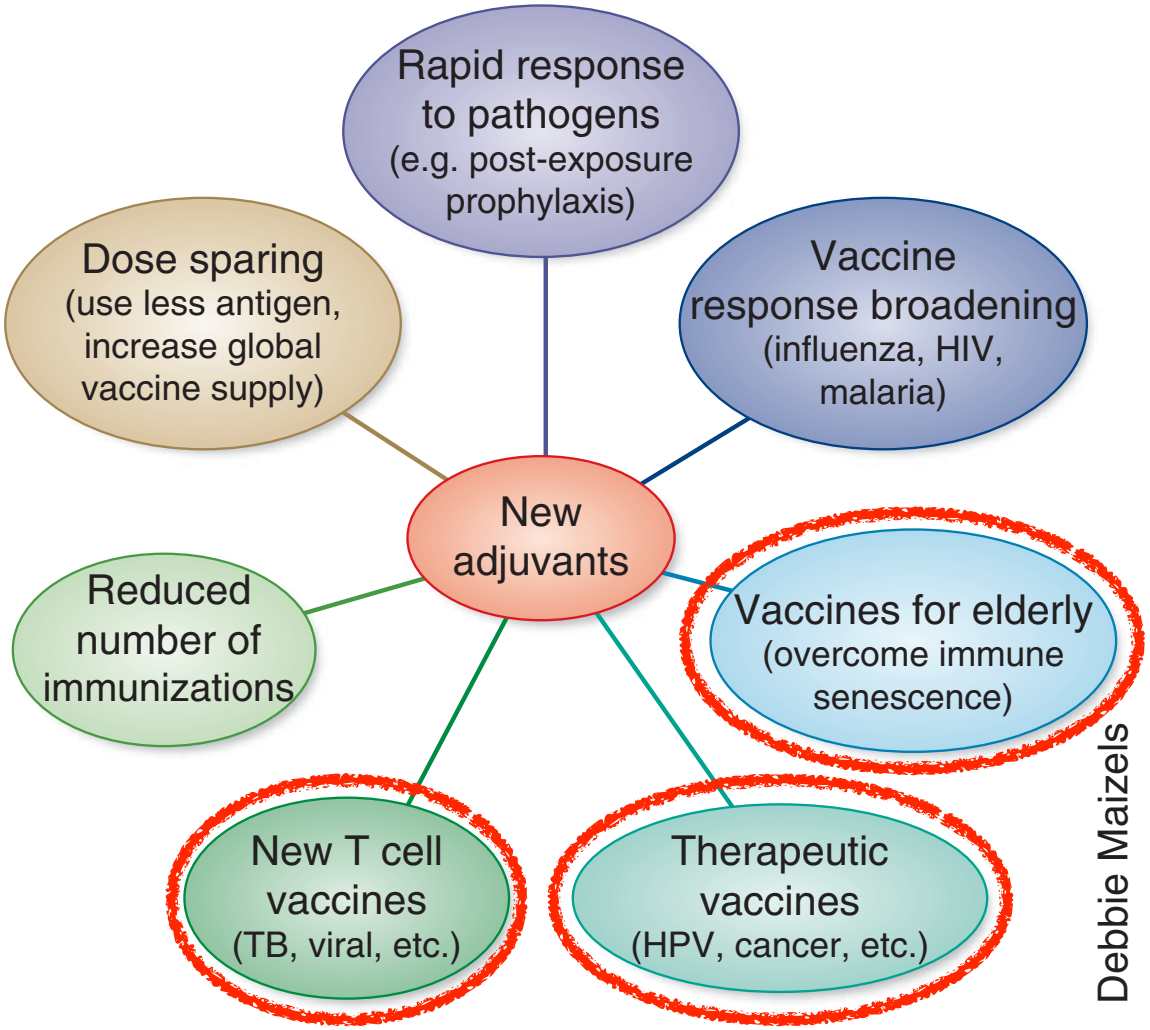
- A component that **potentiates** the immune responses to an antigen and/ or **modulates** it towards the desired immune responses {Adjuvare (in Latin) = help or add}



Rationale for Including Adjuvants in Vaccines

Low immunogenicity: e.g., Subunit vaccine	Low immune response population: e.g., Elderly	Time window: immunization / protection	Amount of antigen	Th1/Th2 balance	Need for booster
↕	↕	↕	↕	↕	↕
Increase immunogenicity, breadth of response	Increase immune response	Decrease number of doses	Antigen sparing	Direct desired immune response	Longevity immune response

Potential benefits of adjuvants



Considerations for ideal adjuvants

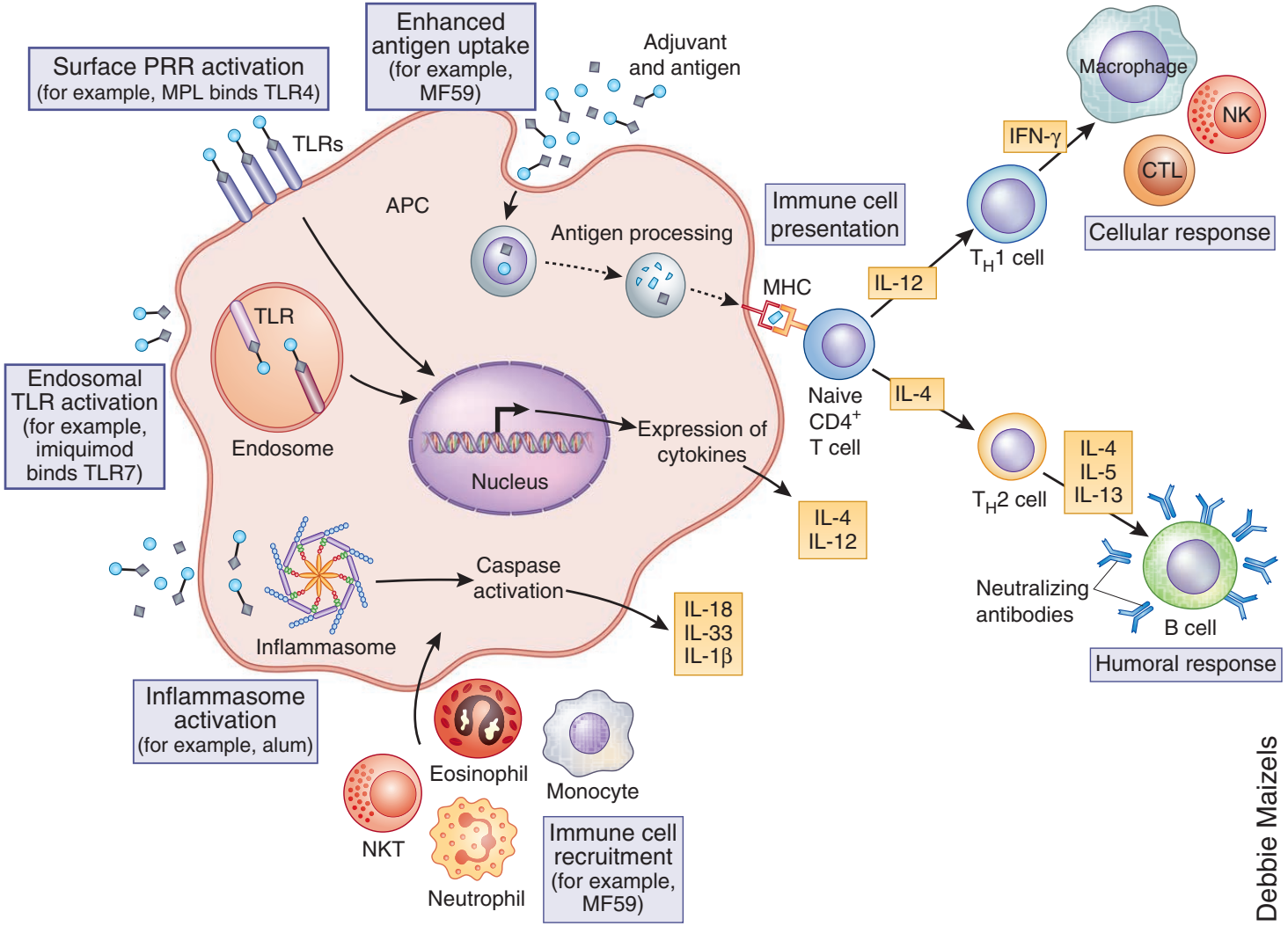
Category	Subcategory	Considerations
Biological activity	Safety	Formulation must be safe and effective in all age groups; metabolizable components preferred; adjuvant activity should be localized and transient; adjuvant should not have direct effect on lymphocytes: no nonspecific B or T cell responses
		Each immunization route may have different formulation requirements
		Adjuvant should enable reduction in required antigen dose or number of immunizations
		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

Vaccine adjuvants

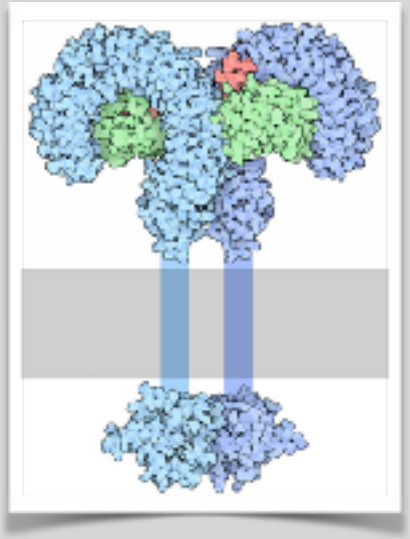
Adjuvant name	Class	Mechanism or receptor	Type of immune response	Clinical phase or licensed product name
dsRNA analogues (for example, poly(I:C))	IM	TLR3	Ab, T_H1, $CD8^+$ T cells	Phase 1
Lipid A analogues (for example, MPL, RC529, GLA, E6020)	IM	TLR4	Ab, T_H1	Cervarix, Supervax, Pollinex Quattro, Melacine
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

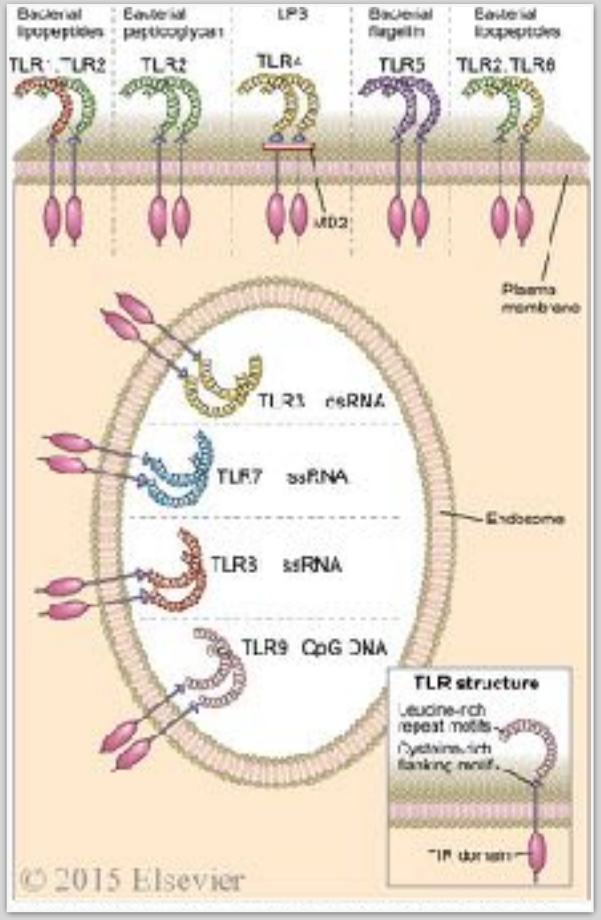
Ab, antibody; Ag, antigen; ASC, apoptosis-associated speck-like protein containing caspase recruitment domain; C, combination of immunomodulatory molecule and particulate formulation; dsRNA, double-stranded RNA; IM, immunomodulatory molecule; ITAM, immunoreceptor tyrosine-based activation motif; PF,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

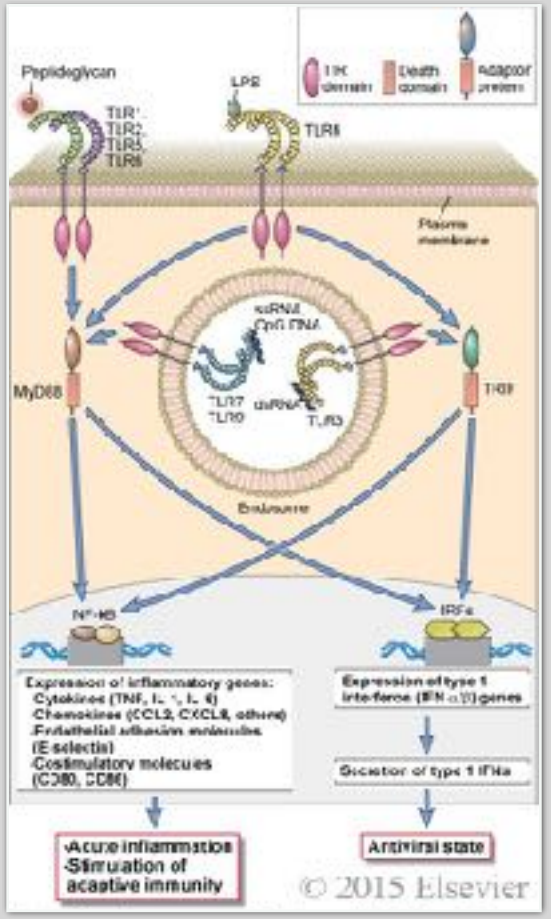
Vaccine adjuvant: putative mechanism of action



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
Versatile Adjuvant

Flagellin

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

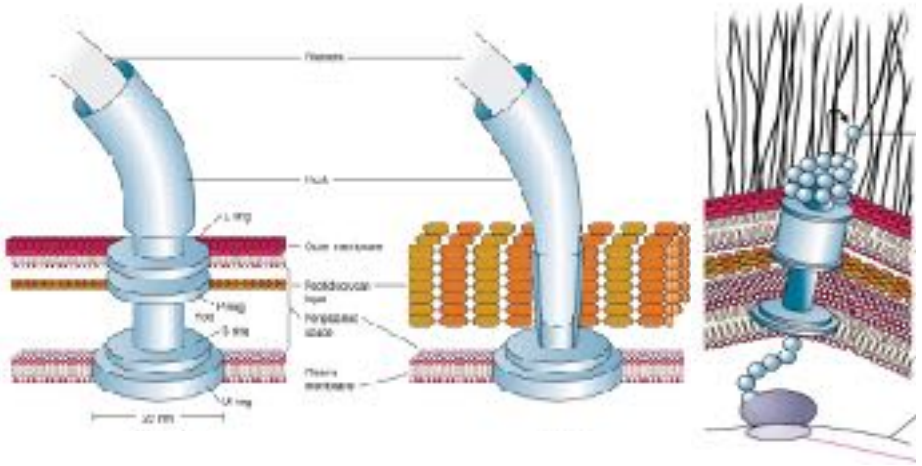
Fumitaka Hayashi*, Kelly D. Smith*‡, Adrian Ozinsky†, Thomas R. Hawn†§, Eugene C. Yi*, David R. Goodlett†, Jimmy K. Eng†, Shizuo Akira||, David M. Underhill† & Alan Aderem†

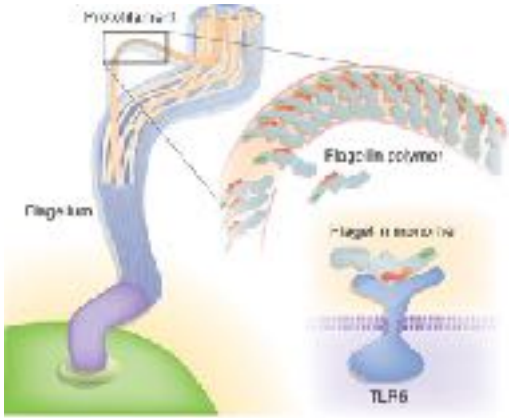
Nature. 2001 Apr 26;410(6832):1099-103.

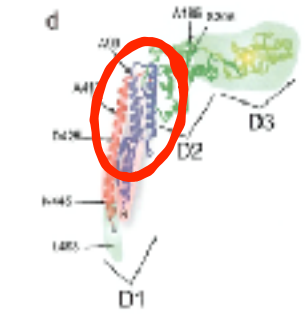
Structure of the bacterial flagellar protofilament and implications for a switch for supercoiling

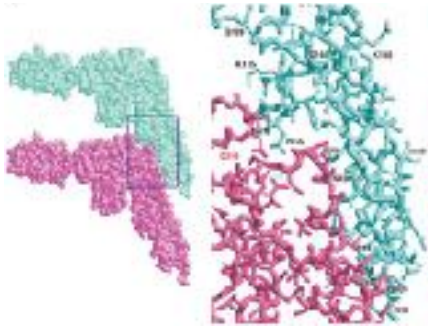
Fadel A. Samatey*, Kaoru Imada*, Shigehiro Nagashima*, Farone Vonderviszt*, Takashi Kumasaka†, Masaki Yamamoto† & Keiichi Namba*§

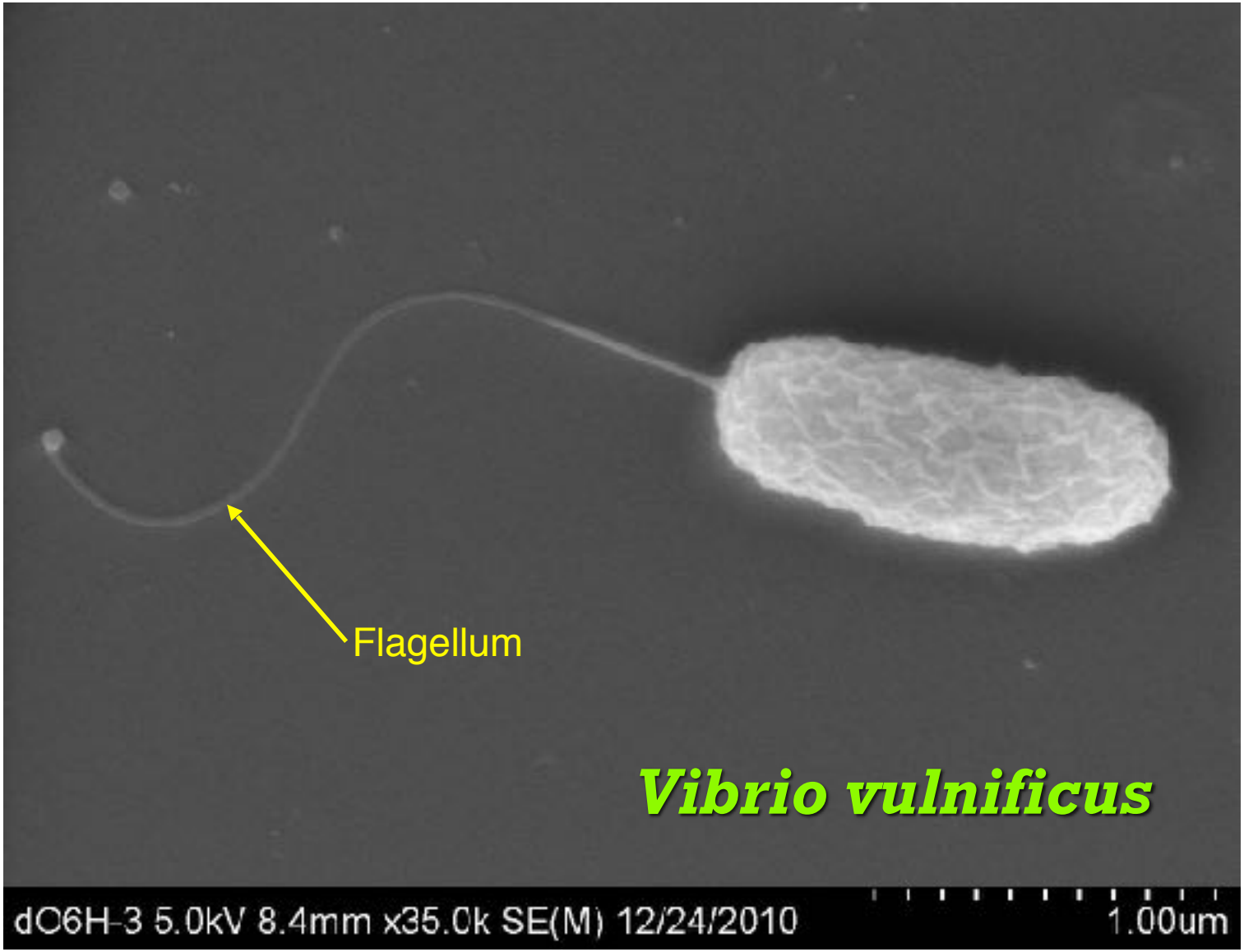
Nature. 2001 Mar 15:410(6826) 331-7.



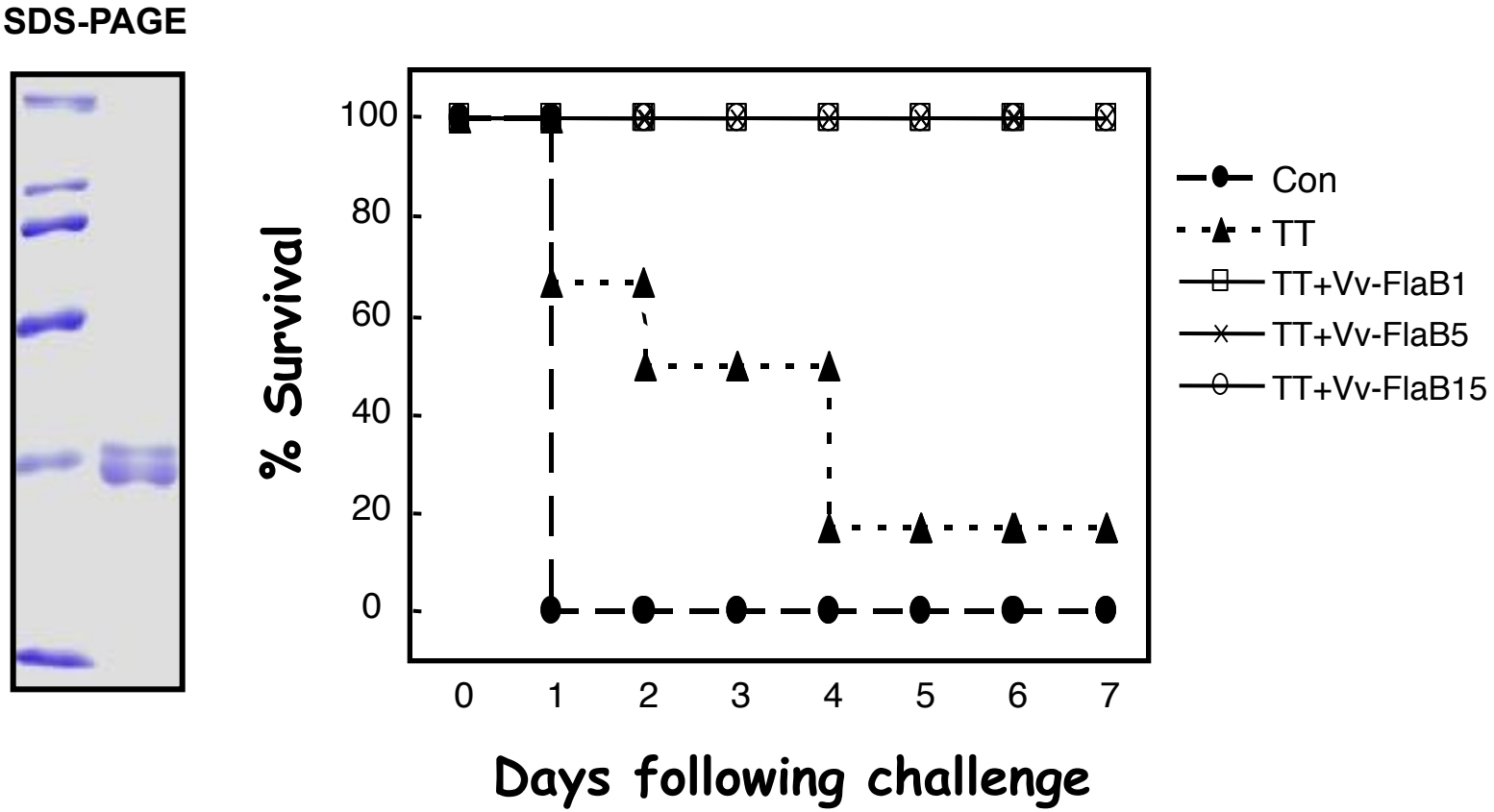







Flagellum
Vibrio vulnificus
dC6H-3 5.0kV 8.4mm x35.0k SE(M) 12/24/2010
1.00um

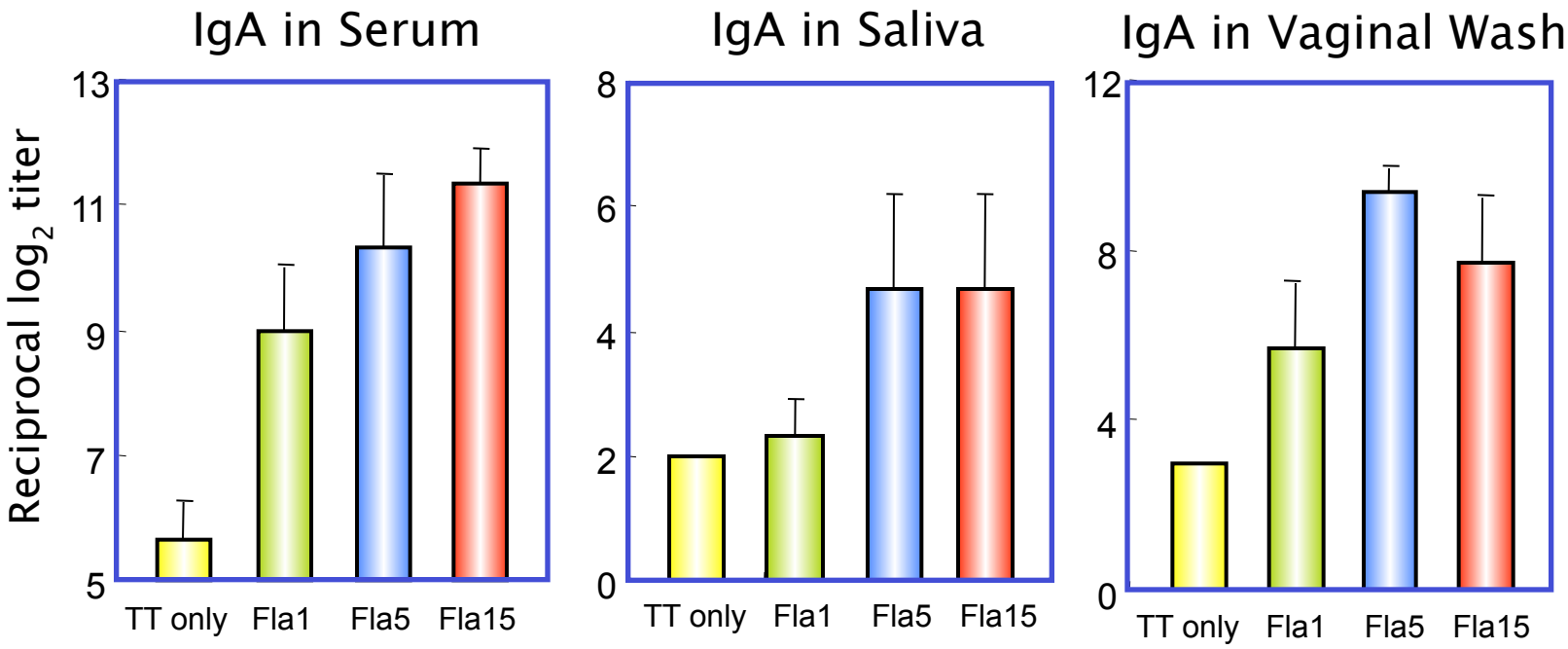
Nasal Vv-FlaB potentiates protective immune response



Nasal immunization with tetanus toxoid -> Tetanus toxin challenge

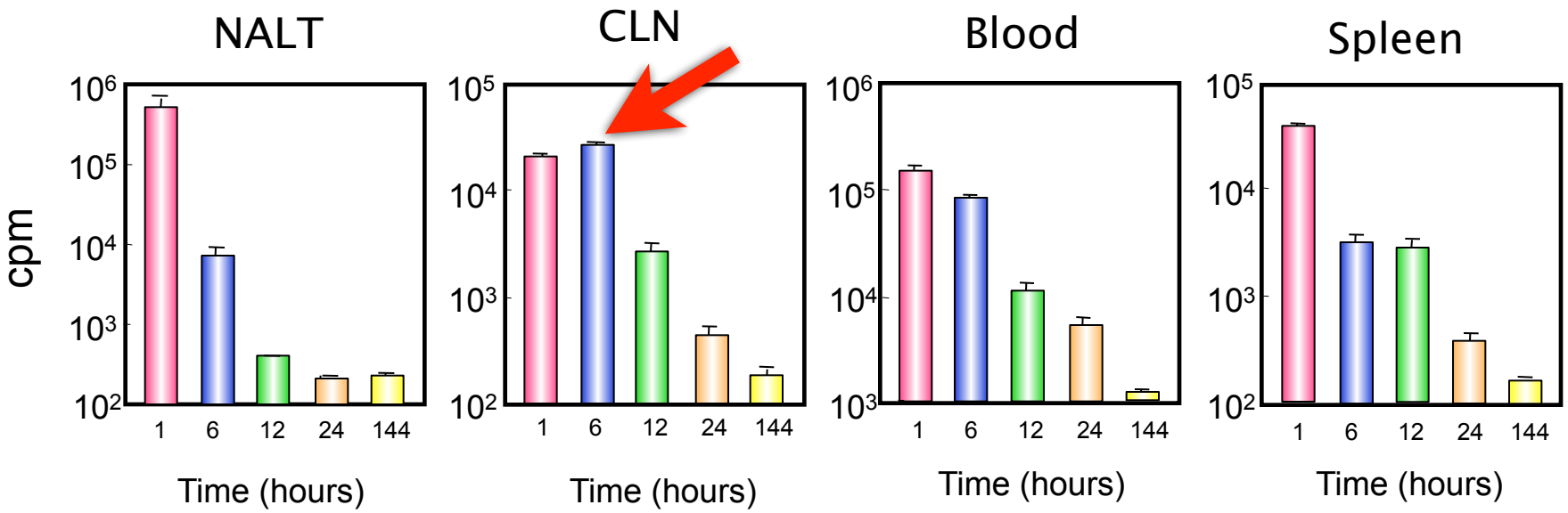
IAI 74:694-702, 2006

Antigen specific antibody production



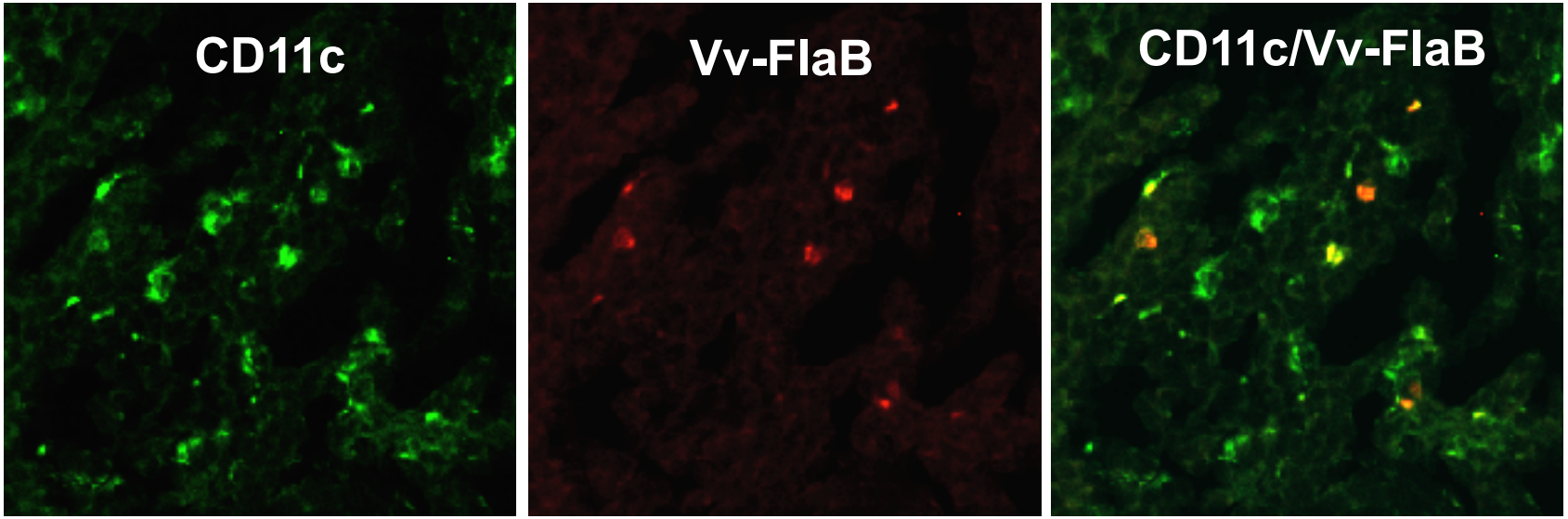
Intranasally administered Vv-FlaB enhanced antigen-specific systemic & mucosal IgA

Trafficking of ^{131}I-Vv-FlaB



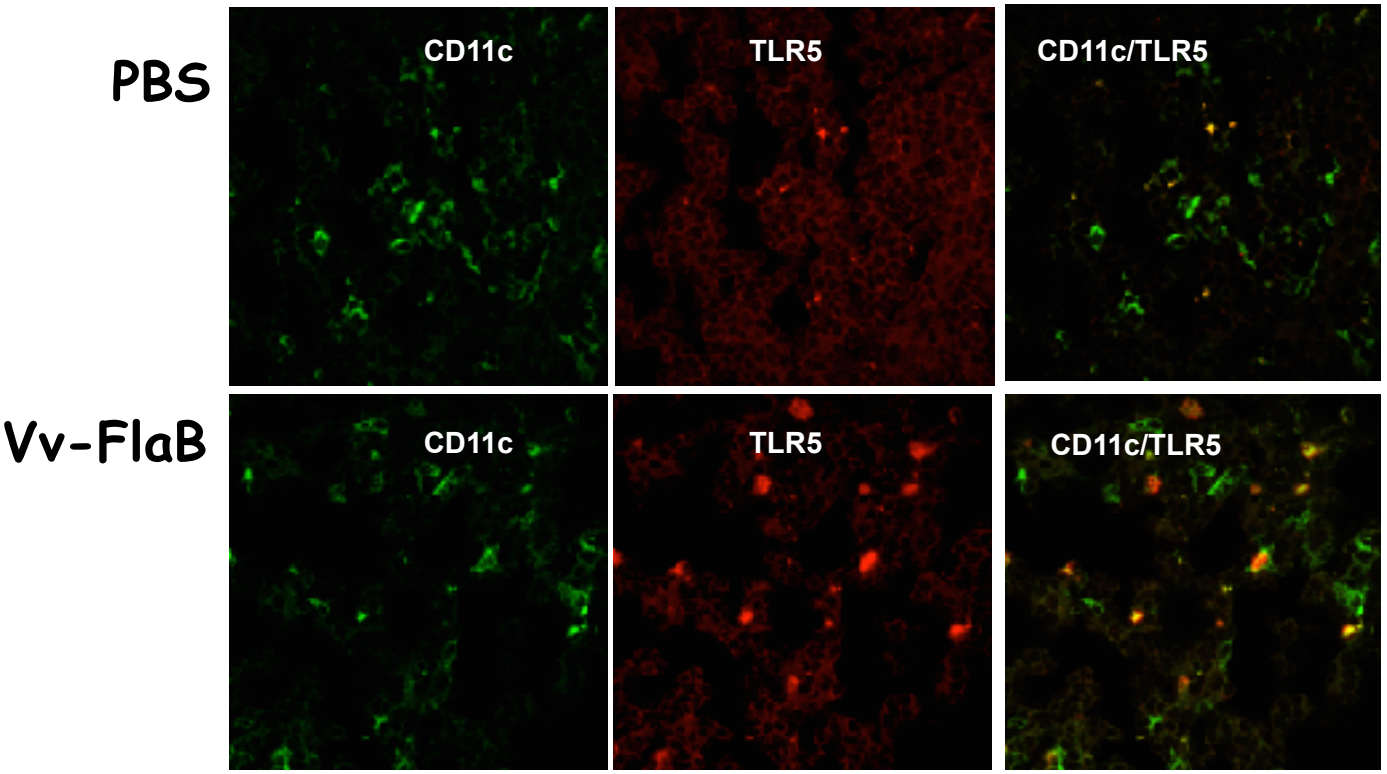
Intranasally administered ^{131}I-Vv-FlaB readily reached systemic circulation while the regional draining cervical lymph nodes retained the adjuvant protein relatively longer than spleen.

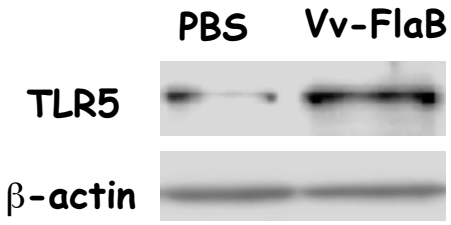
In vivo colocalization of Vv-FlaB with DCs in cervical lymph node

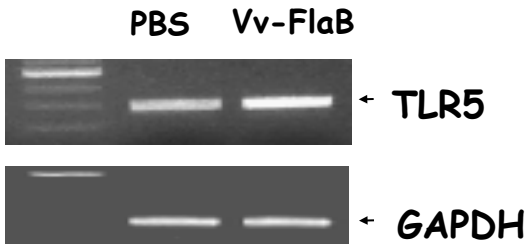


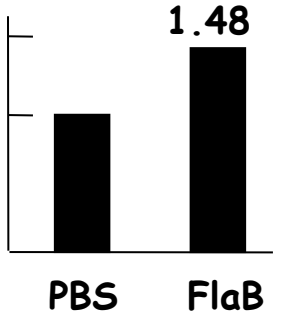
Intranasally administered Vv-FlaB colocalized with CD11c in the draining cervical lymph nodes.

Vv-FlaB treatment stimulates TLR5 expression in vivo









Flagellin as an effective adjuvant for elderly vaccines.

Biochemical evidence

Jae Sung Lim, Shee Eun Lee, and Kyoung A Cho

How to overcome ineffectiveness of elderly vaccines?

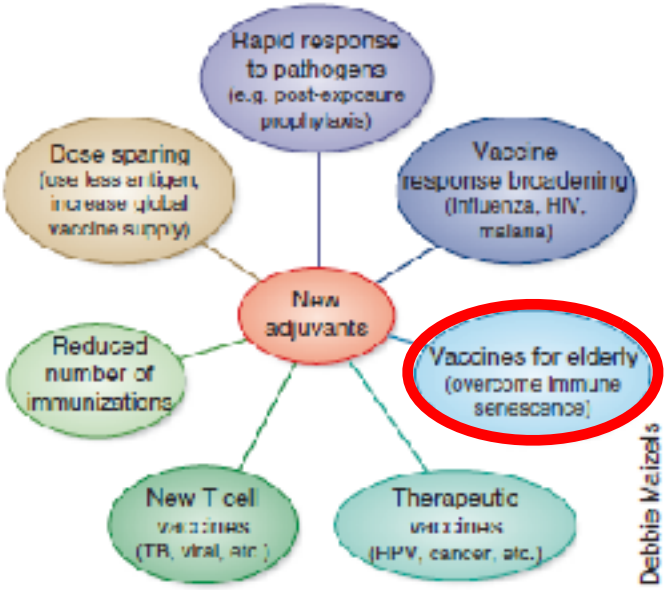
Many countries vaccinate elderly by NIP, but…

- **Immunosenescence – both innate and adaptive immunity**
- **Increase dosage? – Side effects inevitable…**
- **Need for effective adjuvant – Then what adjuvant?**

Seniors *(over age 65)*

You may need one or more vaccines, even if you received vaccines as a child or as a younger adult. Ask your doctor which ones are right for you. Vaccines recommended for older adults can prevent:

- Influenza (Flu)
- Shingles (Herpes Zoster)
- Diphtheria/Tetanus
- Pertussis (Whooping Cough)
- Pneumococcal (Pneumonia)

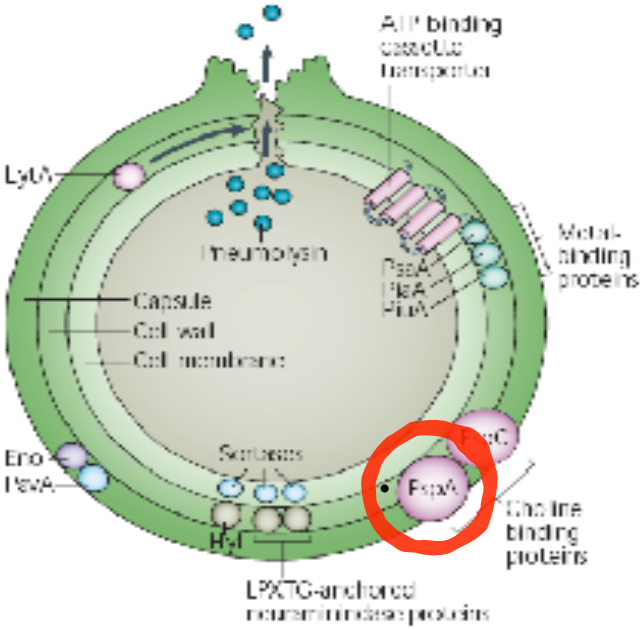


Nat Med 2013

PspA

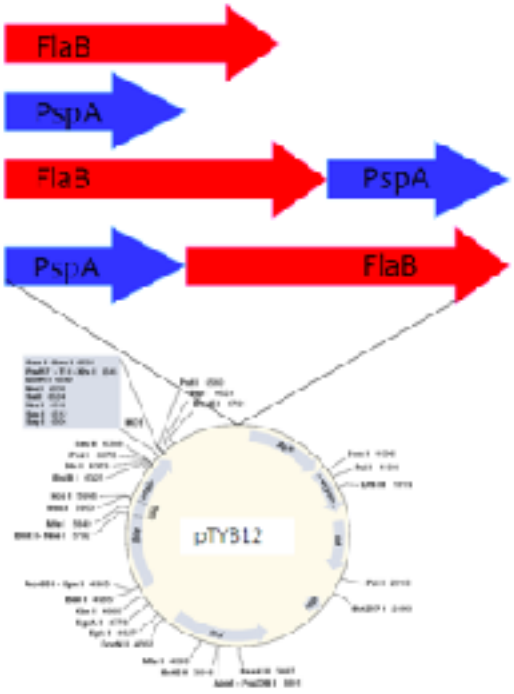
(pneumococcal surface protein A)

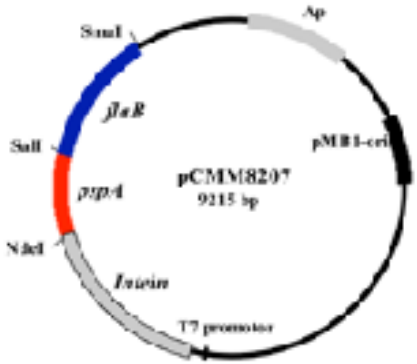
- Present on all strains of *S. pneumoniae*
  - expressed during invasion
  - expressed during colonization
- Virulence factor for
  - invasion
  - colonization
- Elicits protection against
  - bacteremia, sepsis, and pneumonia
  - colonization
- Inhibits
  - C3 activation
  - killing of pneumococci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

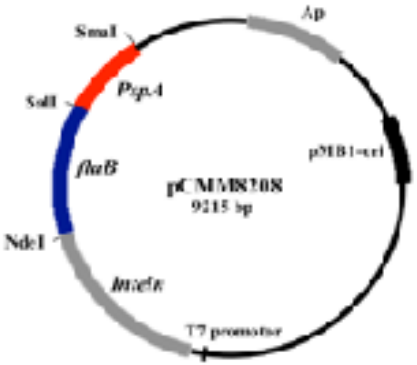


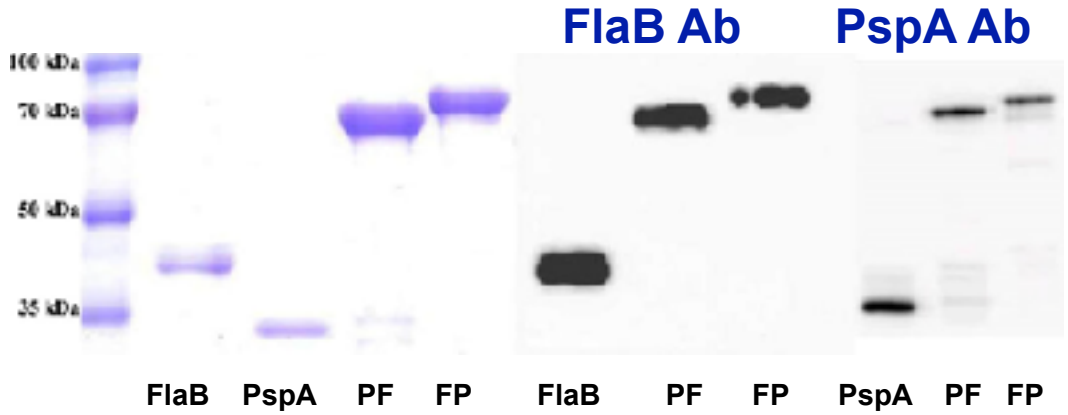
- **Aras Kadioglu, *et al.*** Nat Rev Micro 6(4): 288-301

Recombinant fusion proteins



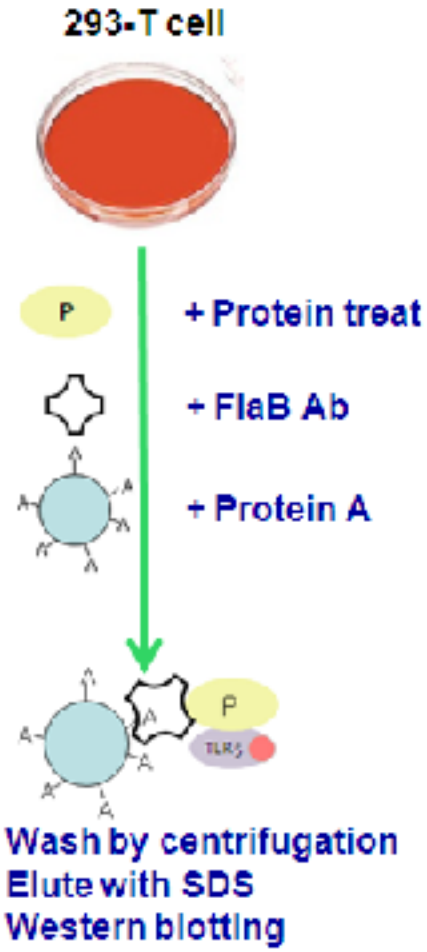


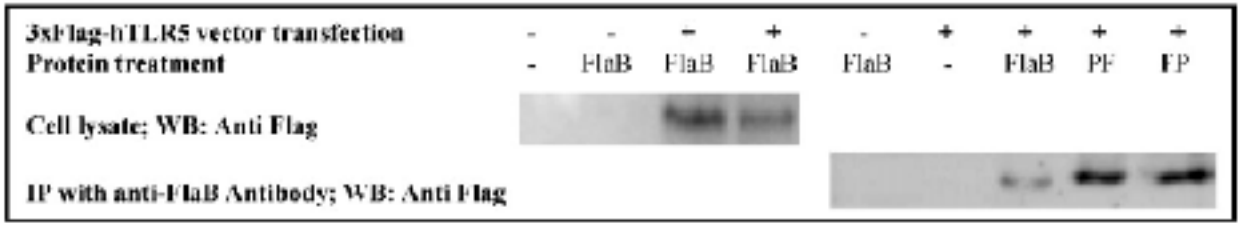




Vaccine 29:5731-9, 2011

Direct association of recombinant fusion protein with TLR5

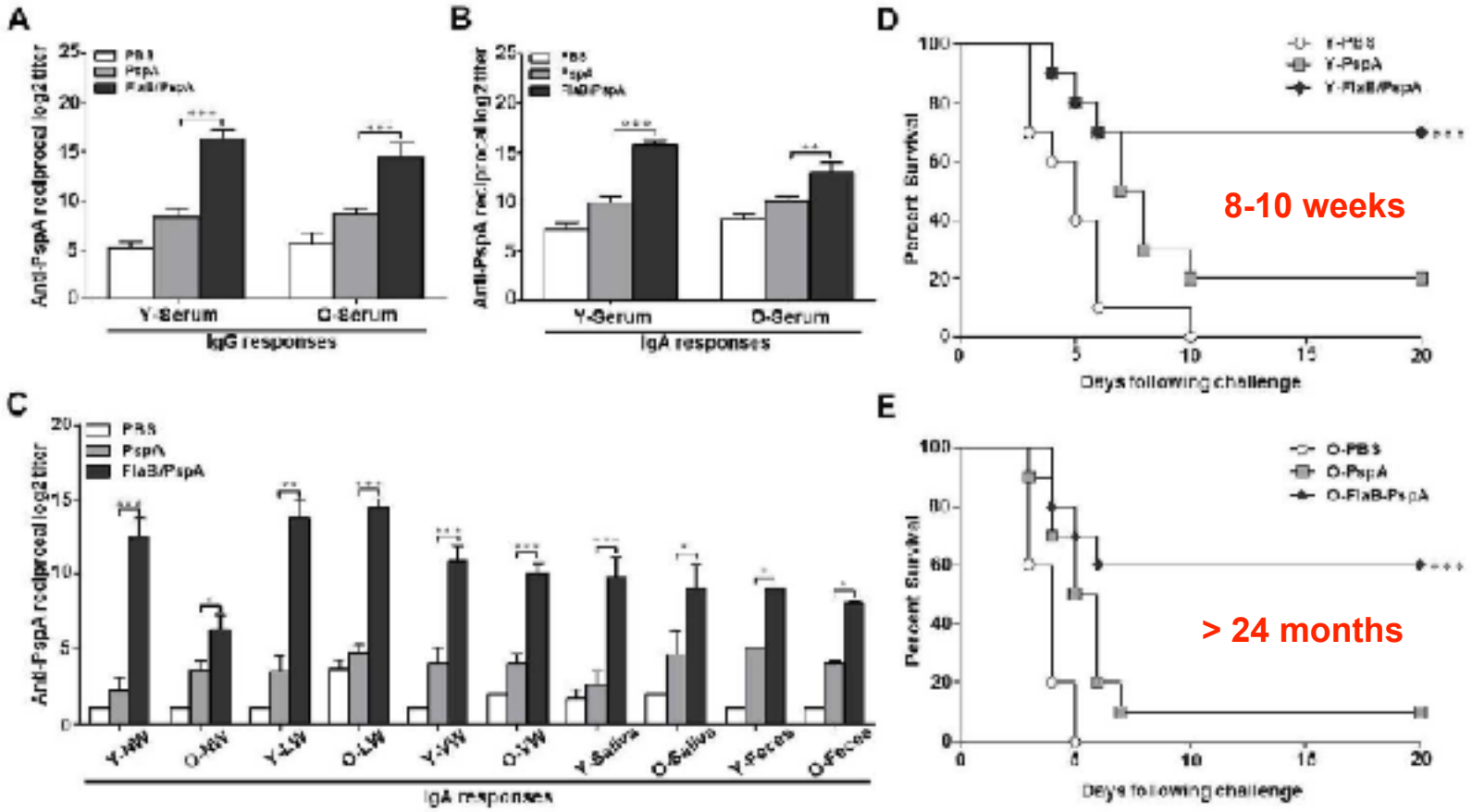




Recombinant fusion proteins directly interacted with TLR5 expressed in epithelial cells

Vaccine 29:5731-9, 2011

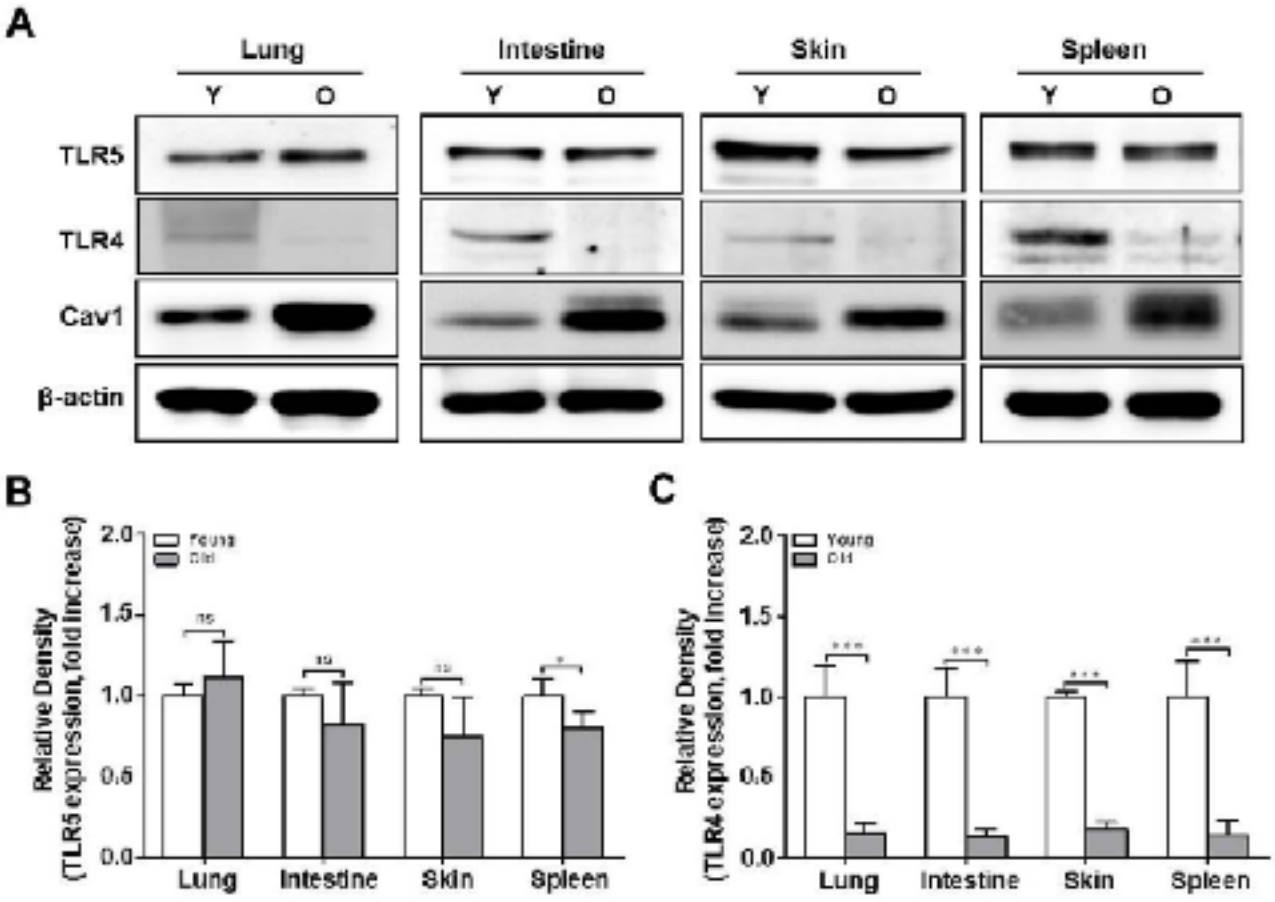
Flagellin-dependent TLR5/caveolin-1 as a promising immune activator in immunosenescence



FlaB-PspA is a promising candidate as the pneumococcal vaccine for elderly

Aging Cell 14:907-915, 2015

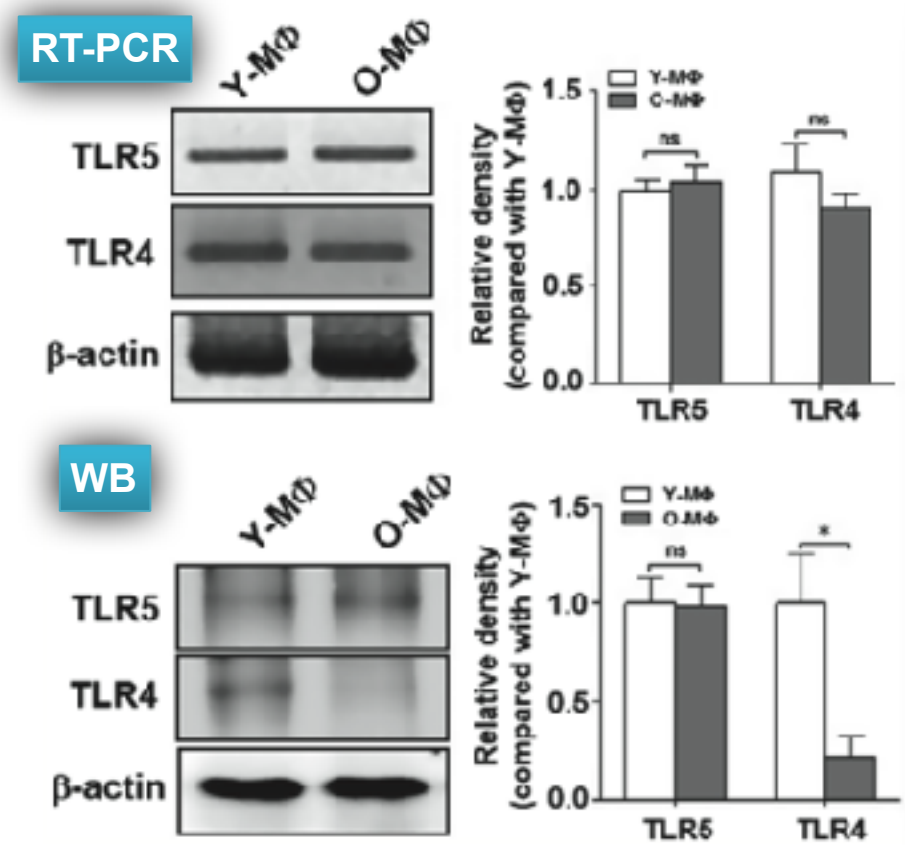
Flagellin-dependent TLR5/caveolin-1 as a promising immune activator in immunosenescence

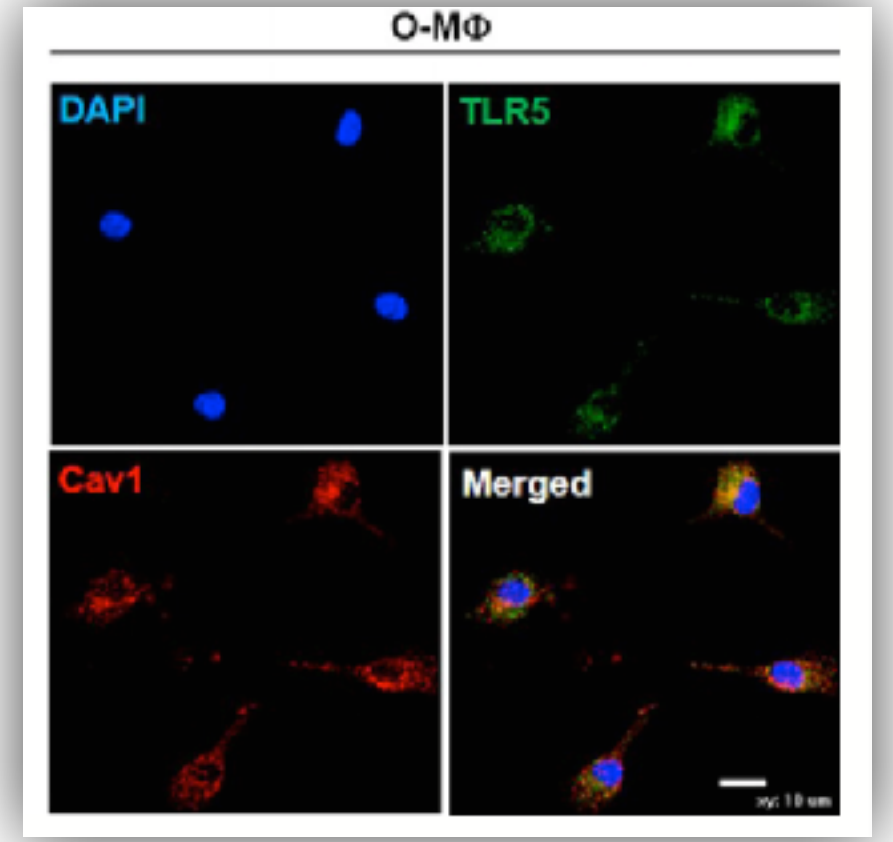


Expression levels of TLRs in various tissues from young and old mice

Aging Cell 14:907-915, 2015

Flagellin-dependent TLR5/caveolin-1 as a promising immune activator in immunosenescence

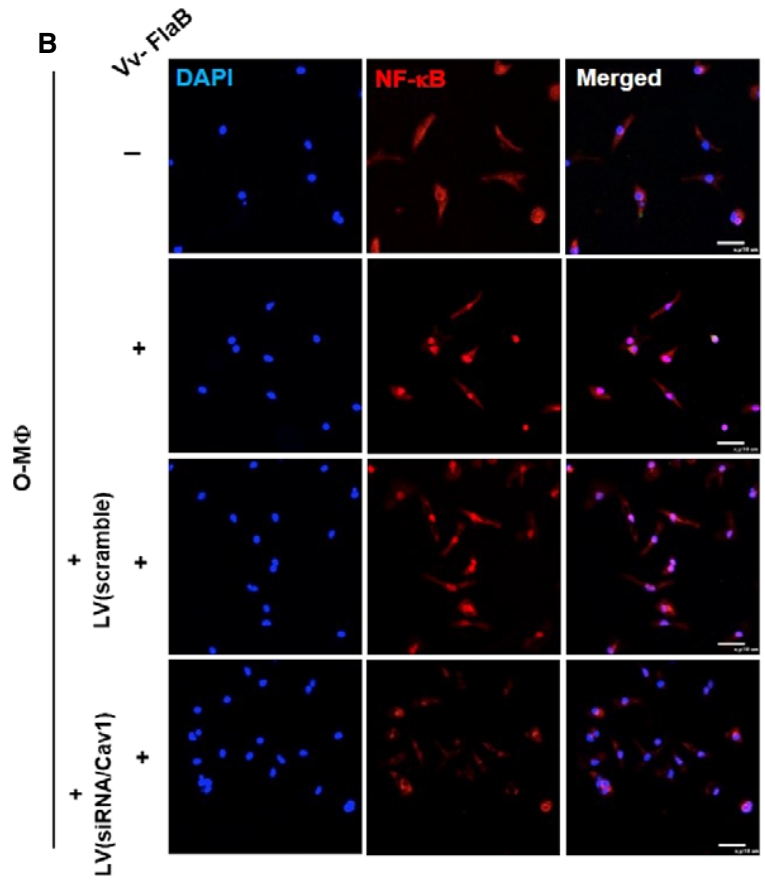


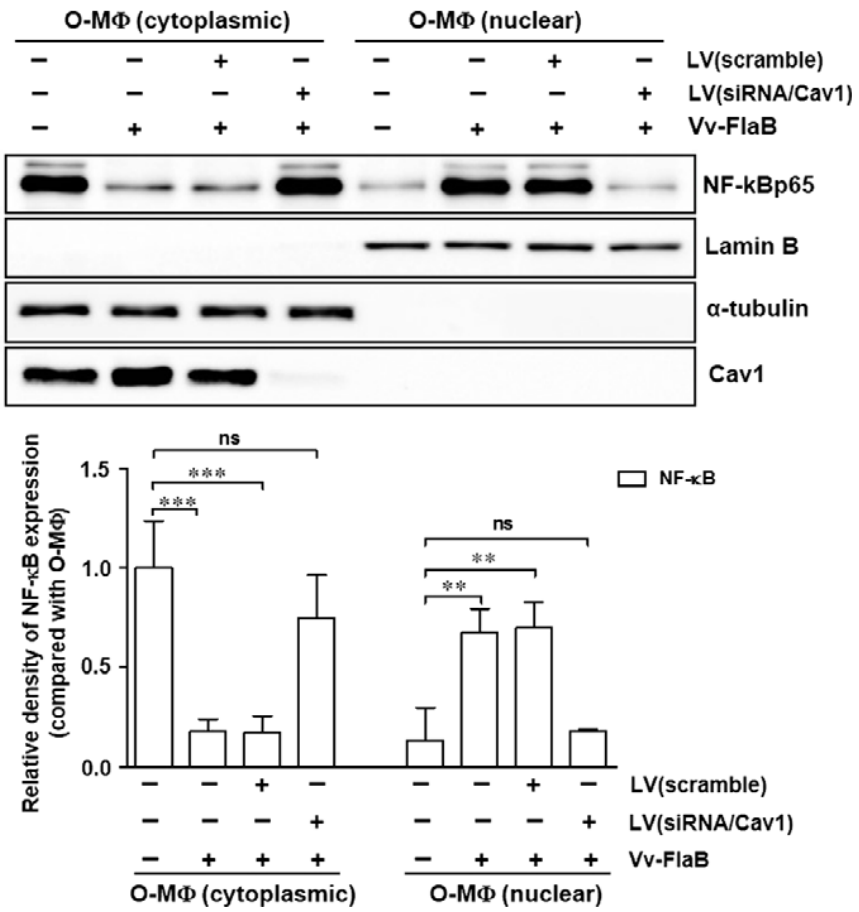


Expression of TLR5 was maintained in old Mϕs in relation to Cav1

Aging Cell 14:907-915, 2015

Flagellin-dependent TLR5/caveolin-1 as a promising immune activator in immunosenescence

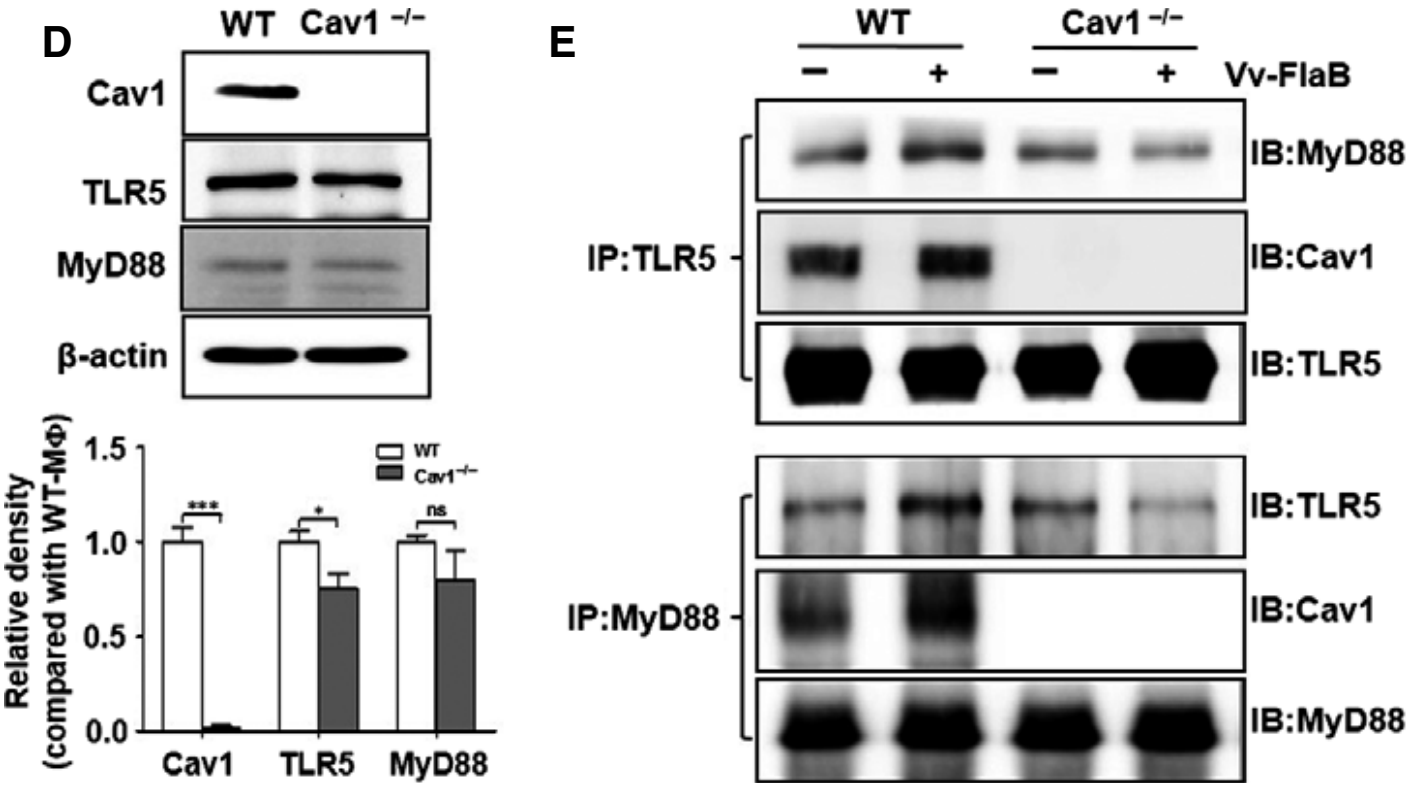




Cav1 KD resulted in abrogation of TLR5-mediated NF-kB activation

Aging Cell 14:907-915, 2015

Flagellin-dependent TLR5/caveolin-1 as a promising immune activator in immunosenescence



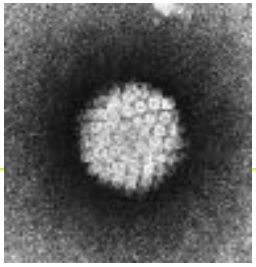
TLR5/MyD88/Cav1 interact physically in the cell membrane

Aging Cell 14:907-915, 2015

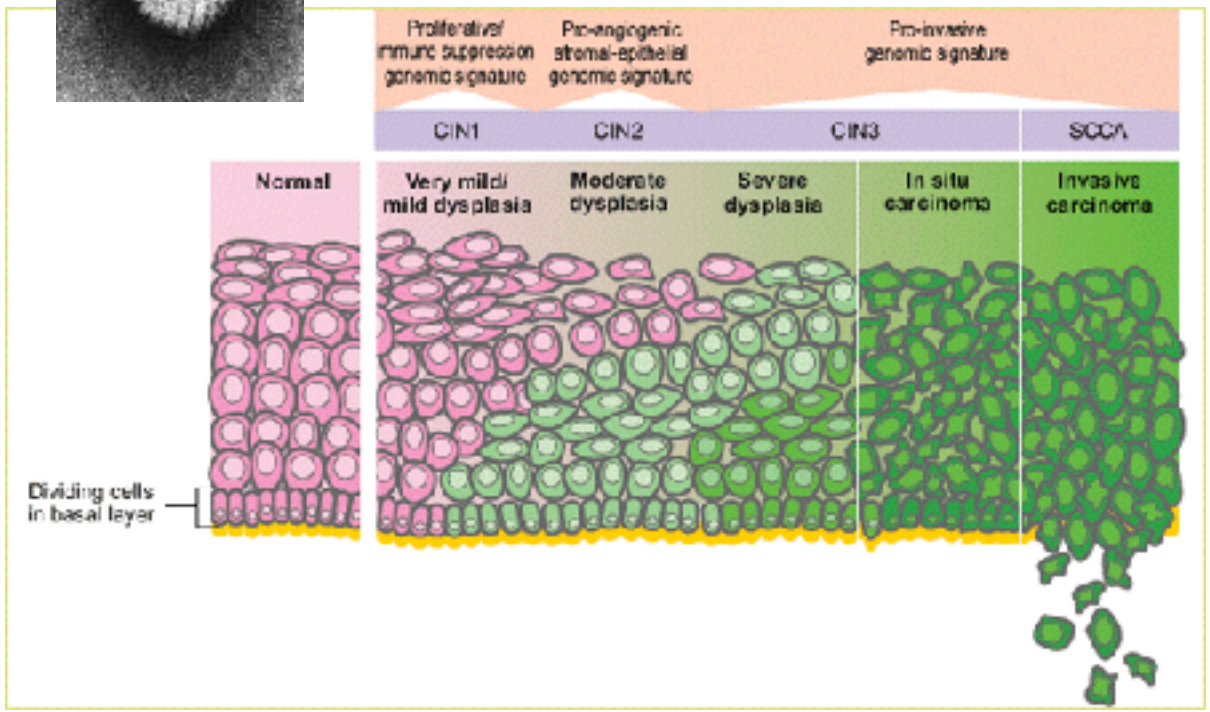
Then, can flagellin be used for a topical immunotherapy of mucosal cancers?

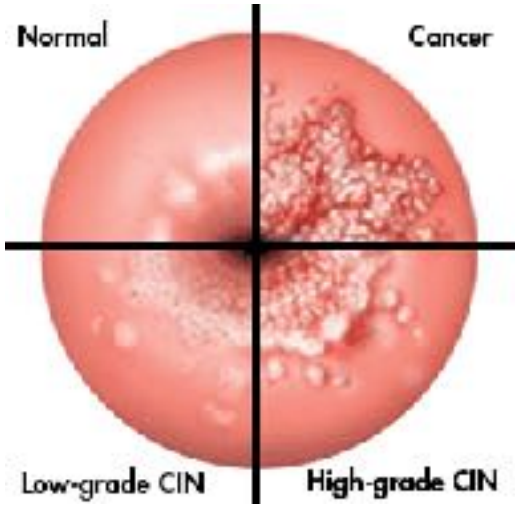
Mucosa is a frequent site of carcinogenesis, and most cancers are the illness of aged…

Shee Eun Lee, Sul Hee Hong, Vivek Verma



HPV, CIN and cervical cancer

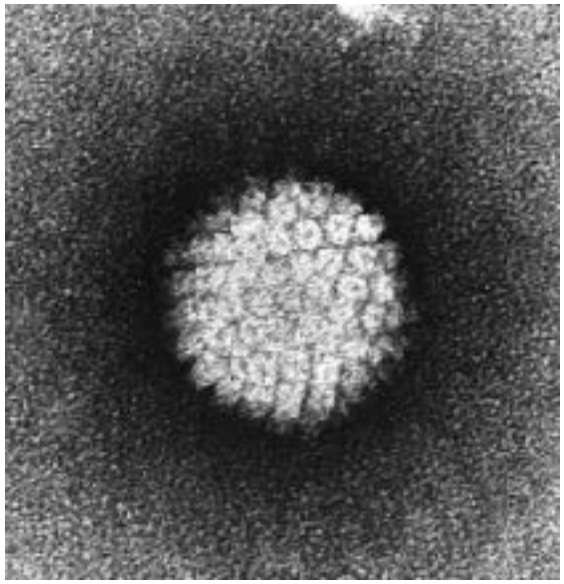


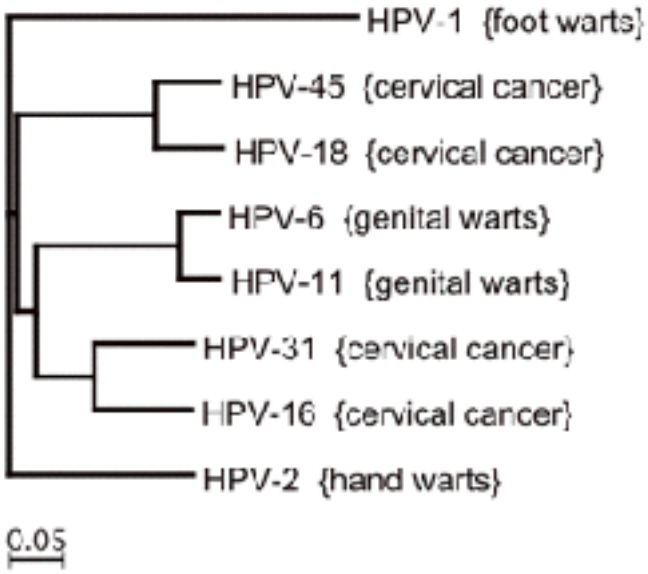


- HPV associated cervical cancer **develop in mucosal organ**
- The type 16 oncogenic proteins **E6 and E7** are **consistently expressed in most cervical cancer**
- E6 and E7 peptides: **promising target** for development of anticancer vaccine in cervical cancer.

HPV

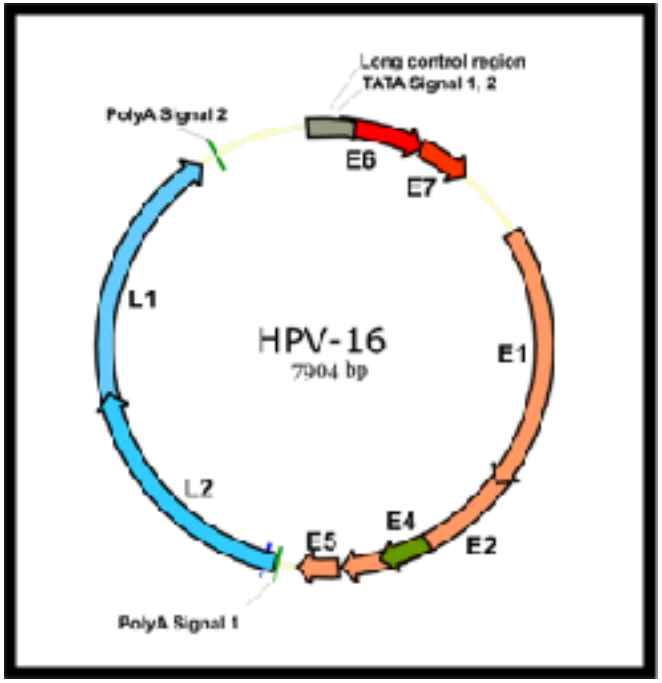
Disease	HPV type
Common warts	*2, 7*
Plantar warts	*1, 2, 4, 63*
Flat warts	*3, 10, 8*
Anogenital warts	*6, 11, 42, 44 and others*
Anal lesions	*6, 16, 18, 31, 53, 58*
Genital cancers	*•Highest risk: 16, 18, 31, 45* *•Other high-risk: 33, 35, 39, 51, 52, 56, 58, 59* *•Probably high-risk:26, 53, 66, 68, 73, 82*
Epidermodysplasia verruciformis	*more than 15 types*
Focal epithelial hyperplasia (oral)	*13, 32*
Oral papillomas	*6, 7, 11, 16, 32*
Oropharyngeal cancer	*16*
Verucous cyst	*60*
Laryngeal papillomatosis	*6,11*





- Over 120 HPV types
- Types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 are carcinogenic
- Cervical Intraepithelial Neoplasia (CIN), Vulvar Intraepithelial Neoplasia (VIN), Penile Intraepithelial Neoplasia (PIN), and/or Anal Intraepithelial Neoplasia (AIN).

Genome organization of human papillomavirus type 16



- HPV type 16: one of the subtypes known to cause cervical cancer
- Early genes: E1-E7
- Late genes: L1-L2 (capsid)
- E6/E7 proteins inactivate tumor suppressor proteins
  E6 - p53 / E7 - pRb

HPV Vaccines: prophylactic

- Merk/Sanofi-Aventis
  - HPV Types 6, 11, 16, and 18
  - Alum adjuvant
  - Recombinant VLPs: assembled from the L1 proteins (capsid) of HPV types 6, 11, 16 and 18.
  - *Saccharomyces cerevisiae* system
  - No DNA: cannot induce cancer.

- GSK
  - HPV Types 16, and 18
  - AS04 (MPL + alum) adjuvant
  - Recombinant VLPs: assembled from the L1 proteins (capsid) of HPV types 16 and 18.
  - Baculovirus system
  - No DNA: cannot induce cancer

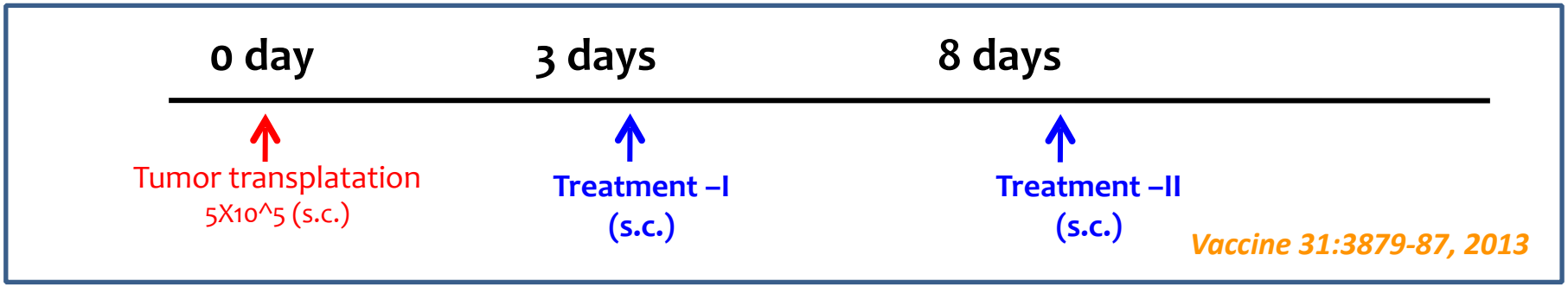
Flagellin & Cancer Immunotherapy: MODEL SYSTEM

- Cancer type
  - Cervical cancer model
    - Well established model
    - TA: E6/E7

- Cancer immunotherapy (Cancer Vaccine)
  - DNA vaccine
  - Protein vaccine
  - Peptide vaccine
  - Cell-based vaccine: ex> DC
  - Other new-generation vaccines

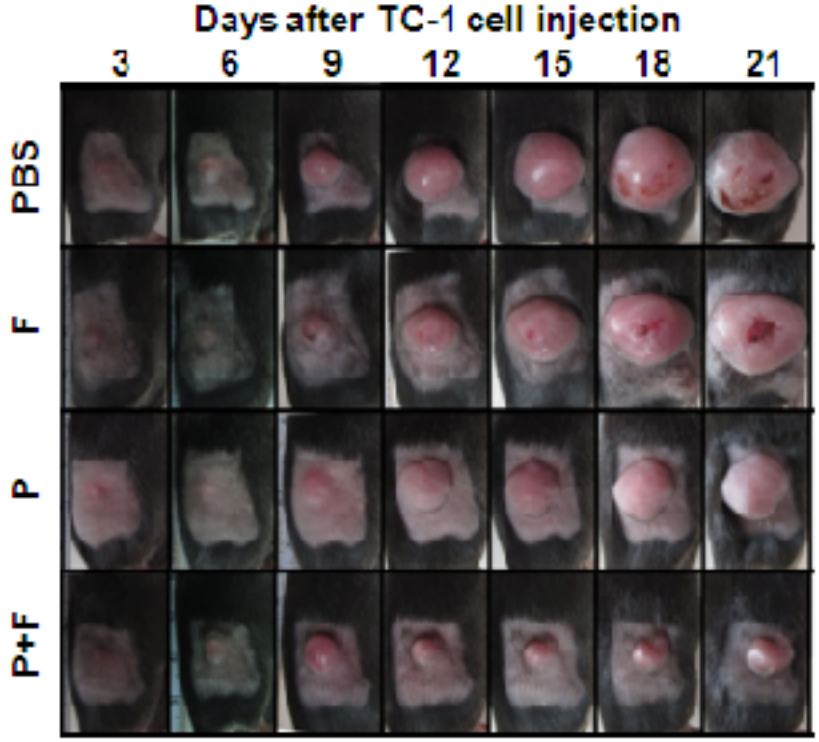
Therapeutic cancer vaccine

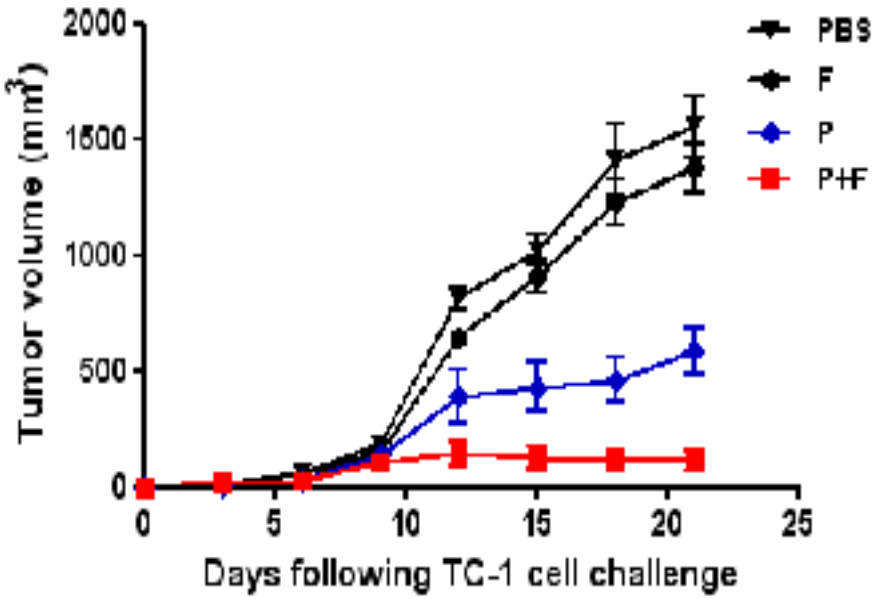
- **Animal model: peptide vaccine model**
  - C57BL/6 female
  - TC-1 cell line
  - Tumor transplantation: $5x10^5$ cells/200μl/mouse (s.c.)
  - **E6: YDFAFRDL (8mer)**
  - **E7: RAHYNIVTF (9mer)**
  - Treatment: peritumorial s.c.
    - **PBS**: PBS only
    - **F**: FlaB 4 μg/mouse
    - **P**: E6/E7 peptide 100μg each/mouse
    - **P F**: E6/E7 peptide 100μg each + FlaB 4 μg/mouse



Therapeutic Cancer Vaccine

-TC-1 cell model: tumor growth-



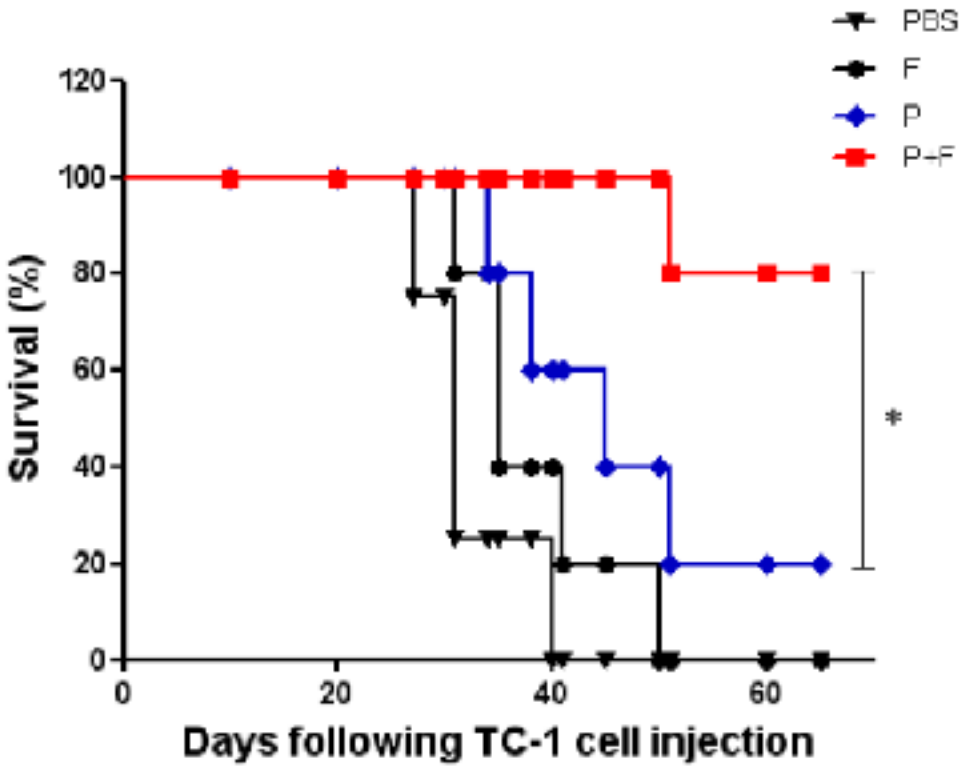


E6/E7 + Flagellin => Ag-specific immune response ↑

=> tumor suppression

Vaccine 31:3879-87, 2013

Therapeutic Cancer Vaccine:
-Survival-



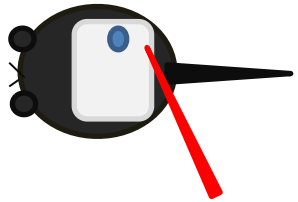
E6/E7 + Flagellin => Ag-specific immune response ↑

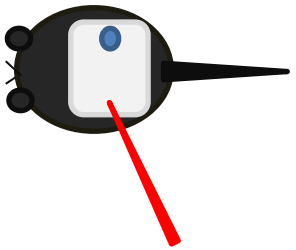
=> tumor suppression => survival ↑

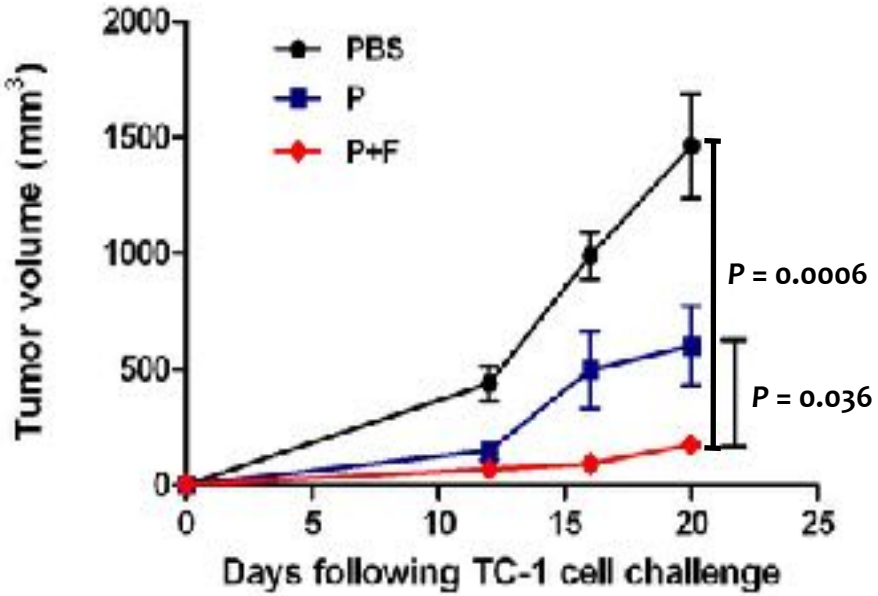
Vaccine 31:3879-87, 2013

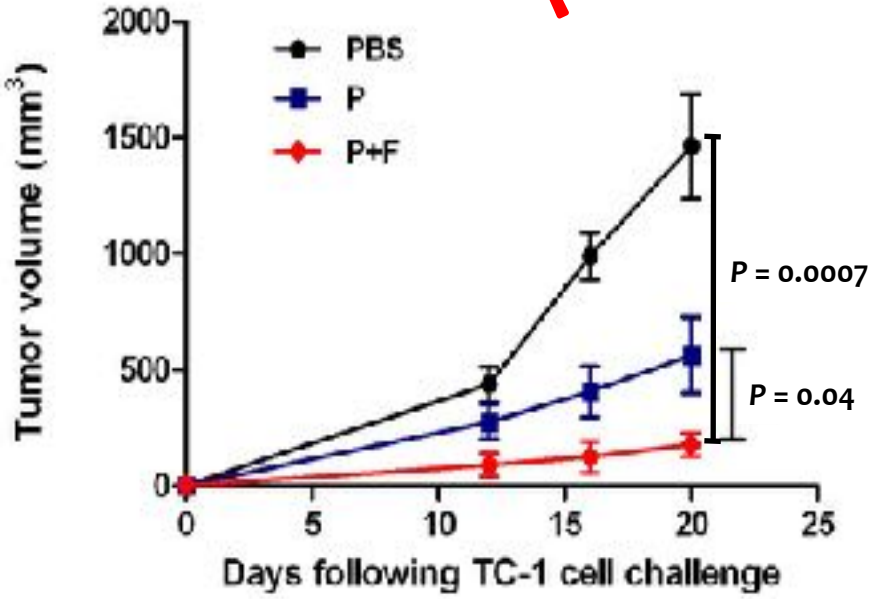
Therapeutic Cancer Vaccine

-Contralateral Vaccination Model-





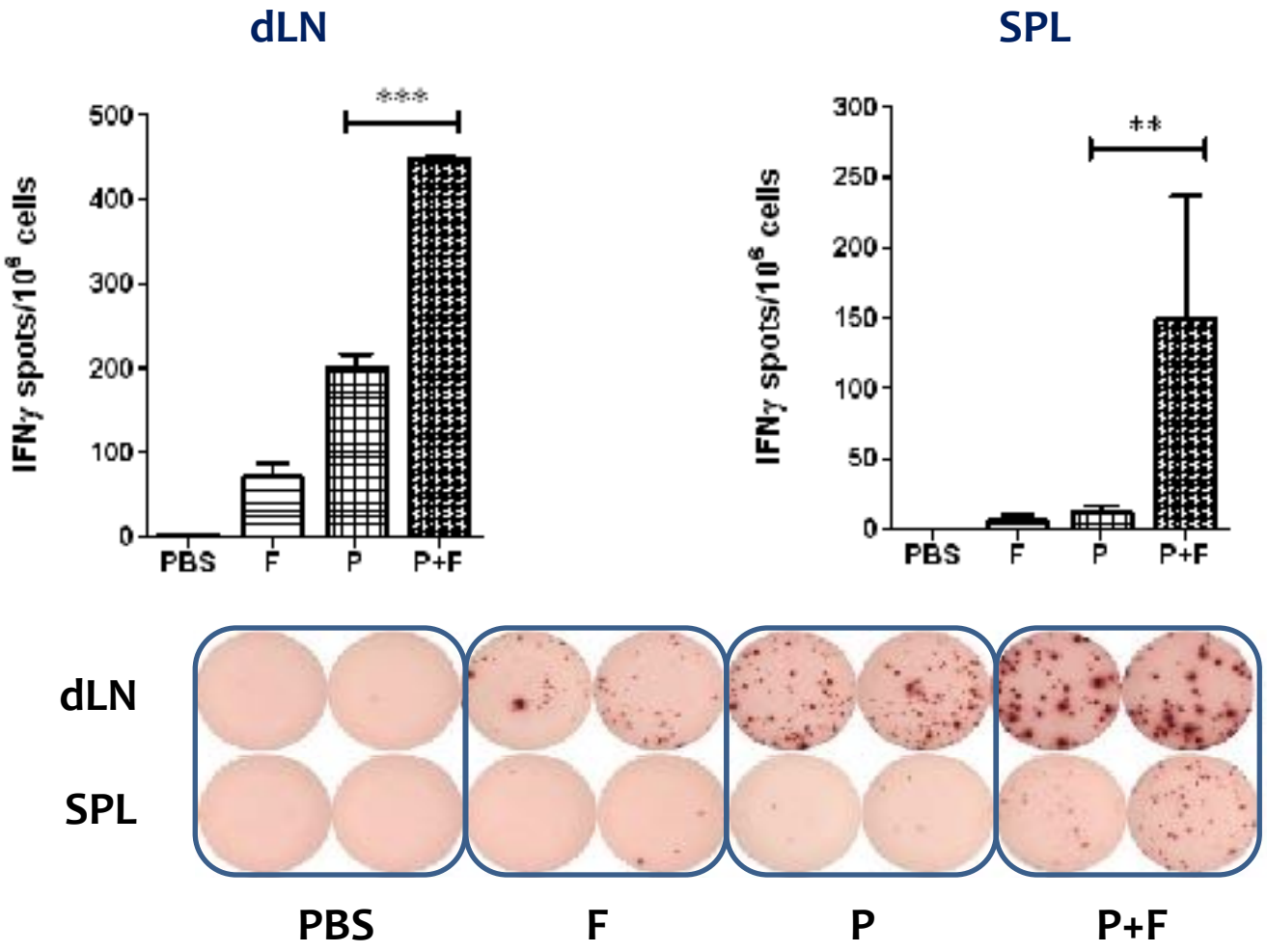




Flagellin enters circulation and acts as systemic immune modulator

Therapeutic Cancer Vaccine

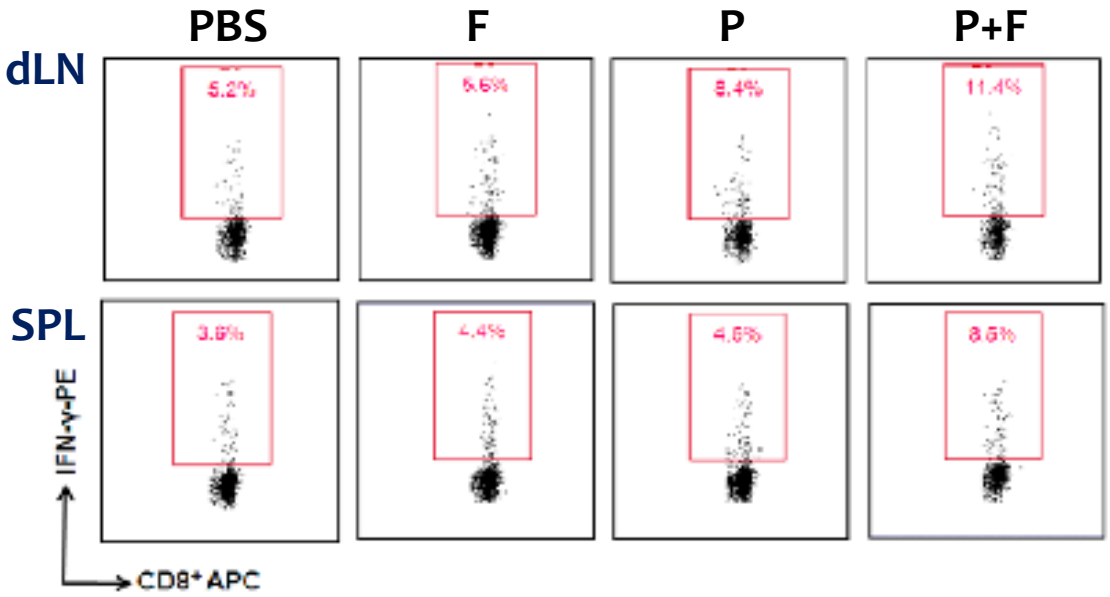
-ELISPOT: E6, E7 peptide re-stimulation-

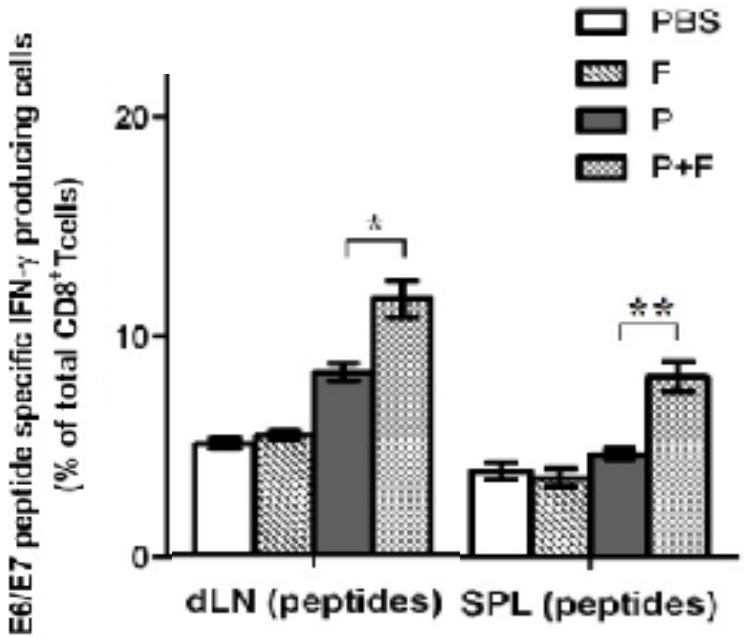


FlaB enhance peptide-specific IFN-γ producing cells

Therapeutic Cancer Vaccine

-ICC Staining and Flow Cytometry-



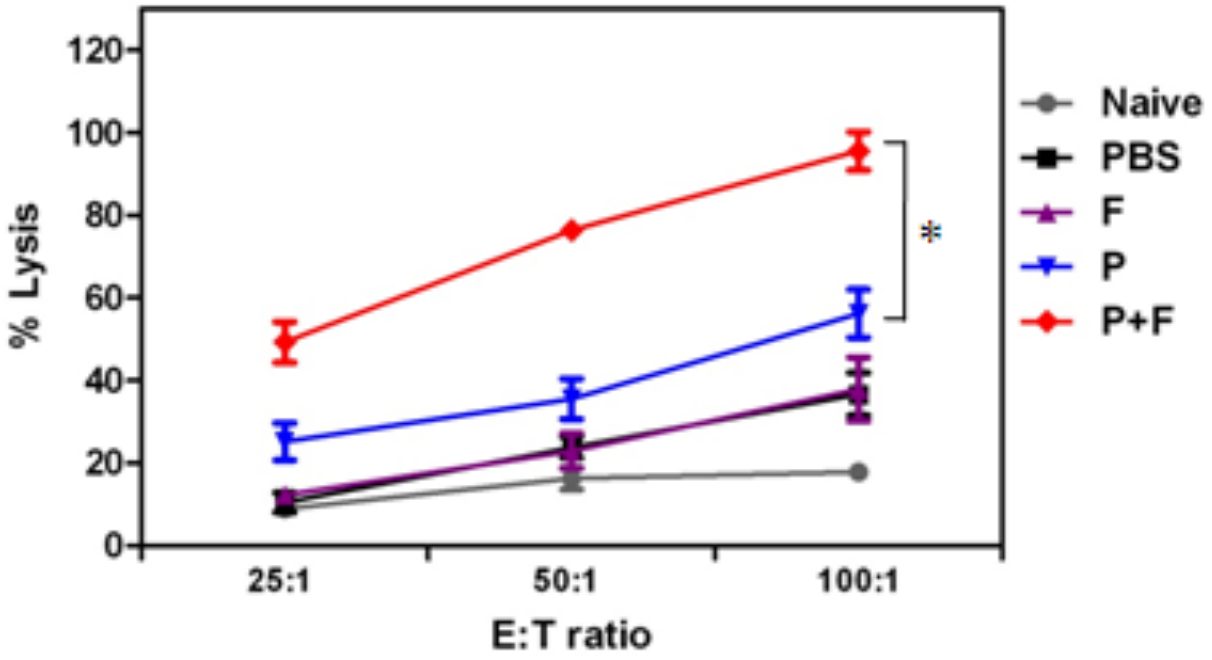


FlaB enhance peptide-specific IFN-γ production in CD8⁺Tcells

Therapeutic Cancer Vaccine

-Peptide-specific CTL activity-

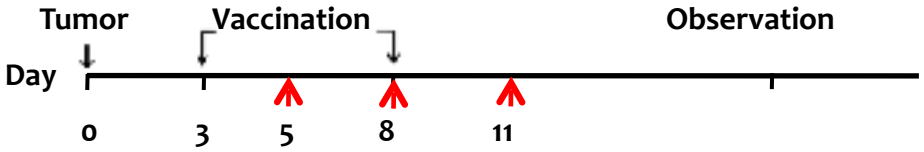
- Effector cell : SPL from vaccinated mice
- Target cell : Cisplatin-treated TC-1
- Cytotoxicity : LDH assay

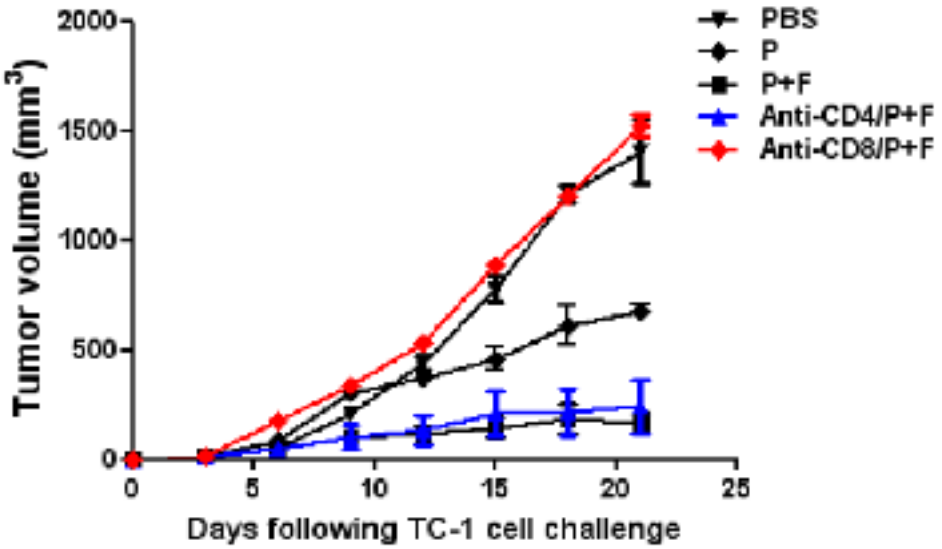


FlaB enhanced TC-1 cell-specific CTL activity

Therapeutic Cancer Vaccine

-CD4+/CD8+ T cell depletion assay-



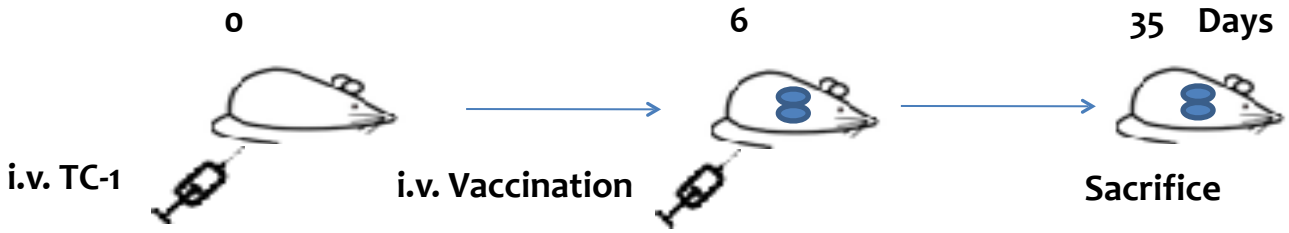


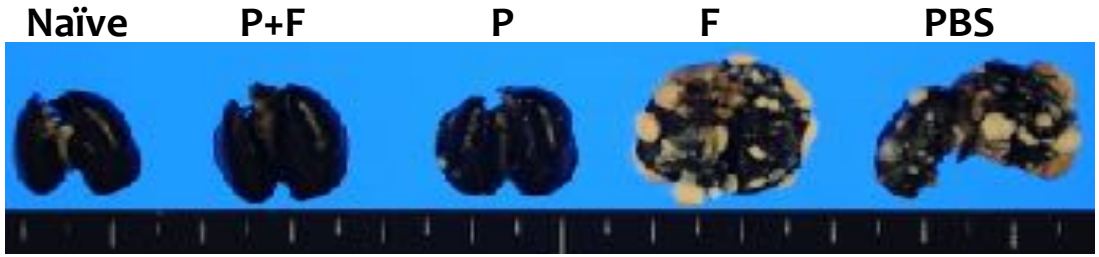
CD8+ CTL response is responsible for the antitumor immunity in this model.

Vaccine 31:3879-87, 2013

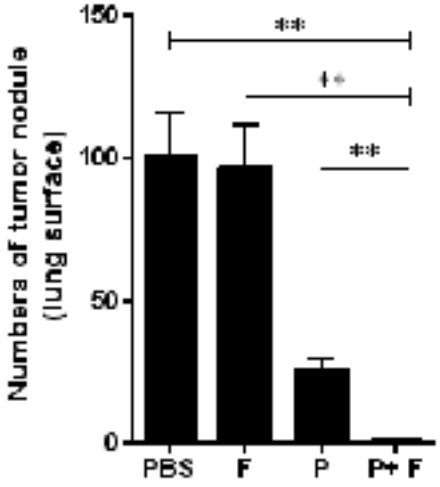
Therapeutic Cancer Vaccine

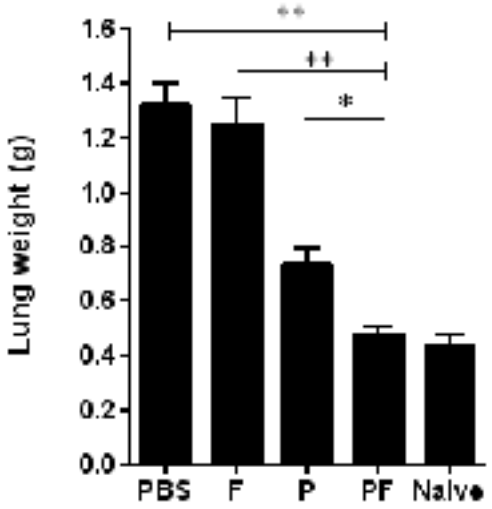
-Metastasis?-





India ink assay





Flagellin could be applicable to various phases of cancer immunotherapy.

Vaccine 31:3879-87, 2013

FlaB: Adjuvant for peptide-based therapeutic cancer vaccine

- FlaB potentiates E6/E7 peptide-mediated anti-tumor efficacy.
- FlaB enhances E6/E7-specific CTL responses and IFNγ production.
- In vivo adjuvant activity of FlaB is mediated by $CD8^+$ cells.
- The adjuvant activity of FlaB was abolished in TLR5 KO mice.
- FlaB significantly suppresses tumor development in the TC-1 lung metastasis model.

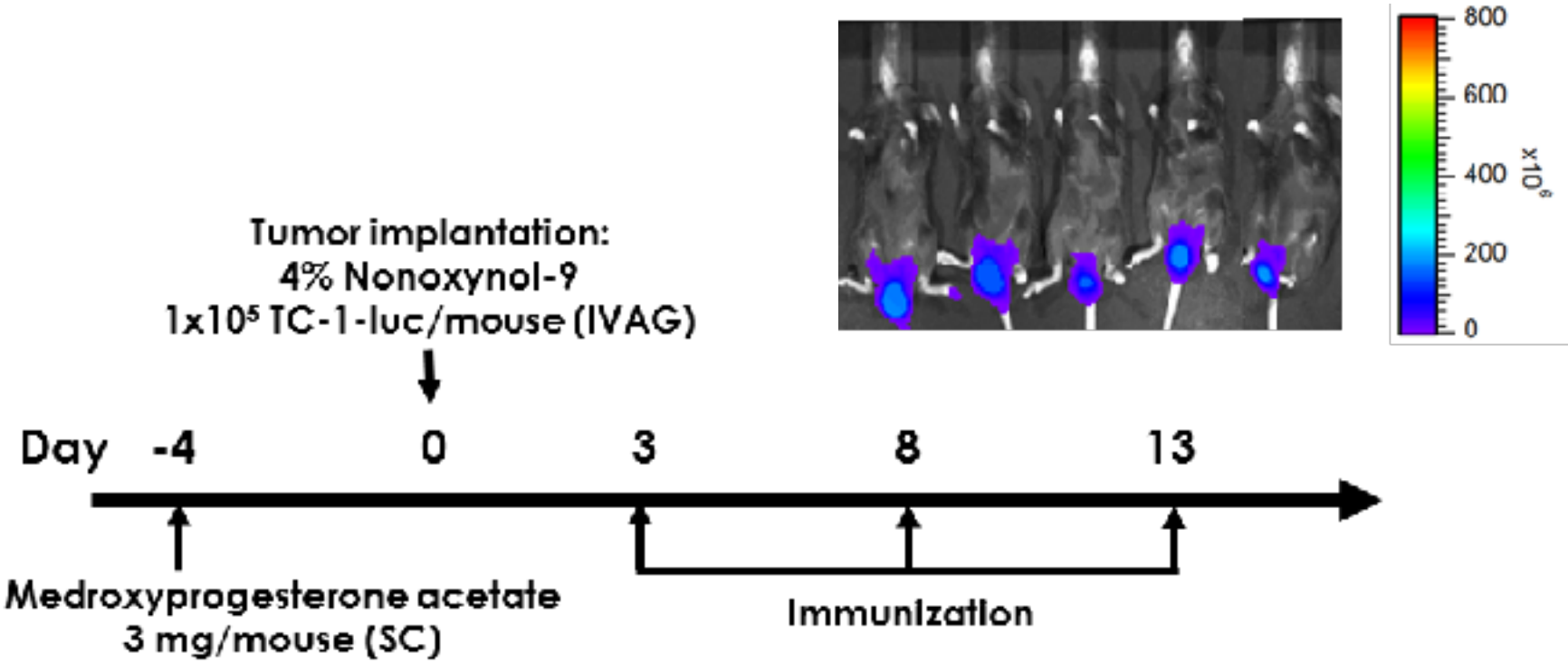
Orthotopic genital cancer model

- Genital organ: portal entry site for HPV
- Therapeutic topical vaccine for cervical cancer?

- **Considerations…**
  - Compartmentalized genital organ
  - Unique immune system in genital organ
  - Inductive site-specific anti-tumor immune response?

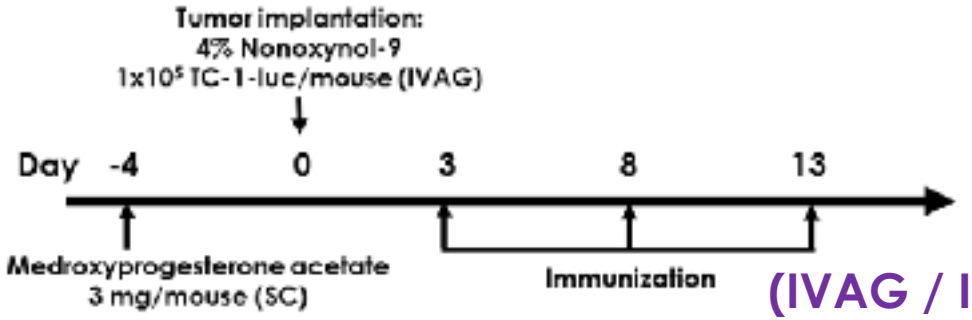
Orthotopic therapeutic cancer vaccine

- **TC-1-luc cell line**
  - Monitoring tumor growth by luciferase

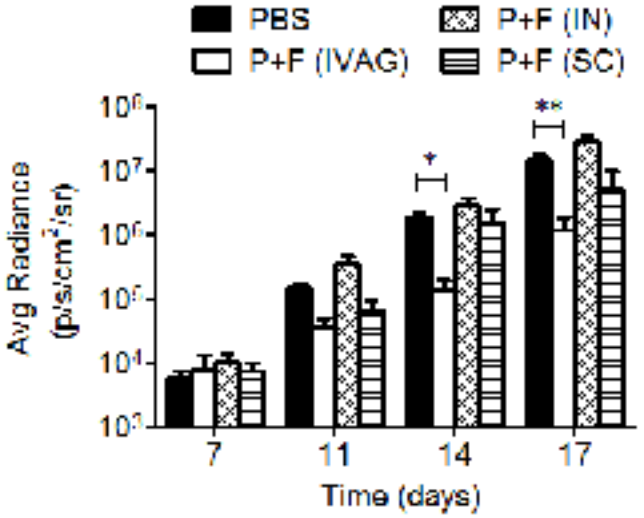


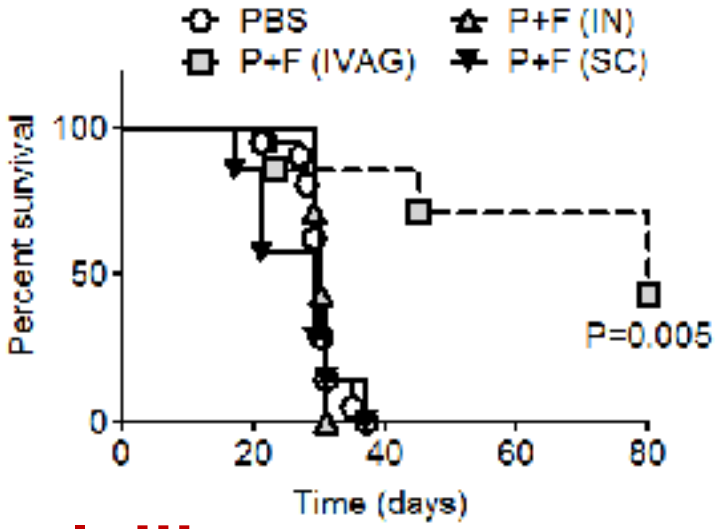
Lee et al, *Oncoimmunology 2016*

Orthotopic therapeutic cancer vaccine
- Immunization route -



(IVAG / IN / SC)



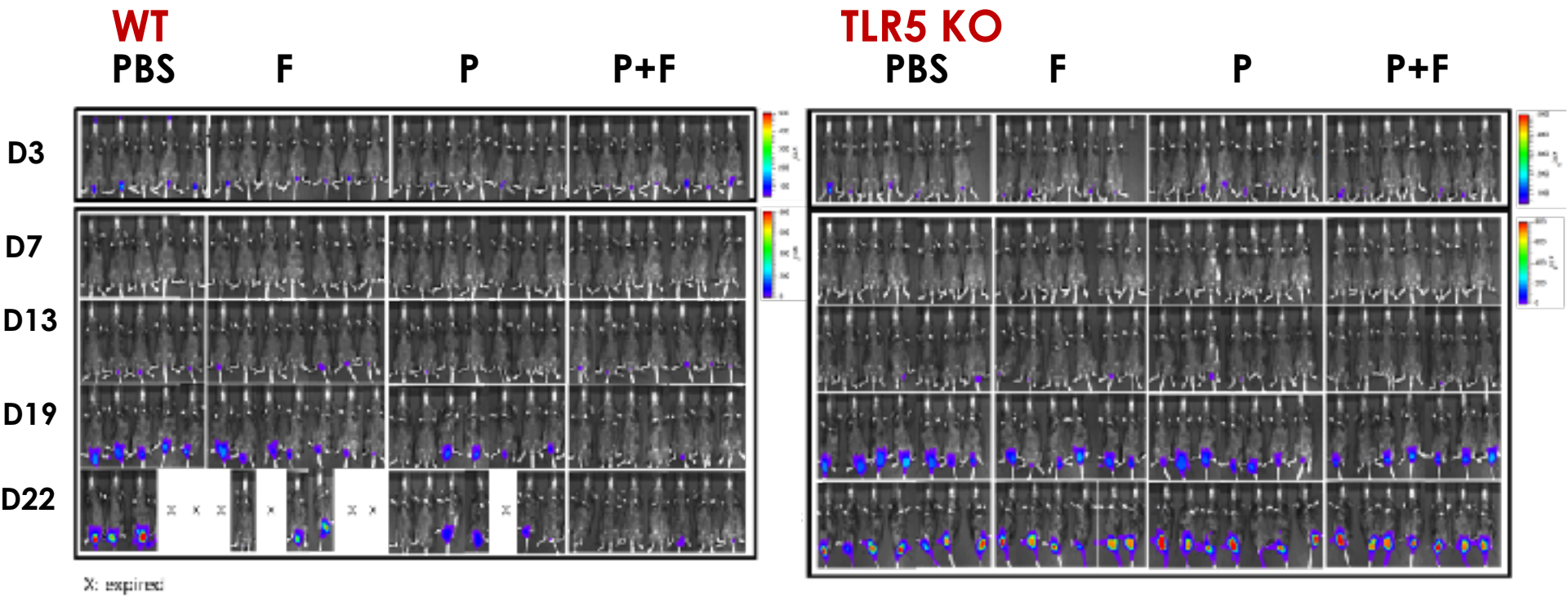


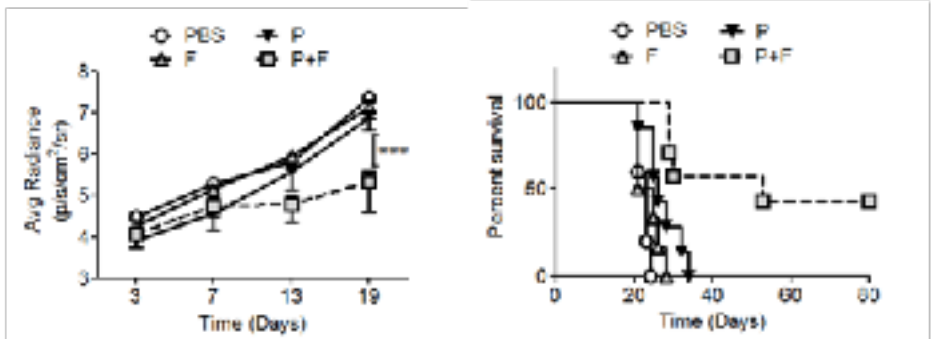
IVAG only!!!

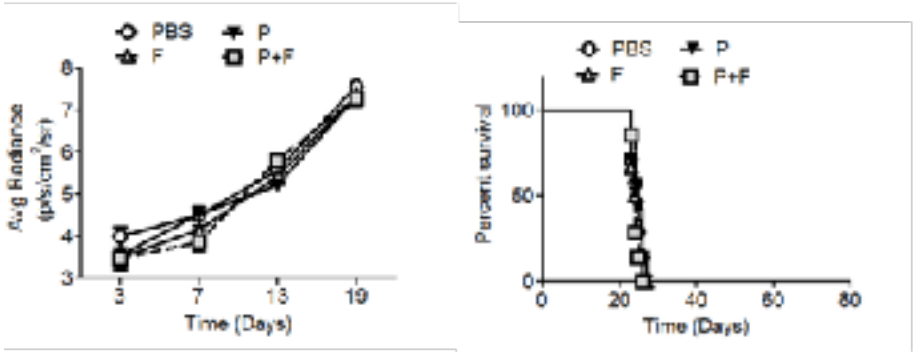
Lee et al, *Oncoimmunology 2016*

Orthotopic therapeutic cancer vaccine

- TC-1-Luc -

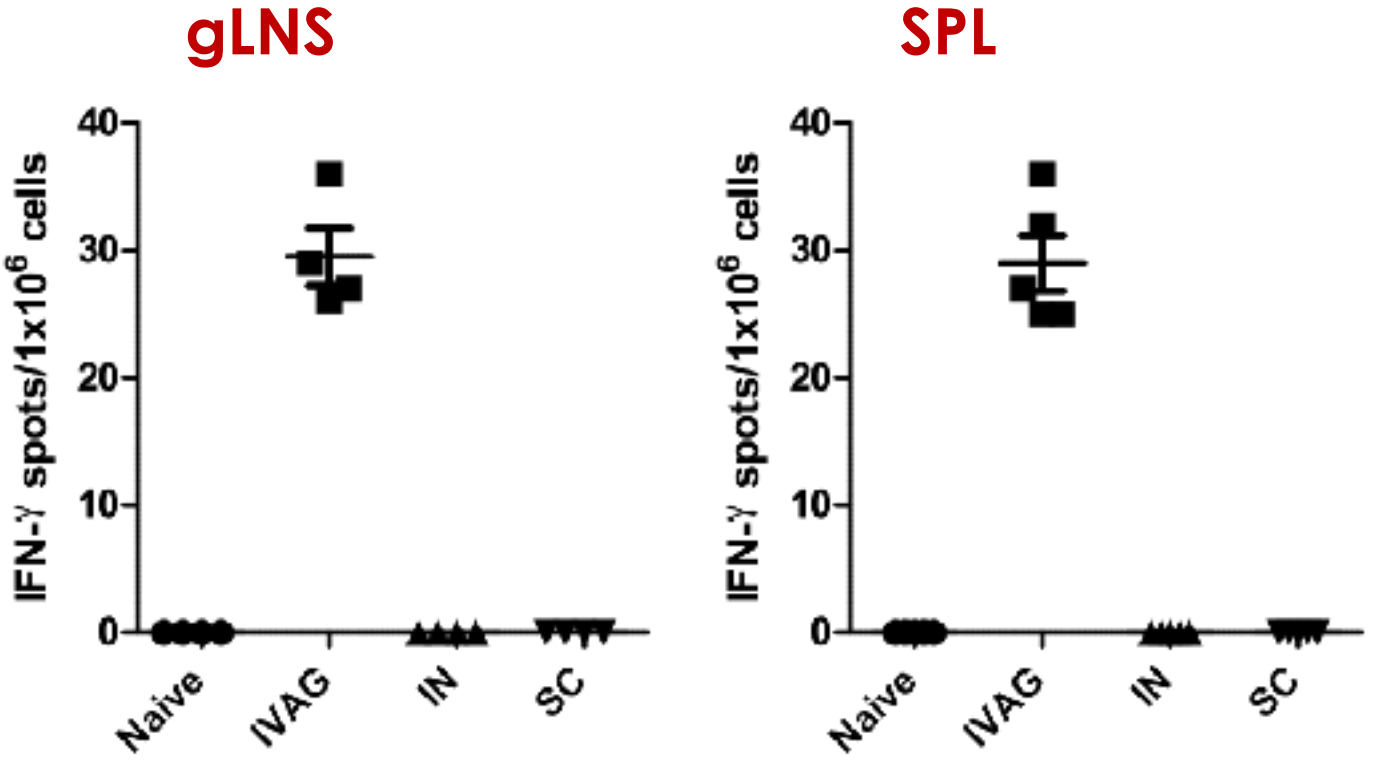






Lee et al, *Oncoimmunology 2016*

Orthotopic therapeutic cancer vaccine
- Immunization route -

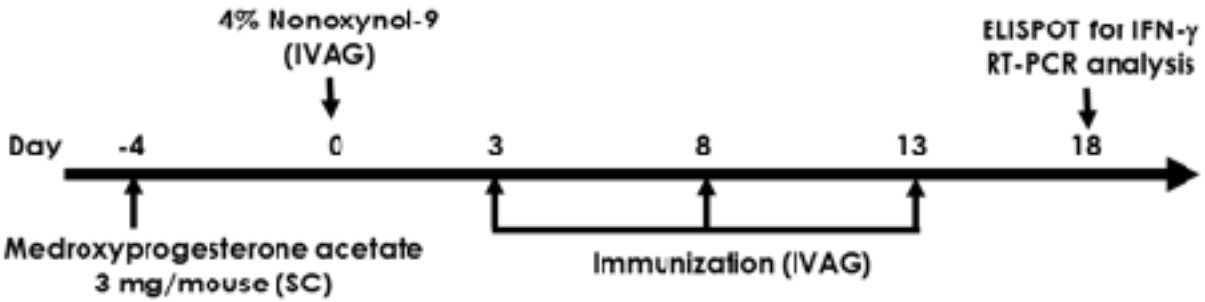


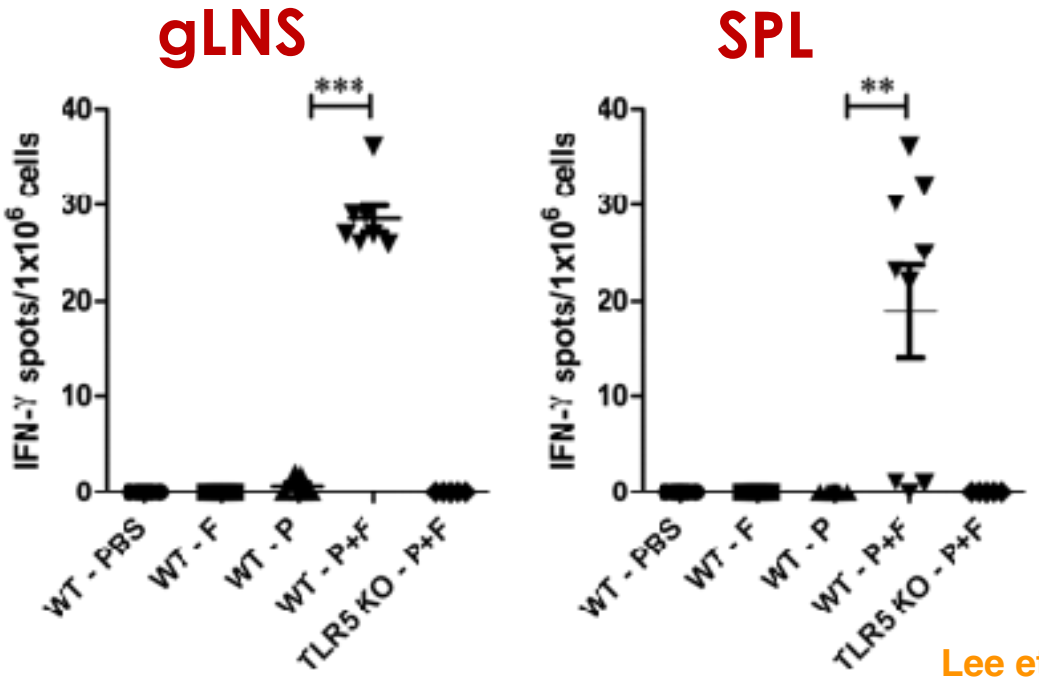
IFNγ production gLNs & SPL - only IVAG

Lee et al, *Oncoimmunology 2016*

Orthotopic therapeutic cancer vaccine

- Immune responses? -

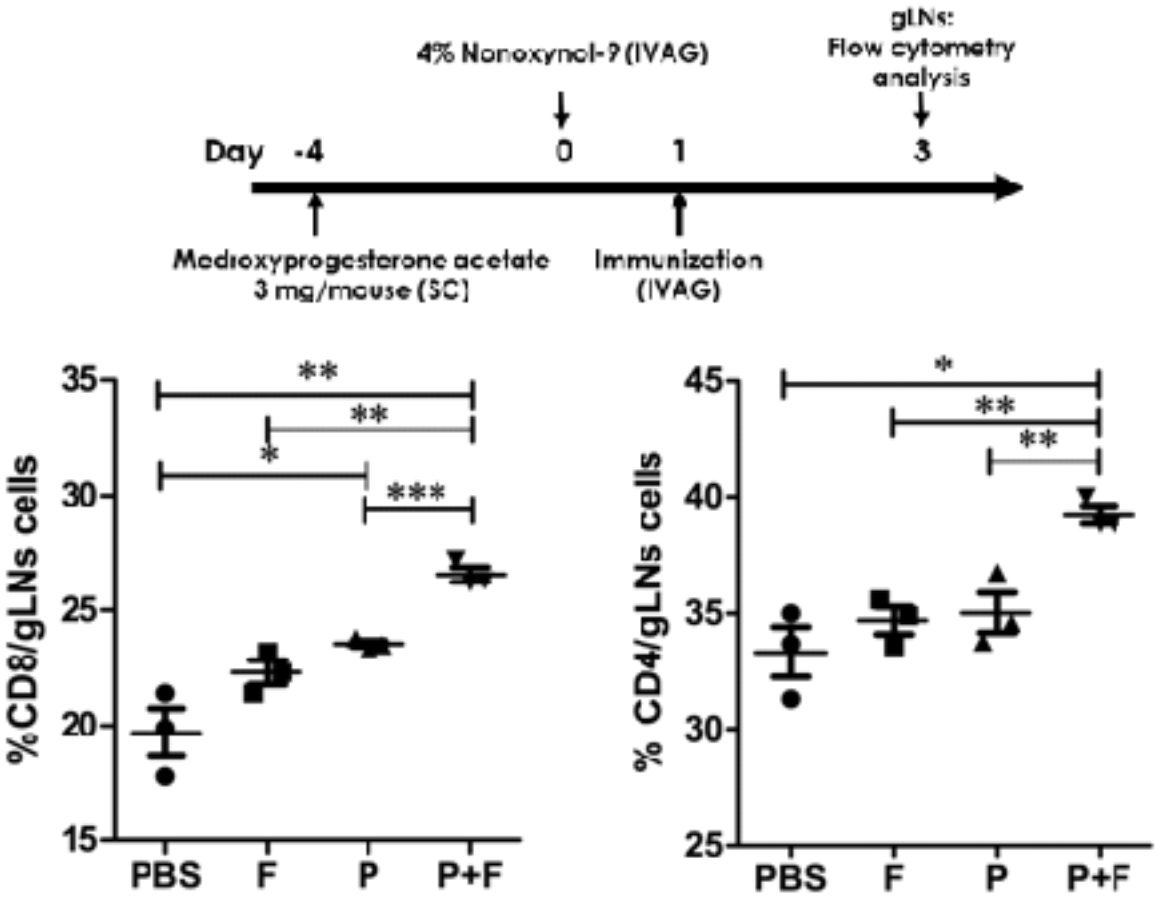




Lee et al, *Oncoimmunology 2016*

Orthotopic therapeutic cancer vaccine

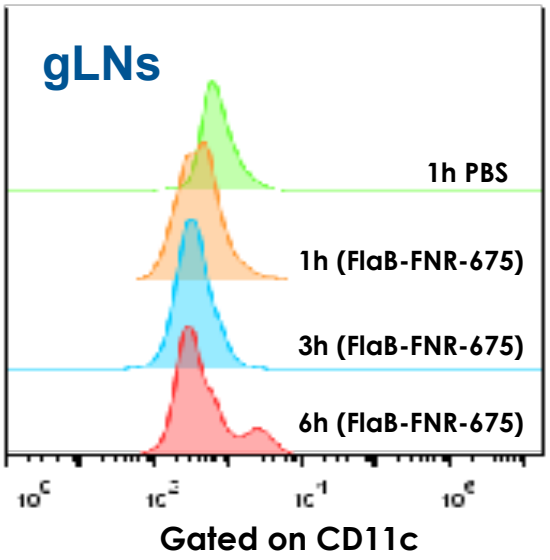
- Immune responses? -

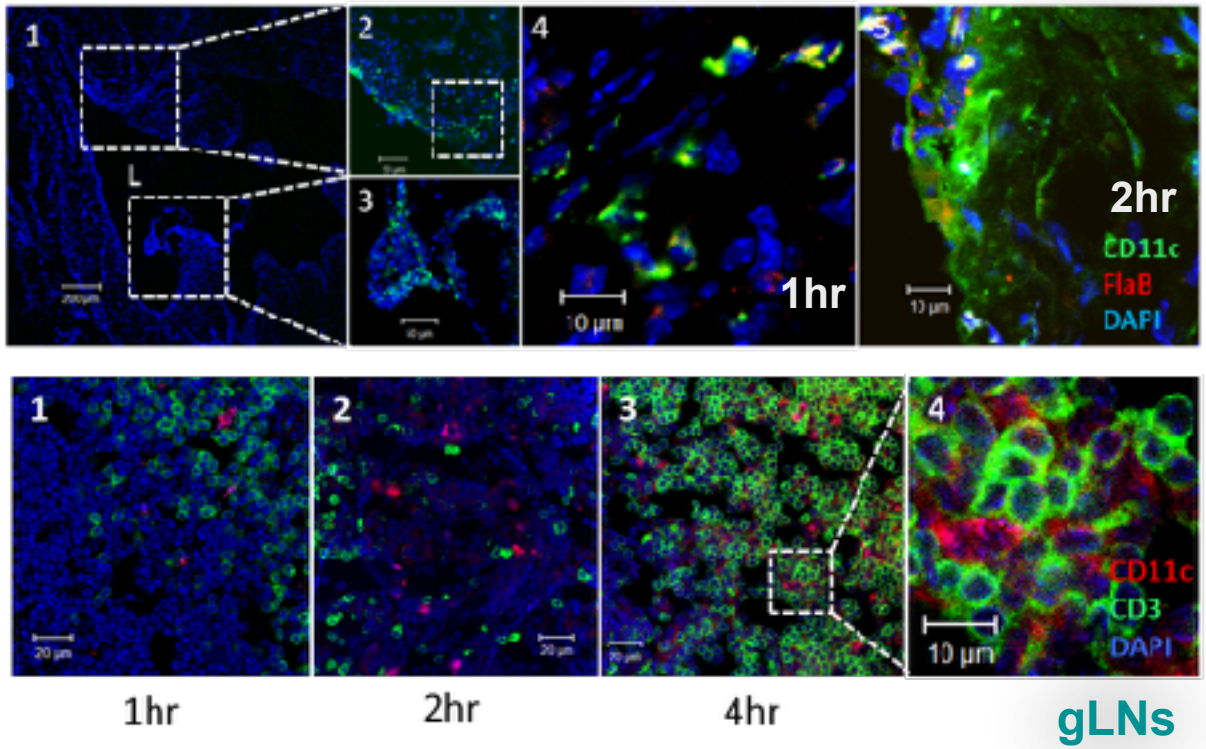


Cell components

Lee et al, *Oncoimmunology 2016*

Orthotopic therapeutic cancer vaccine
- IVAG Flagellin: tracking -

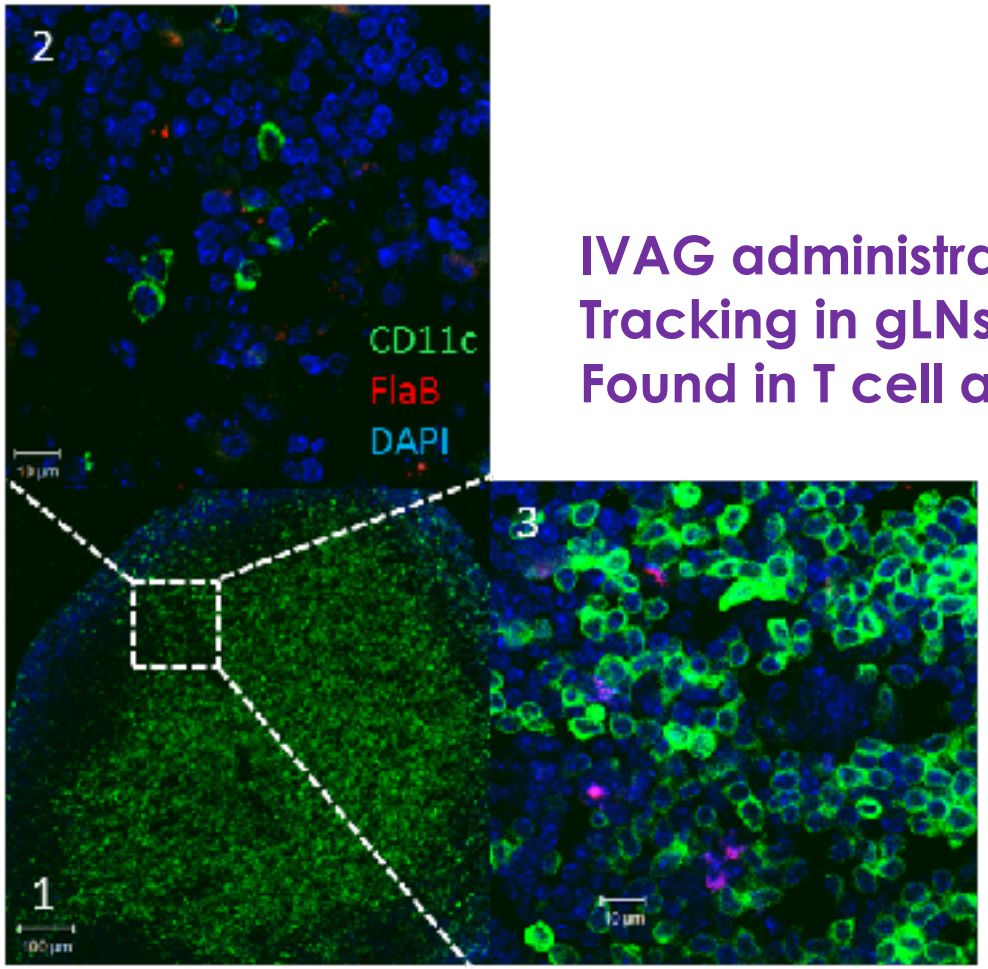




Recruitment of DCs in the vaginal wall
Trafficking of IVAG-administered FlaB to gLNs

Lee et al, *Oncoimmunology 2016*

Orthotopic therapeutic cancer vaccine
- IVAG Flagellin: tracking -

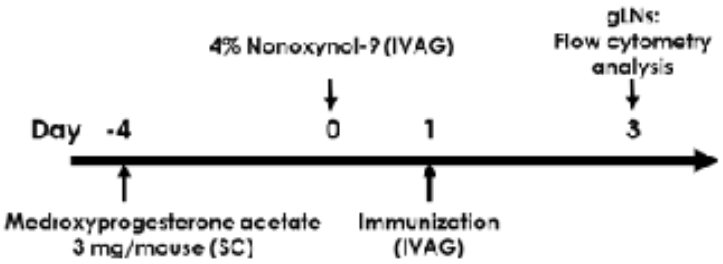


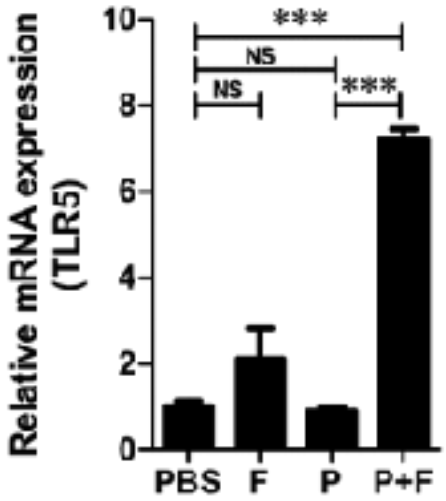
IVAG administration
Tracking in gLNs
Found in T cell area

Lee et al, *Oncoimmunology 2016*

Orthotopic therapeutic cancer vaccine

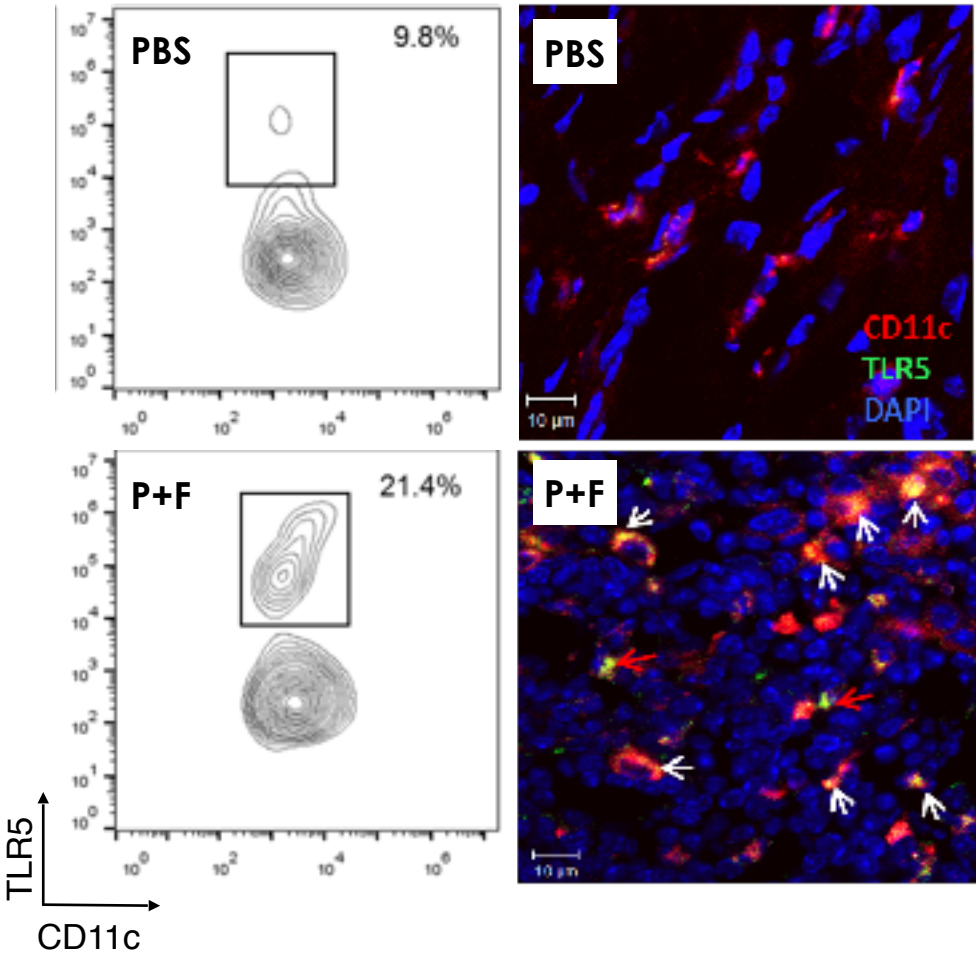
- Immune responses? -





TLR5 expression ↑

CD11c+ cells in gLNs



Lee et al, *Oncoimmunology 2016*

Conclusions

- ✔ IVAG flagellin exerted excellent adjuvant activity in combination with peptide antigens in an orthotopic cervical cancer model.
- ✔ IVAG flagellin induced tumor-specific cell mediated immunity in both local and systemic compartments.
- ✔ IVAG flagellin induced TLR5 pathways.

Final conclusion

Flagellin serves
a potent adjuvant for

cancer

vaccines/immunotherapy.

Thank you for your attention !

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

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연세대학교

부산대학교