Innate immunity in livestock and adjuvants

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DGVAC Consultancy

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- **“Adjuvants are the immunologist’s dirty little secret”**
- (Prof. Dr. V. Schijns)

ELSEVIER

One important lesson learned is to apply the KISS principle whenever possible, KISS is an acronym for Keep It Simple Stupid! To encourage success in adjuvant development, unnecessary complexity must be avoided.

The path to a successful vaccine adjuvant – 'The long and winding road'

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New generation vaccines will increasingly comprise highly purified recombinant proteins. Unfortunately, these antigens are often poorly immunogenic. Therefore, adjuvants will be required to enable these proteins to become effective vaccines. Although several novel adjuvants have recently emerged, including formulations comprising more than one adjuvant, the approval of vaccines containing novel adjuvants has been slow, particularly in the US. However, despite significant ongoing concerns, the necessary safety data is now emerging to show that new generation adjuvants can be safely used in diverse human populations. In combination with data showing the positive contributions of the adjuvants to the immune response, this safety data should allow several vaccines containing novel adjuvants to obtain licensure within the next few years.

What are adjuvants, which ones are currently available?

Vaccine adjuvants are defined by the effects that they achieve, so they tend to defy easy descriptions of what they actually are. The earliest definition of vaccine adjuvants describes them as components that are added to vaccine antigens to make them more immunogenic [1]. Because adjuvants are defined so imprecisely, there are many candidates available and new ones are regularly described in the literature. To add to the lack of clarity around adjuvants, many of the 'new' ones are actually variations on old themes, often repackaged to make them more potent. Moreover, as the signaling pathways involved in immune activation are becoming increasingly better defined, many more 'new' adjuvants will emerge, or be rediscovered. This will inevitably add to the complexity. Attempts have been made to group adjuvants into classes, to try better to define how they work. For example, a broad range of adjuvants have been grouped as 'delivery systems', which means that their predominant mechanism of action was thought to be the delivery of antigens to immune cells [2]. In addition, an alternative range of adjuvants have been described as 'immune potentiators', because they exert direct effects on immune cells, leading to their activation [2]. However, this classification system, which was always simplistic, has been largely superceded by recent observations, that highlighted that delivery systems are also immune potentiators [3,4]. An alternative way to define adjuvants is in terms of the signals

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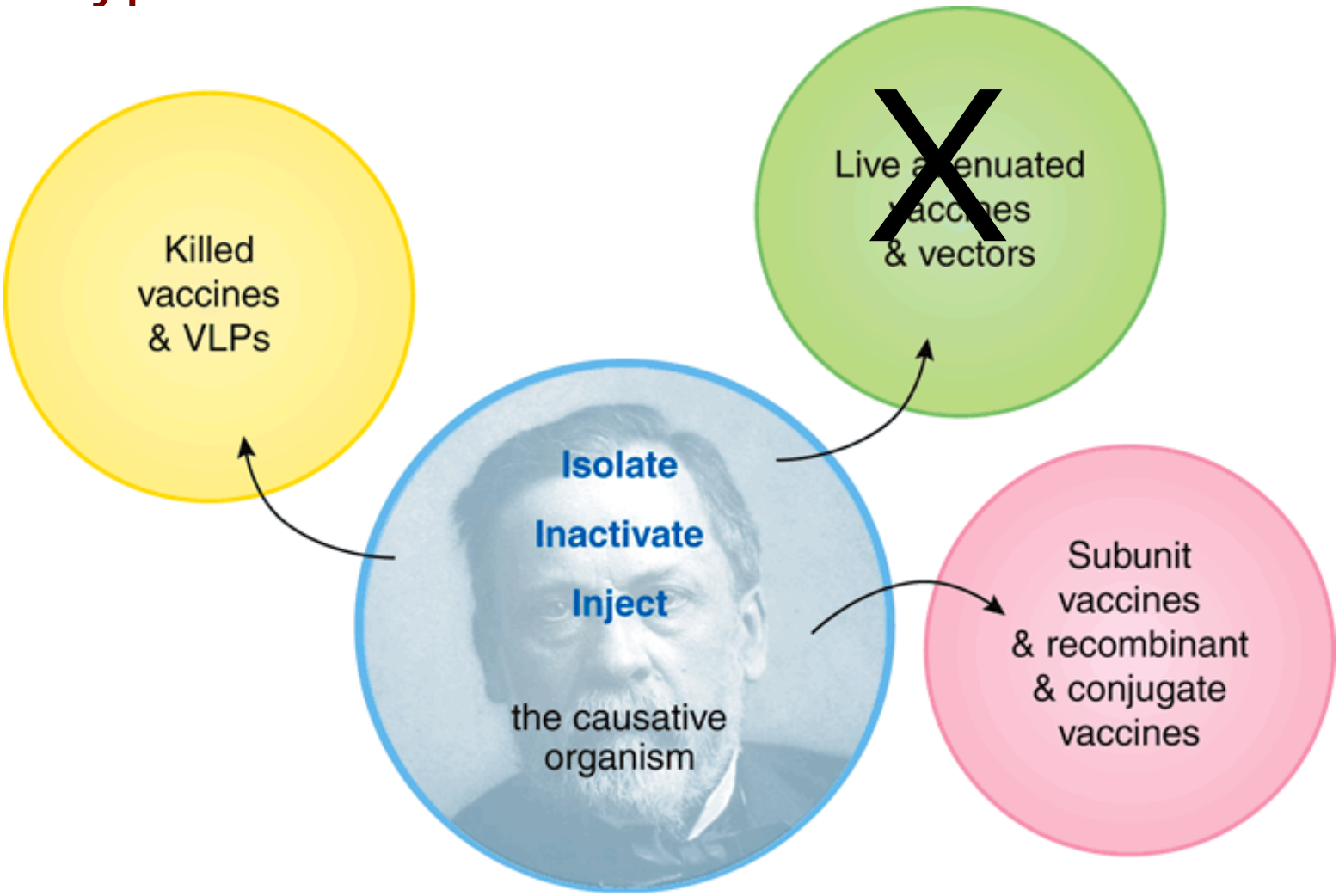
Ennio De Gregorio
Ennio De Gregorio is head of the Immunology Function of the Research Unit, Novartis Vaccines and Diagnostics in Siena, Italy. Before this position he was project leader of two research programs in Novartis Vaccines. Between 2000 and 2003 Dr De Gregorio worked on the innate immune response as a post-doctoral fellow of Human Frontier Science Program at the CNRS, Gif-sur-Yvette, France. Between 1996 and 2000, Dr De Gregorio performed his PhD work at the European Molecular Biology Laboratory (EMBL) Gene Expression Program in Heidelberg, Germany. Dr De Gregorio received his degree on Molecular Biology from the University of Rome, Italy in 1994.

1359-6446/08/$ - see front matter doi:10.1016/j.drudis.2009.02.009

Outline of the "long and winding adjuvant road"

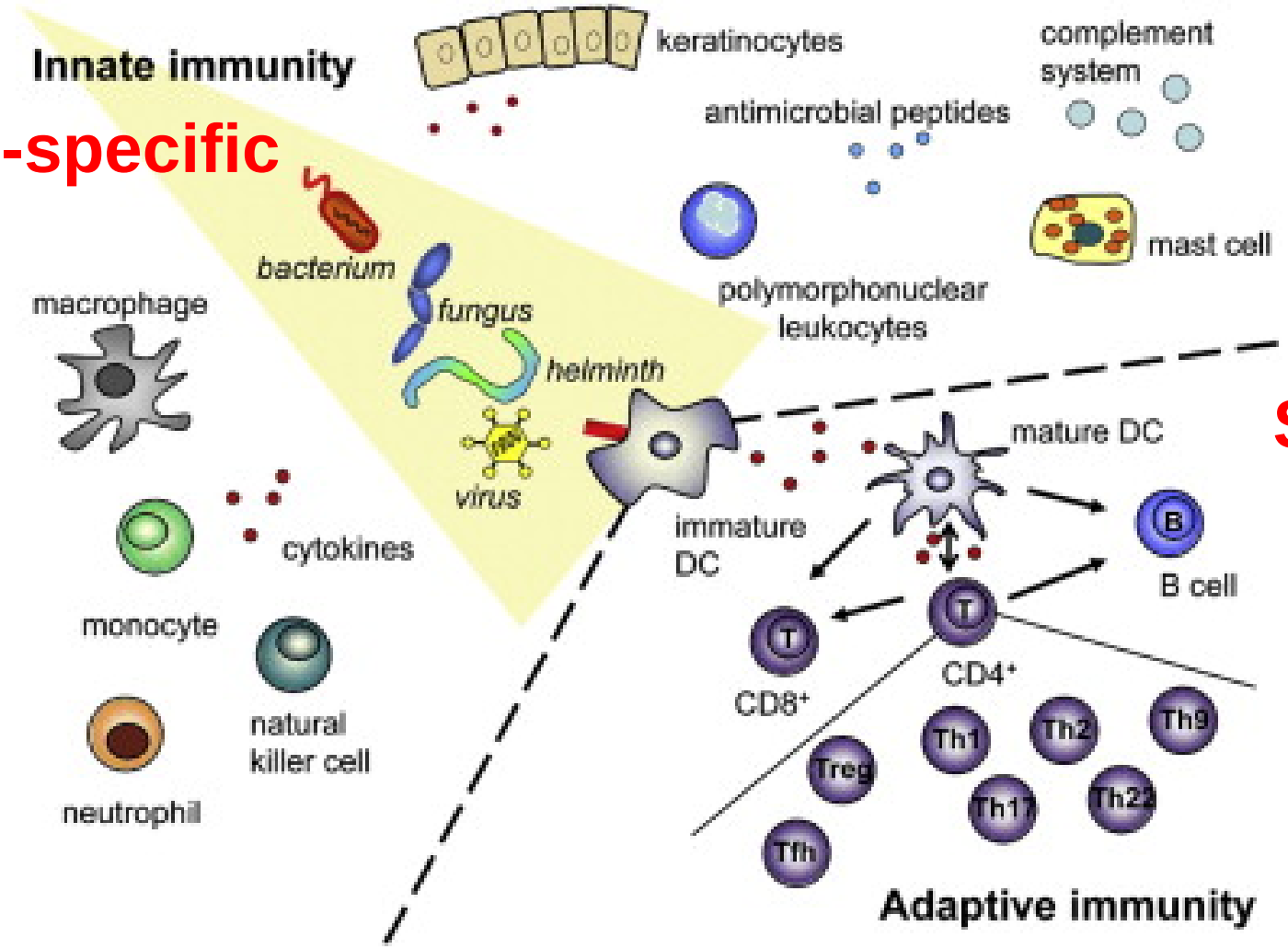
- Focus on inactivated vaccines given by injection
- The immune response: innate versus adaptive
- Need for adjuvants
- History of adjuvants
- Types of adjuvants and their mode of action
- Examples of adjuvants for veterinary and human vaccines
- Regulatory aspects
- Future perspectives

Types of vaccines



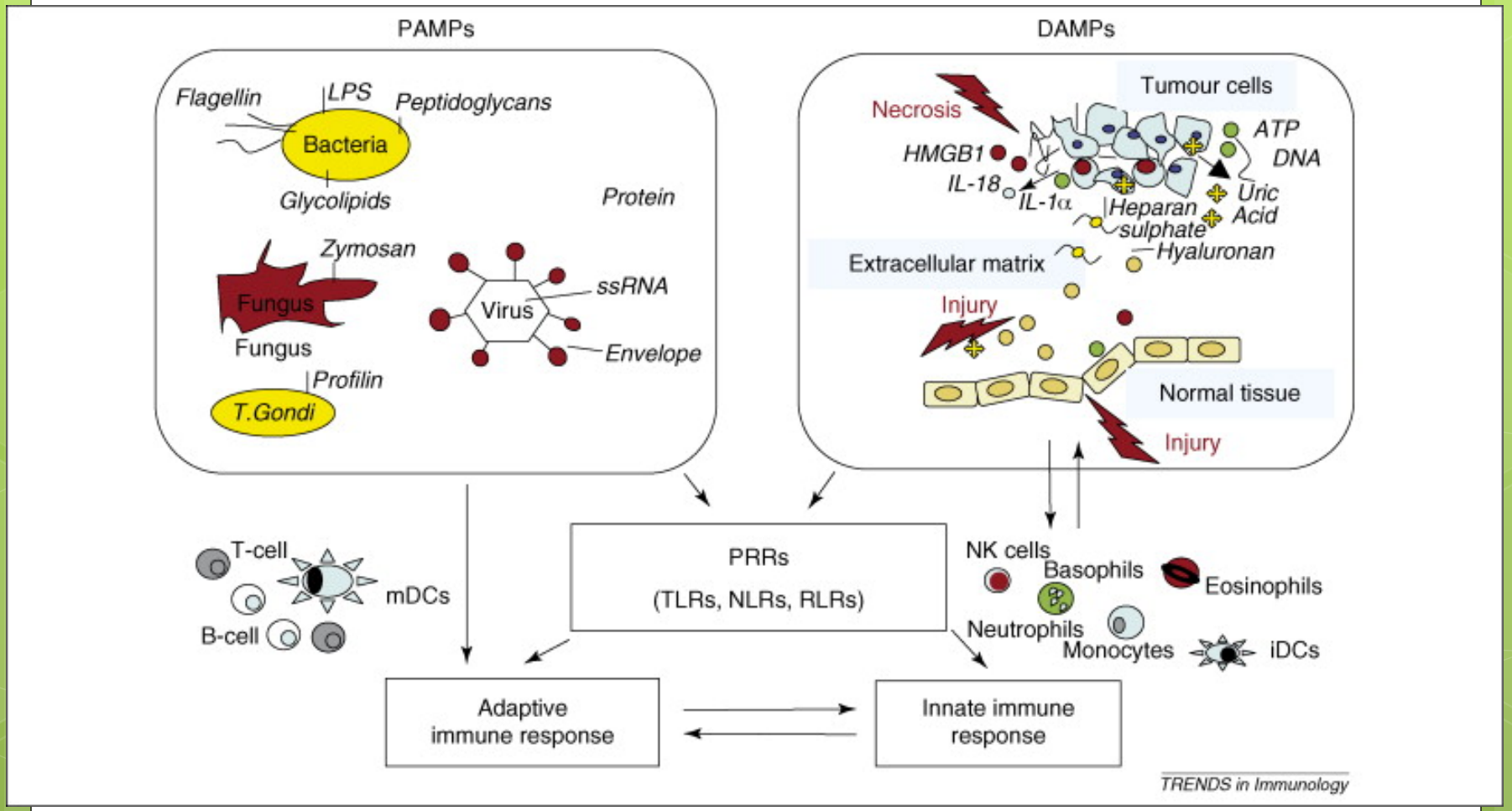
- Focus on injection (sc / im) vaccination

Innate versus adaptive immunity



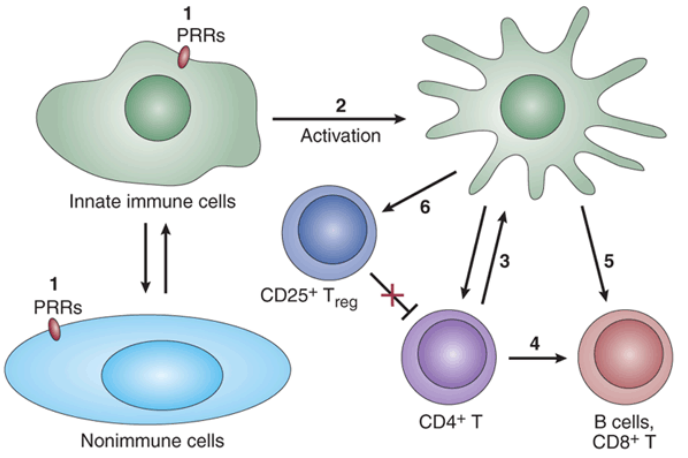
From Bal et al J.Controlled Release,2010

Interactions during an immune response



APC are vital; a crucial role for DC

- Uptake of antigen by DC depends on:
  - Size
  - Charge
  - Hydrophobicity and hydrophylicity
  - Interactions with receptors
- Trafficking of DC to lymph nodes
- DC maturation
- Interaction with T / B lymphocytes

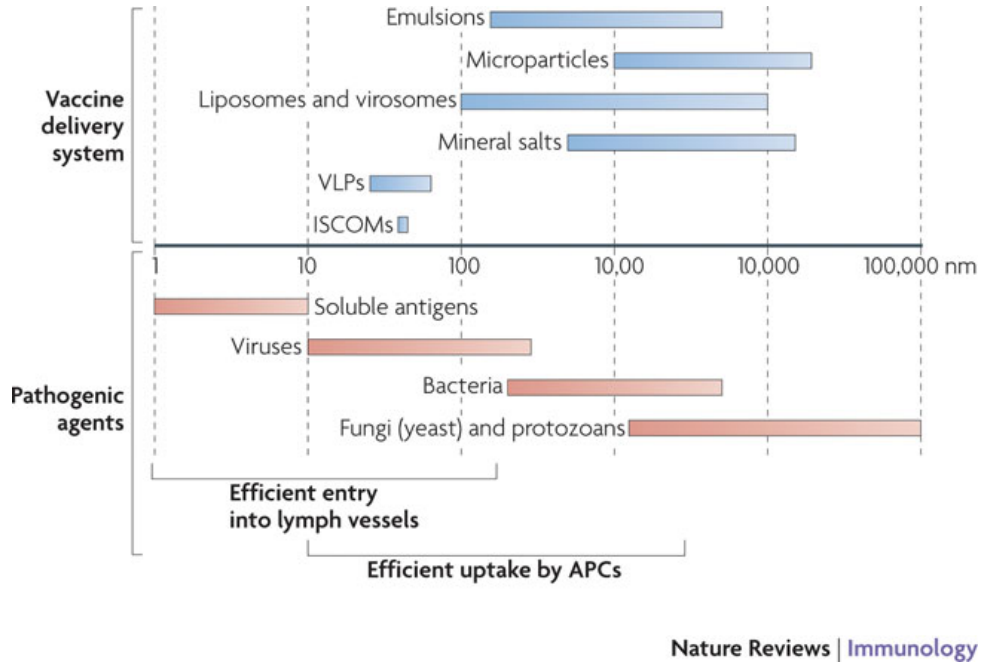


Why do we need an adjuvant in vaccines?

- Due to use of only certain antigen components in vaccines
- In general a weaker immune response is induced
- Therefore a need for an adjuvant to compensate for this
- The term adjuvant is derived from the Latin word

- **“ Adjuvare” = helping or enhancing**

Size of adjuvant delivery systems and pathogens



The ideal combination of adjuvant and antigen in a vaccine should lead to.....

- Stimulation of an efficacious immune response (T cell and antibody responses)
- Induction of memory
- Reduction of antigen dose or number of vaccination(s)
- A broadening of the response
- A stimulation of the response against combined vaccines, reduce ag competition
- Stimulation of the response in young and elderly
- A more rapid induction of immune response
- A sufficient duration of immune response

Risk / benefit of safety versus efficacy should be considered in relation to disease involved

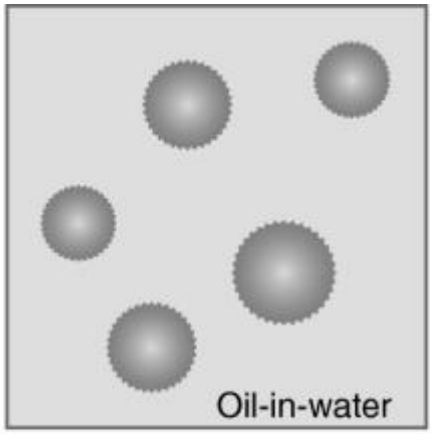
Some historical developments

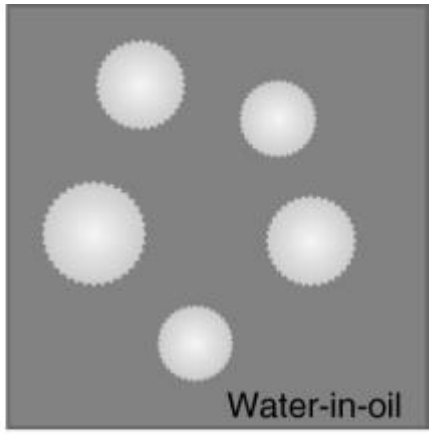
1916	Le Moigne and Pinoy	*S. Typhi* in oil demonstrated enhanced reponses
1926	Ramon	Anti-toxin response by addition of agar, saponin and bread crumbs
1926	Glenney	Adjuvant effect of aluminium hydroxide and diphtheria toxoid
1936	Thibault and Richou	Adjuvant activity of saponins from *Quillaja saponaria*
1937	Freund	Adjuvant effect of Mycobacteria in oil emulsions
1990	Various	Demonstration of CTL induction by different adjuvants and delivery systems
1997	Chiron/Novartis	MF-59 licensed in EU for flu vaccines
2005	GSK	AS04 licensed in EU for HBV
2008	GSK	AS03 licensed in EU for pandemic flu
2009	GSK	AS04 licensed in US

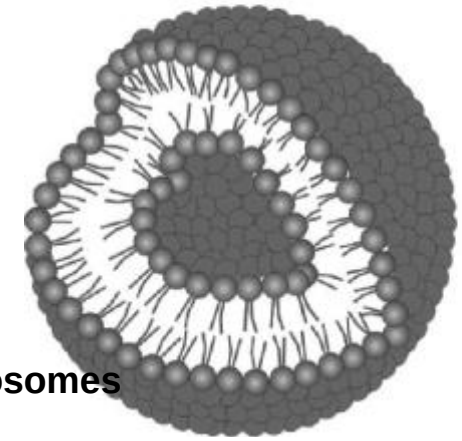
Different types of adjuvants

Vehicles	Immunostimulants
Adsorbants: Aluminium or calcium based **Emulsions:** Freund's type, IFA, O/W, W/O, DOE **Polymers:** Carbopol **Vesicles:** Liposomes/Virosomes Iscoms **Microspheres or nanospheres:** PLAGA	**TLR ligands:** LPS, MPL **Saponins:** QuilA, Qvac **Cytokines:** GM-CSF **Bacterial toxins:** CT and LT **Drugs:** Levamisole

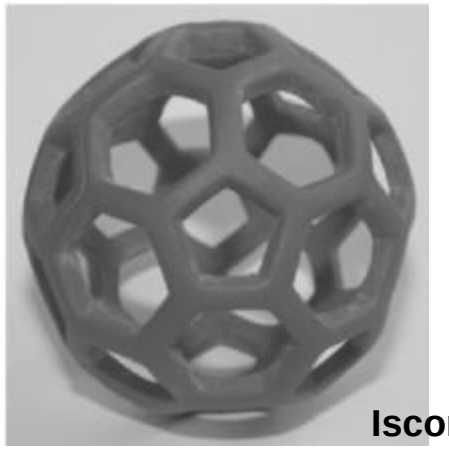
Some adjuvant structures





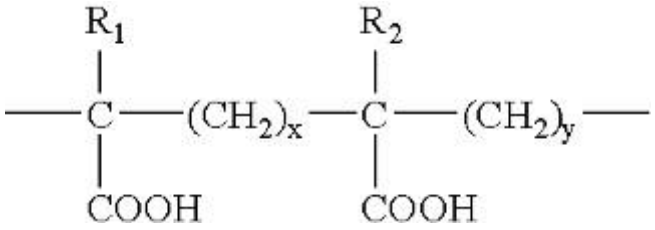


Liposomes

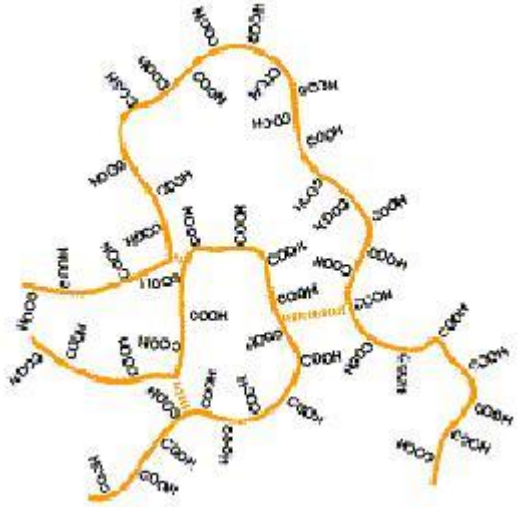


Iscoms

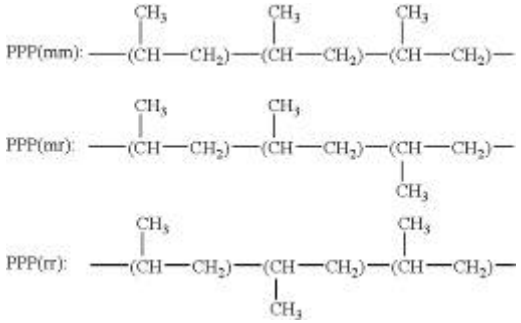
Adjuvants: a variety of structures…….



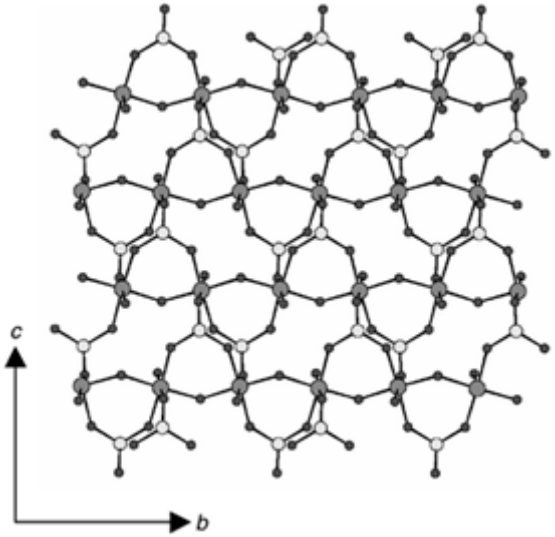
EMA



Carbomer

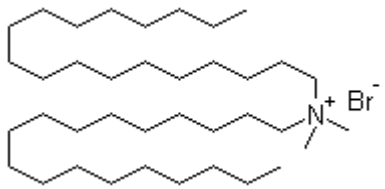


Mineral oil

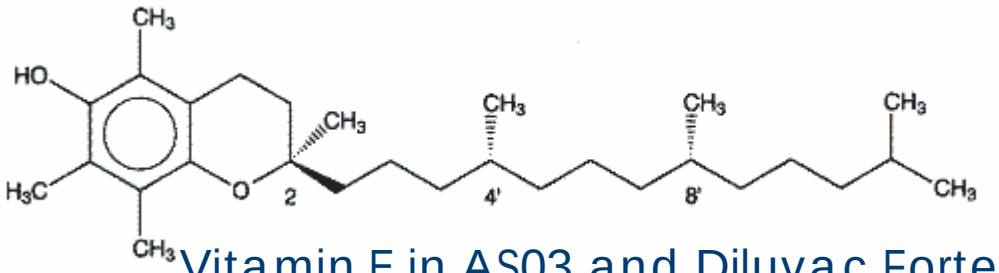


Alhydrogel

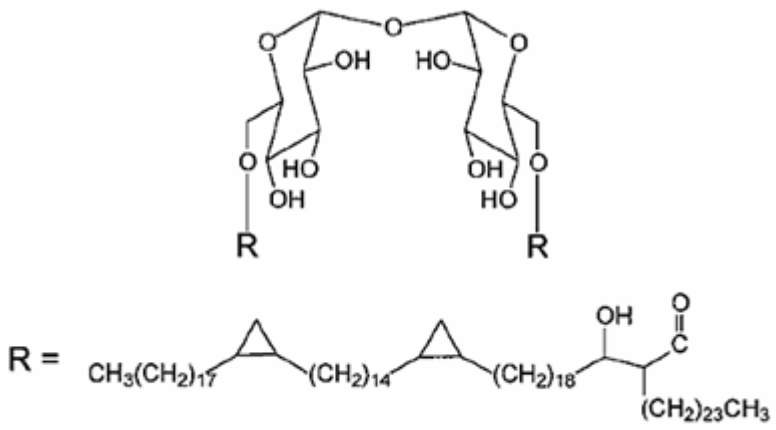
More adjuvant structures



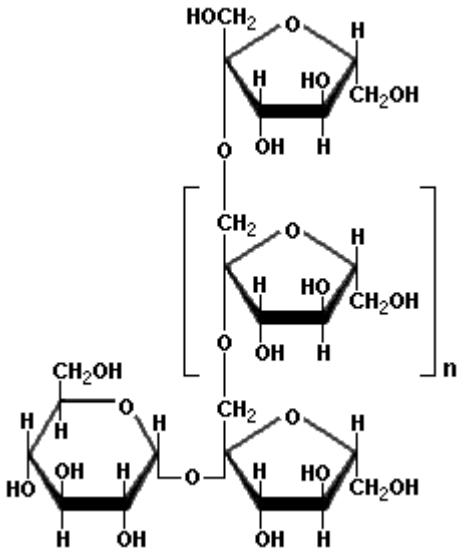
DDA



Vitamin E in AS03 and Diluvac Forte



TDM: Trehalose dimycolate



Inulin (Advax)

Choice of the adjuvant technically depends on:

- **Species**; livestock, companion animal, human
- **Targeted immune response**
- **Antigen**; Gram -, Gram +, virus, purified or not
- **Purpose**: protect animal or progeny, duration or fast response
- No dogmas but a lot of **empirical trial and error.**

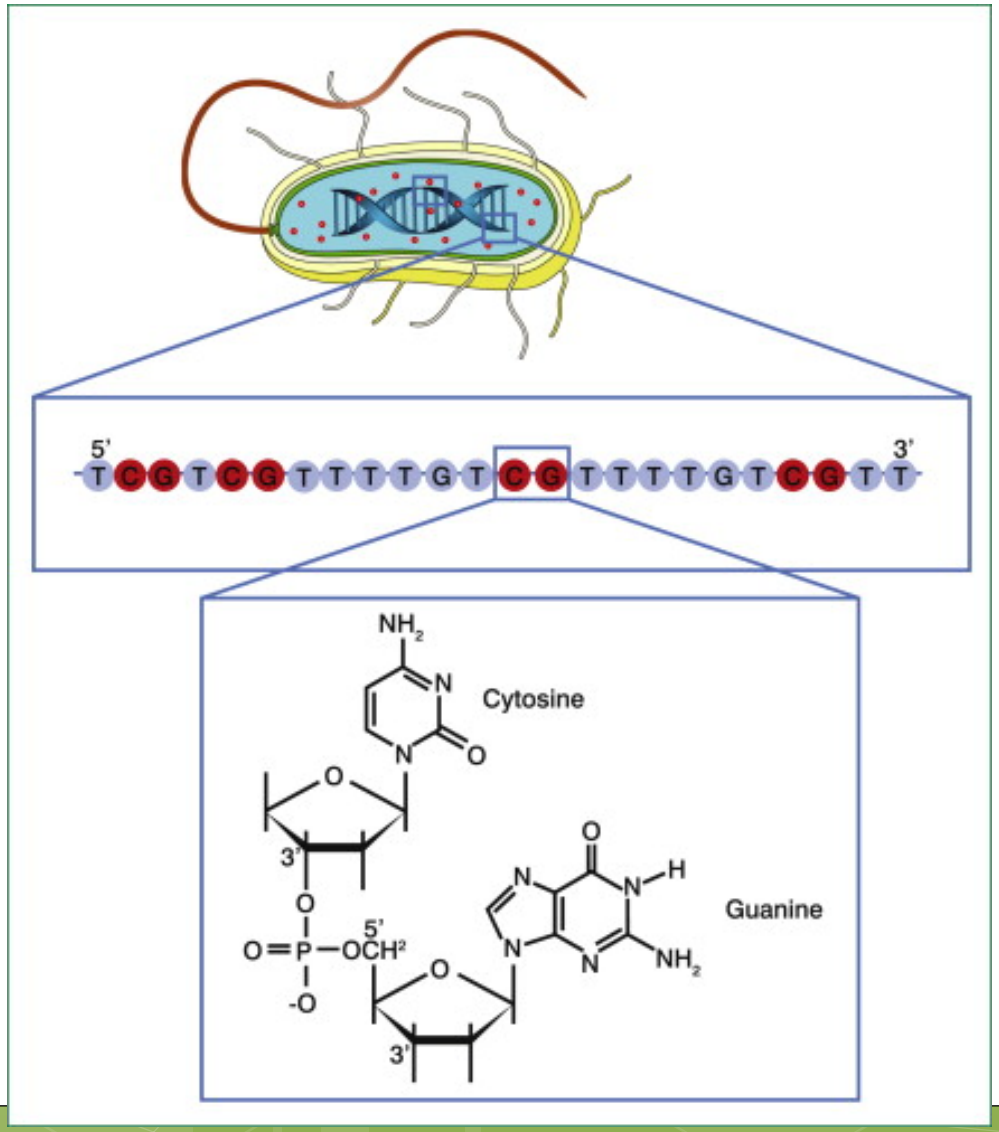
Choice of the adjuvant commercially depends on:

- **Cost;** oil, aluminum, commercial supplier versus in house production, half of the volume can be adjuvant.
- **Supply;** single source manufacturer – in house production
- **% waterphase left;** e.g. ISA70 or ISA50, aluminium
- **Ease of manufacturing;** simply mix or absorb, emulsion or complex packaging
- **Seringibility;** viscosity
- **For livestock**: Food chain ingredients, MRL needed?

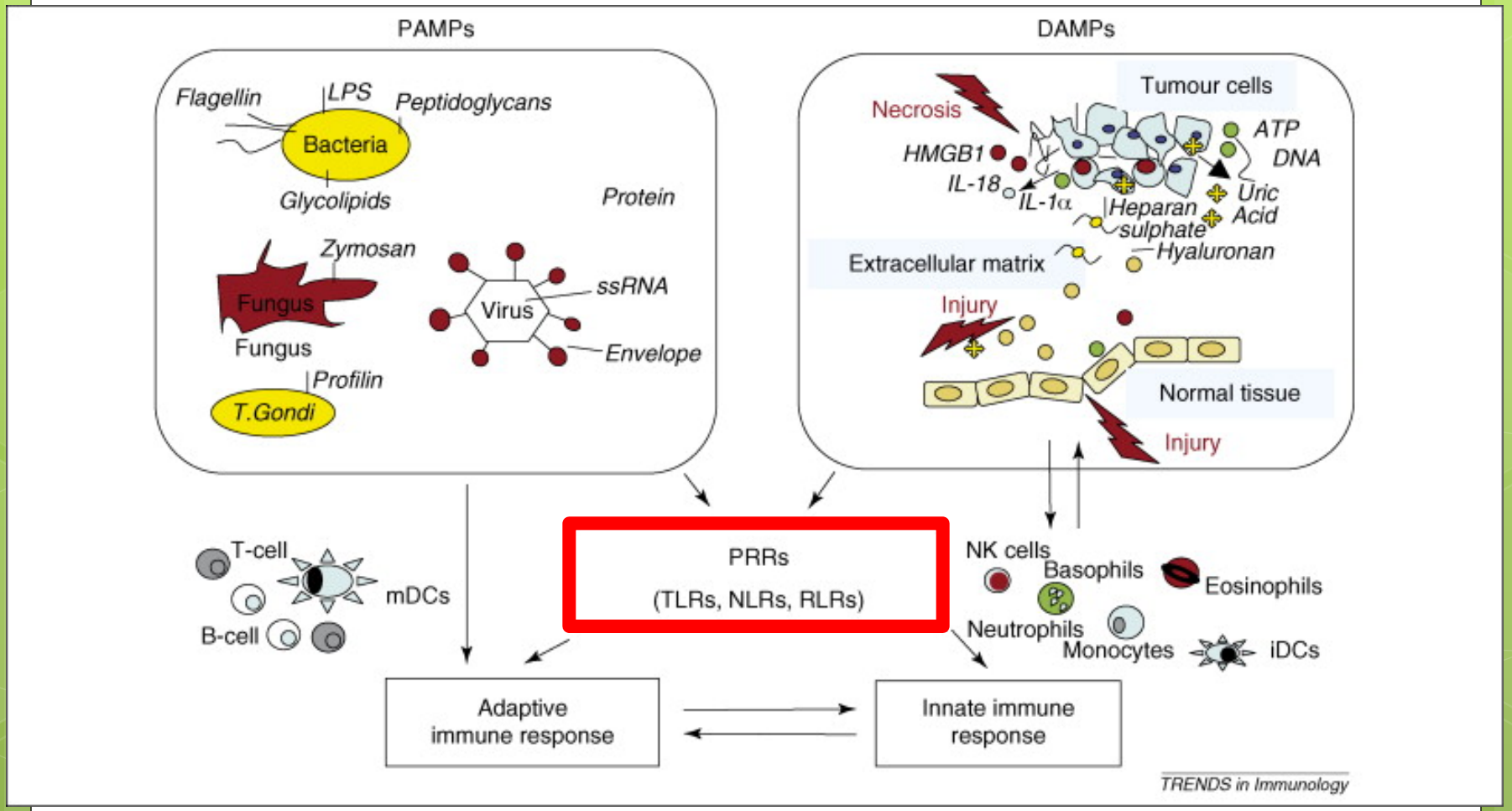
CpG ODN adjuvants – TLR agonists

Unmethylated deoxycytidyl-deoxyguanosin dinucleotides (CpG) oligodeoxynucleotides (ODNs)

CpG as adjuvants originating from DNA



Interactions during an immune response



Vaccine. 2011 Oct 19;29(45):7960-5. doi: 10.1016/j.vaccine.2011.08.072. Epub 2011 Aug 26.

CpG oligodeoxynucleotide and montanide ISA 206 adjuvant combination augments the immune responses of a recombinant FMDV vaccine in cattle.

Ren J[1], Yang L, Xu H, Zhang Y, Wan M, Liu G, Zhao L, Wang L, Yu Y.

Biologicals. 2015 Nov;43(6):437-43. doi: 10.1016/j.biologicals.2015.09.004. Epub 2015 Oct 20.

Comparative efficacy of virus like particle (VLP) vaccine of foot-and-mouth-disease virus (FMDV) type O adjuvanted with poly I:C or CpG in guinea pigs.

Int Immunol. 2016 Apr 7. pii: dxw017. [Epub ahead of print]

CpG-mediated augmentation of CD8+ T-cell responses in mice is attenuated by a water-in-oil emulsion (Montanide ISA-51) but enhanced by an oil-in-water emulsion (IDRI SE).

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Bioconjug Chem. 2016 Apr 13. [Epub ahead of print]

Optimization, Production and Characterization of a CpG-Oligonucleotide-Ficoll Conjugate Nanoparticle Adjuvant for Enhanced Immunogenicity of Anthrax Protective Antigen.

Milley B, Kiwan R, Ott GS, Calacsan C, Kachura M, Campbell JD, Kanzler H, Coffman RL.

Methods Mol Biol. 2016;1404:289-98. doi: 10.1007/978-1-4939-3389-1_20.

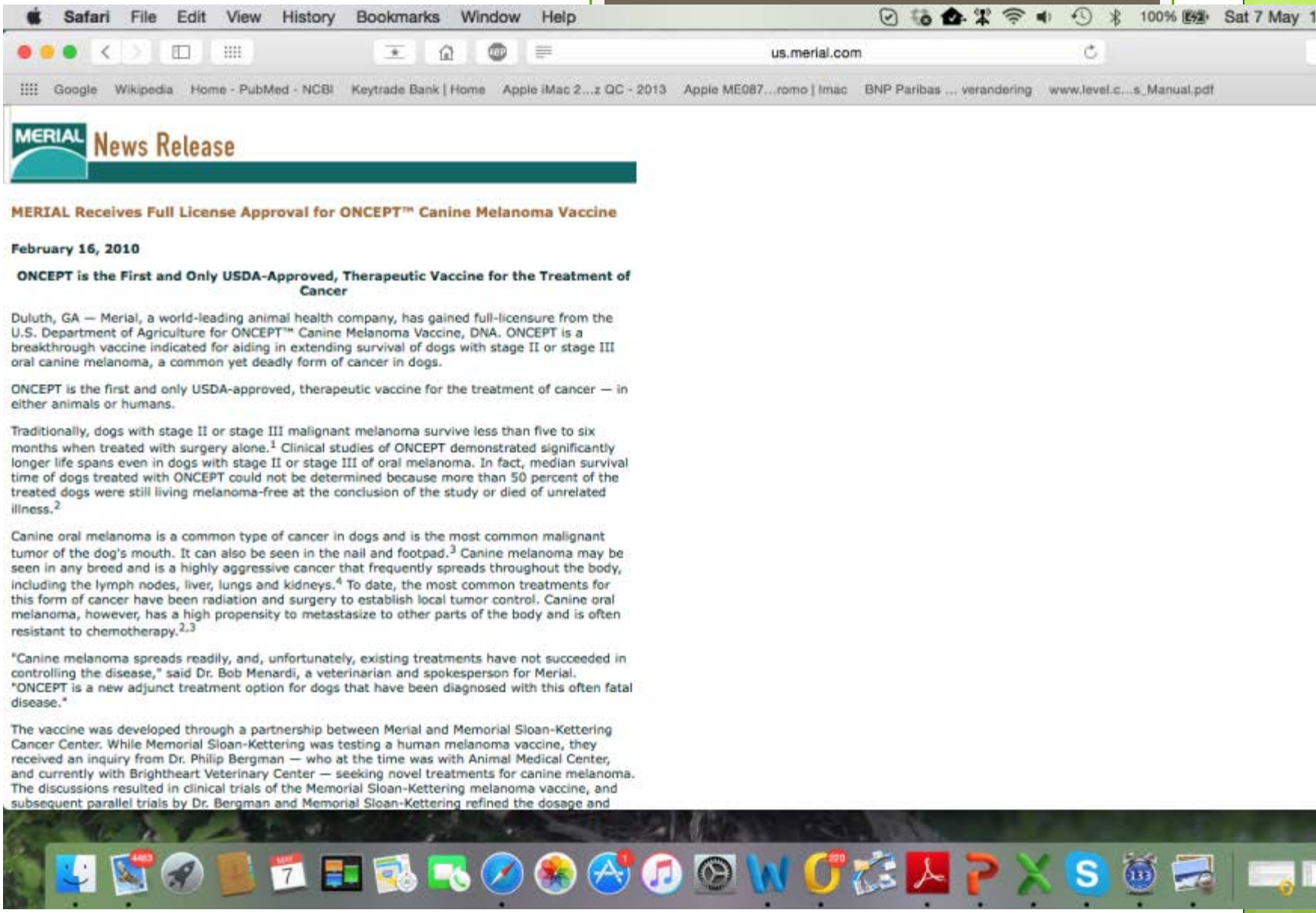
Development of CpG ODN Based Vaccine Adjuvant Formulations.

Gursel M[1], Gursel I[2].

Viral Immunol. 2016 Apr 14. [Epub ahead of print]

Characterization of Immune Responses to an Inactivated Avian Influenza Virus Vaccine Adjuvanted with Nanoparticles Containing CpG ODN.

Singh SM[1], Alkie TN[1], Abdelaziz KT[1,2], Hodgins DC[1], Novy A[1], Nagy É[1], Sharif S[1].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MERIAL News Release
MERIAL Receives Full License Approval for ONCEPT™ Canine Melanoma Vaccine
February 16, 2010
ONCEPT is the First and Only USDA-Approved, Therapeutic Vaccine for the Treatment of Cancer
Duluth, GA — Merial, a world-leading animal health company, has gained full-licensure from the U.S. Department of Agriculture for ONCEPT™ Canine Melanoma Vaccine, DNA. ONCEPT is a breakthrough vaccine indicated for aiding in extending survival of dogs with stage II or stage III oral canine melanoma, a common yet deadly form of cancer in dogs.
ONCEPT is the first and only USDA-approved, therapeutic vaccine for the treatment of cancer — in either animals or humans.
Traditionally, dogs with stage II or stage III malignant melanoma survive less than five to six months when treated with surgery alone.[1] Clinical studies of ONCEPT demonstrated significantly longer life spans even in dogs with stage II or stage III of oral melanoma. In fact, median survival time of dogs treated with ONCEPT could not be determined because more than 50 percent of the treated dogs were still living melanoma-free at the conclusion of the study or died of unrelated illness.[2]
Canine oral melanoma is a common type of cancer in dogs and is the most common malignant tumor of the dog's mouth. It can also be seen in the nail and footpad.[3] Canine melanoma may be seen in any breed and is a highly aggressive cancer that frequently spreads throughout the body, including the lymph nodes, liver, lungs and kidneys.[4] To date, the most common treatments for this form of cancer have been radiation and surgery to establish local tumor control. Canine oral melanoma, however, has a high propensity to metastasize to other parts of the body and is often resistant to chemotherapy.[2,3]
"Canine melanoma spreads readily, and, unfortunately, existing treatments have not succeeded in controlling the disease," said Dr. Bob Menardi, a veterinarian and spokesperson for Merial. "ONCEPT is a new adjunct treatment option for dogs that have been diagnosed with this often fatal disease."
The vaccine was developed through a partnership between Merial and Memorial Sloan-Kettering Cancer Center. While Memorial Sloan-Kettering was testing a human melanoma vaccine, they received an inquiry from Dr. Philip Bergman — who at the time was with Animal Medical Center, and currently with Brightheart Veterinary Center — seeking novel treatments for canine melanoma. The discussions resulted in clinical trials of the Memorial Sloan-Kettering melanoma vaccine, and subsequent parallel trials by Dr. Bergman and Memorial Sloan-Kettering refined the dosage and

Vaccine 29 (2011) 1289–1296

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

The introduction of multi-copy CpG motifs into an antiviral DNA vaccine strongly up-regulates its immunogenicity in fish

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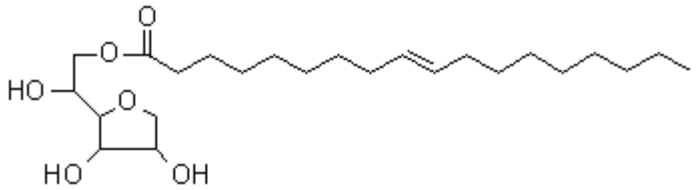
ABSTRACT

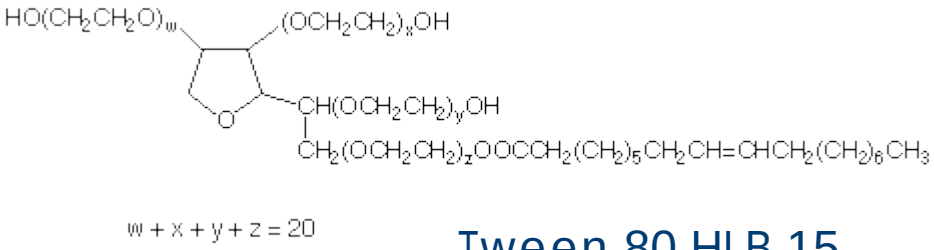
The protection conferred by antiviral DNA vaccines in fish is known to rely greatly on innate immune responses. Since oligodeoxynucleotides (ODNs) containing unmethylated CpG dinucleotides (CpG motifs)

Emulsions

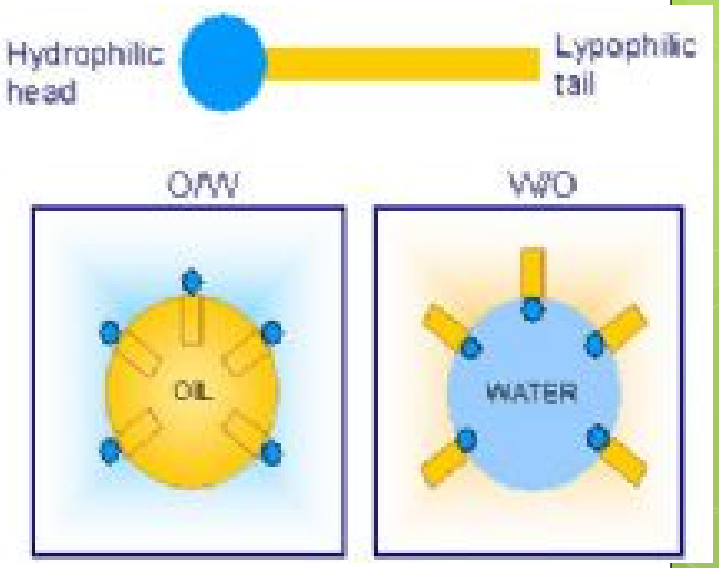
Emulsions: importance of oil and surfactants



Span 80 HLB 4.3



Tween 80 HLB 15



HLB = hydrophilic lipophilic balance

Emulsions: advantages

W/O	O/W	W/O/W
Duration Efficacious with most ags Slow release of the waterphase No systemic reactions	Low viscosity Early onset Fast release of the waterphase No residues	Duration Early onset possible Efficacious with most ags No residues

Emulsions: disadvantages

W/O	O/W	W/O/W
Duration Efficacious with most ags Slow release of the waterphase No systemic reactions	Low viscosity Early onset Fast release of the waterphase No residues	Duration Early onset possible Efficacious with most ags No residues
Viscous **No early onset** **Local reactions** **Residues**	**No duration** **Systemic reactions** **Ag dependent efficacy**	**Not stable**

Example FMDV Adjuvants

Adjuvant	Cattle			Sheep			Pigs		
	Eff	Saf	Syr	Eff	Saf	Syr	Eff	Saf	Syr
O/W	+++	+	-	+++	+	-	+++	-	-
W/O/W	++	++	++	++	++	++	+++	+++	++
Gel	+	++	++	+	++	++	-	++	++

Eff, efficacy; Saf, safety; Syr, Syringablility

Formulation, Formulation, Formulation

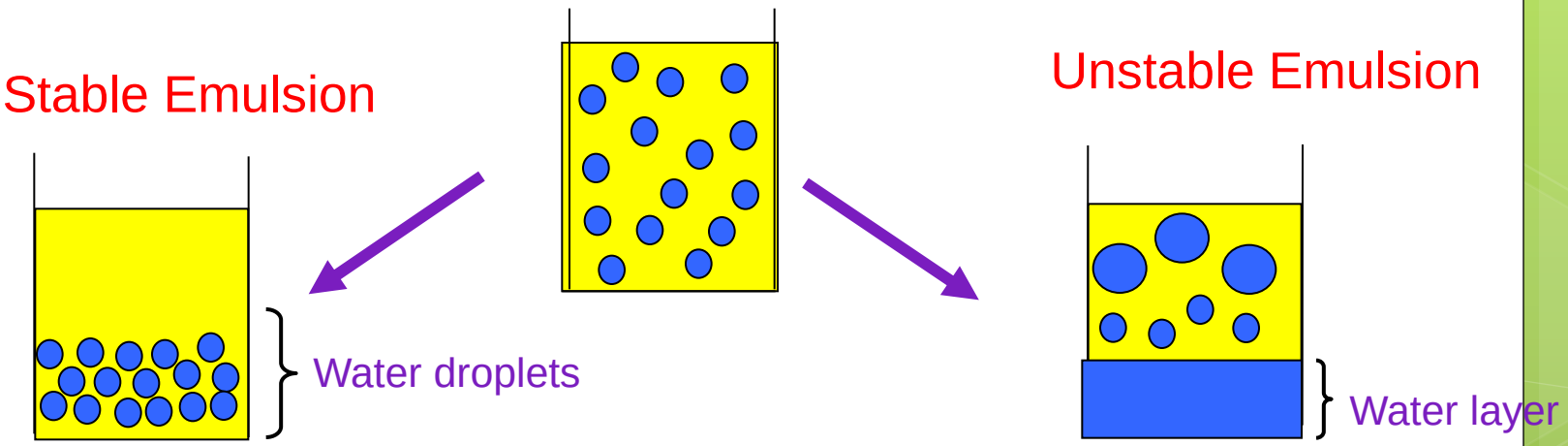
- Conditions of formulation at least as important than the actual ingredients
- Multitude of suppliers, not all have equal quality, emulsifiers VG, HLB of oil and emulsifiers
- Mineral oil; Marcol, Drakeol, Vegetal oil, Squalene
- Order and speed of adding ingredients
- Production parameters (pressure, droplet size, sheer force, temperature, charge, time, nature, combination and % emulsifiers…)

Formulation, Formulation, Formulation

- Specifications and physicochemical parameters;

- Macroscopic or visual appearance
- Microscopic appearance; x% droplets smaller than X um
- Viscosity test at 25^0C
- Drop dispersal test
- Stability at 4^0C
- Accelerated stability at 37^0C

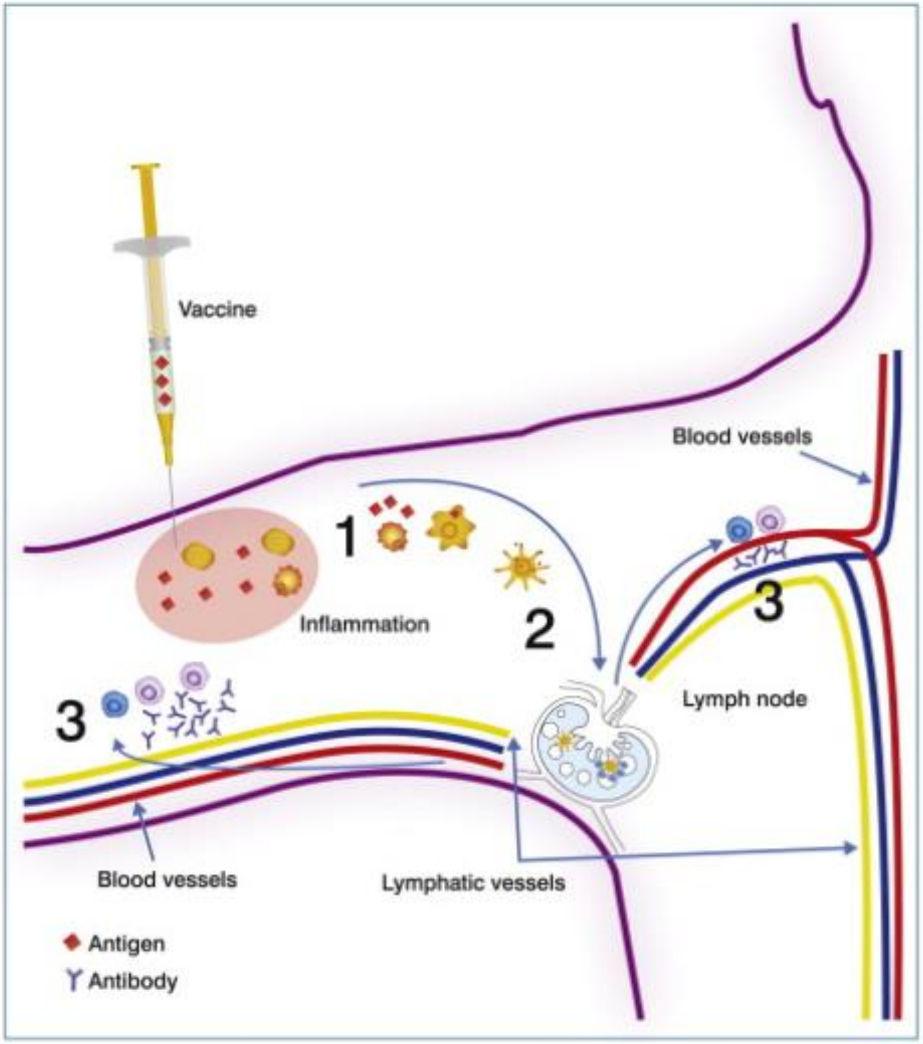
Stability of an emulsion W/O



sedimentation

breaking

Working mechanism of oil emulsions



From Oberdan, et al. 2011

Emulsification equipment

Small scale

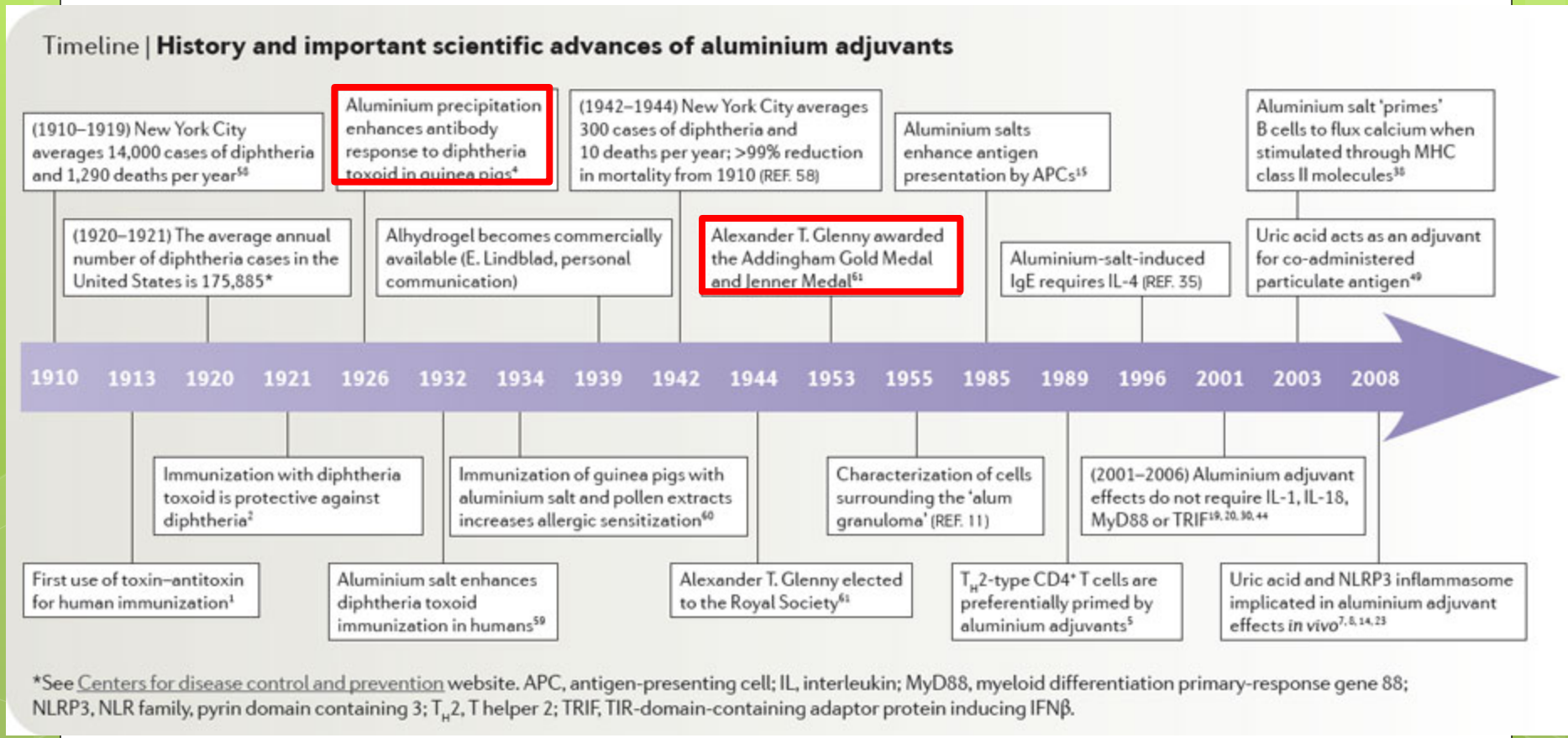
Large scale

Emulsification equipment

Microfluidizers

Aluminium adjuvants

Historical developments Aluminium adjuvants



From Marrack et al, Nature Reviews Immunology 9, 287-293, 2009

Aluminium-based adjuvants available

Name	Chemical formula	Chemical name
Alum	$AlK(SO_4)_2$	Aluminium potassium phosphate
Alhydrogel/Rehydragel	$Al(OH)_3$	Aluminiumhydroxide
Adjuphos/Rehydraphos	$Al(PO_4)_3$	Aluminiumphosphate
Merck Aluminium Adjuvant		Aluminiumhydroxy-phosphatesulfate
Imject Alum	$Al(OH)_3 + Mg(OH)_2$	Aluminiumhydroxide + Magnesiumhydroxide

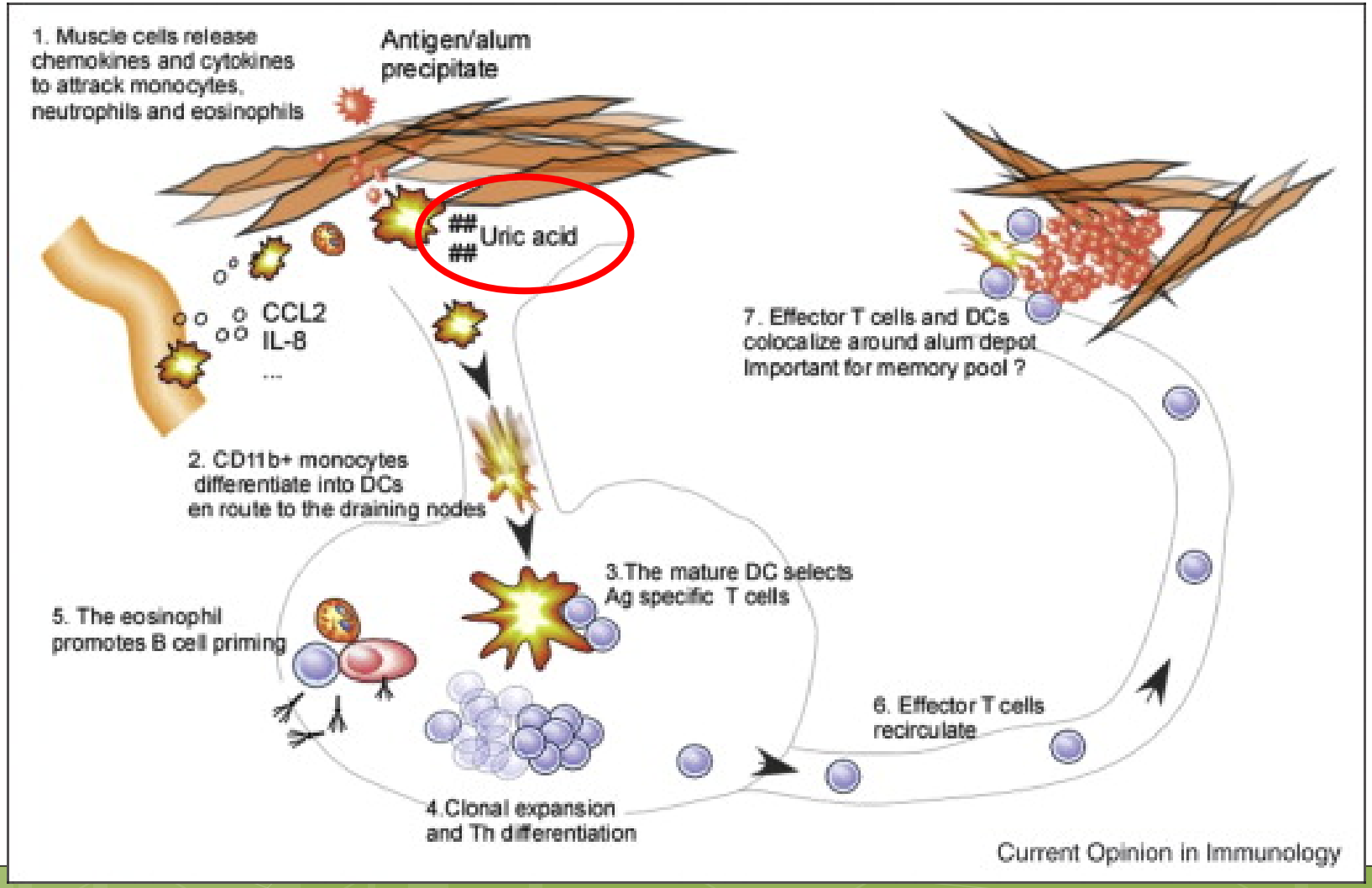
Antigen binding to Aluminium adjuvants

- Various types have different characteristics
  - Alhydrogel = Boehmite structure, fiber-based; gel
  - Alu phosphate = amorphous structure; suspension
- Binding can be influenced by:
  - IEP, pH and buffers
  - Surfactants (desorption)
  - Competing proteins
- No freezing of Alhydrogel □oss of structure

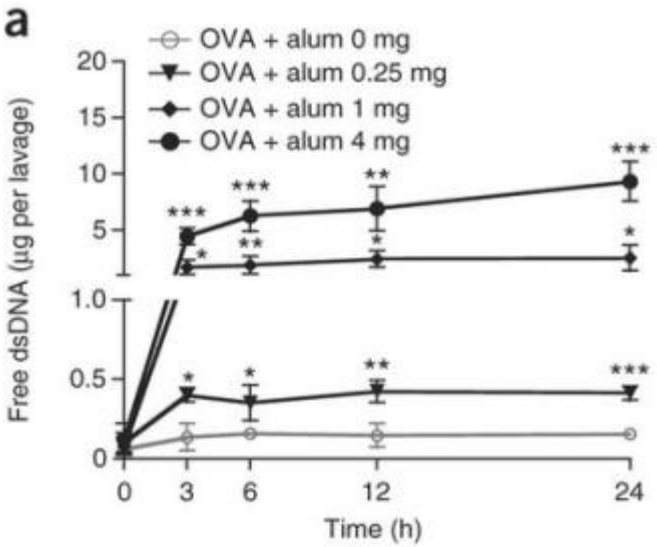
Aluminium adjuvants: proposed mode of action

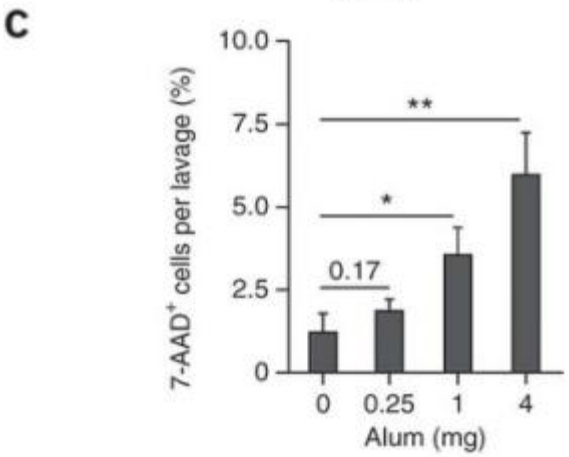
- Depot and slow release
- Increases ag uptake and reduces ag degradation
- Inflammation □ release of uric acid □ inflammasome □ pro-inflammatory cytokines
- Cell toxicity □ DNA release □ rf3 (in) dependent induction of antibody responses
- Interaction with lipids in DC cell membranes □ better (co-stimulatory) signaling process

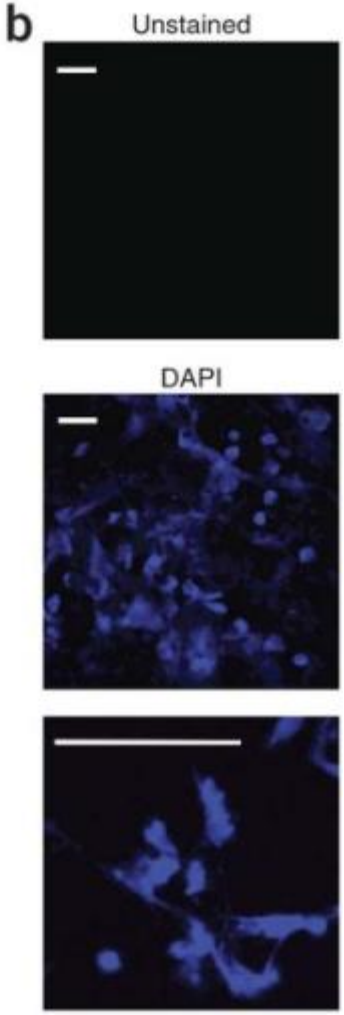
Aluminium adjuvants: mode of action-1



Aluminium adjuvants: mode of action-2



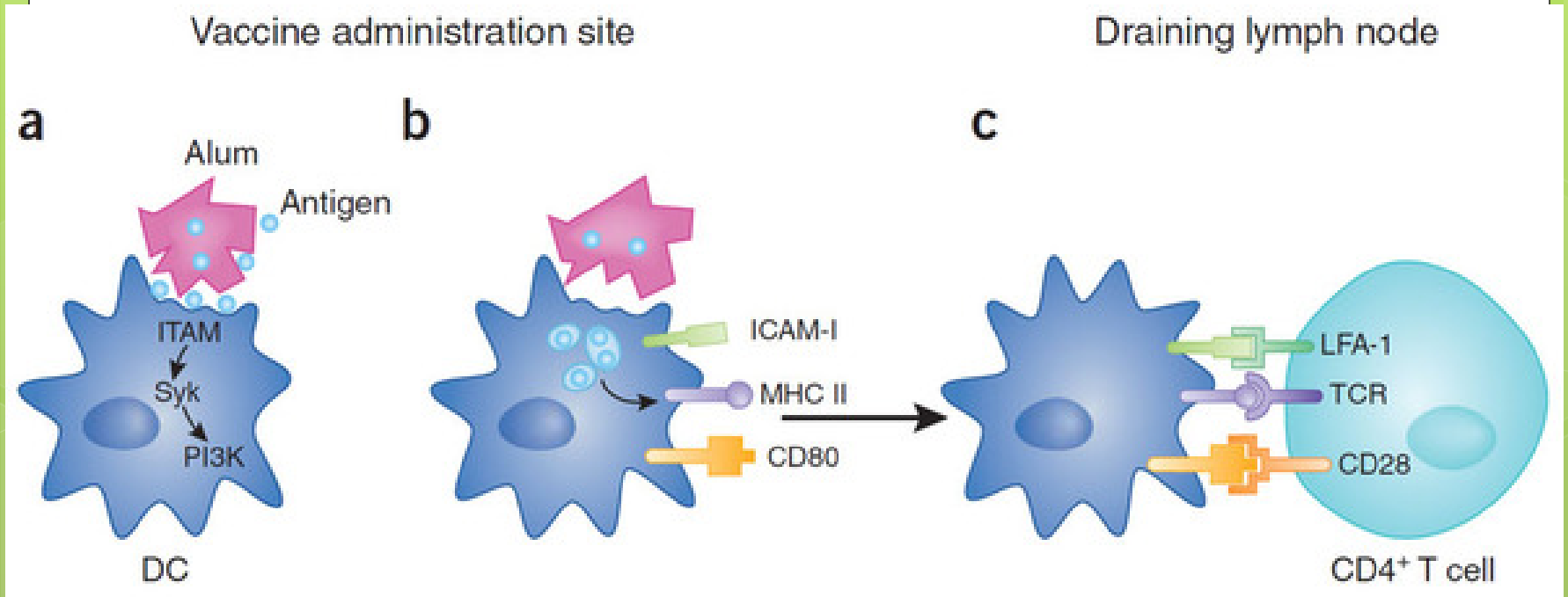




DNA release

From Marichal et al Nature Medicine, 17, 996-1002, 2011

Aluminium adjuvants: mode of action-3



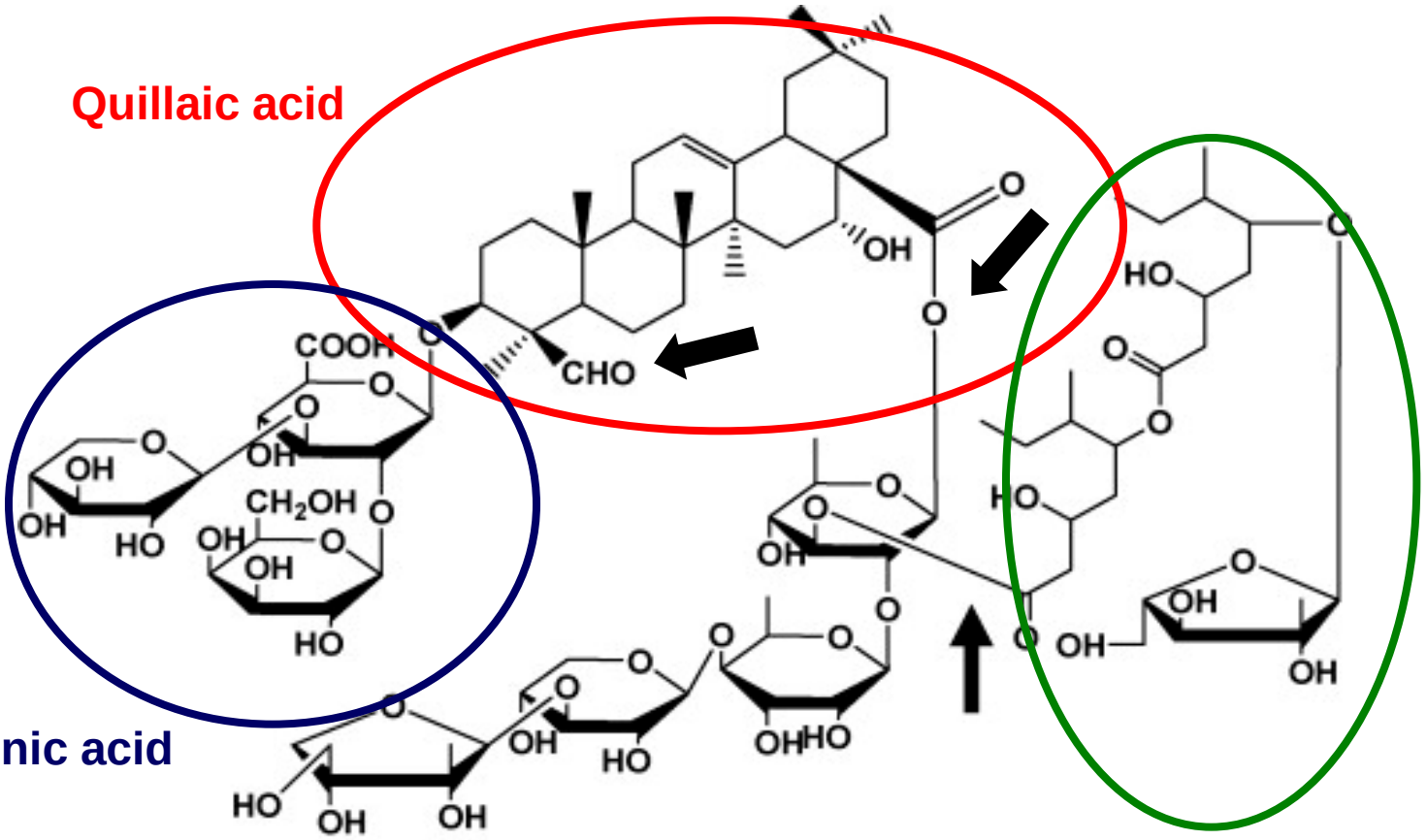
Interaction with cell membranes

From Lamine Mbow et al Nature Medicine 17, 415-416, 2011

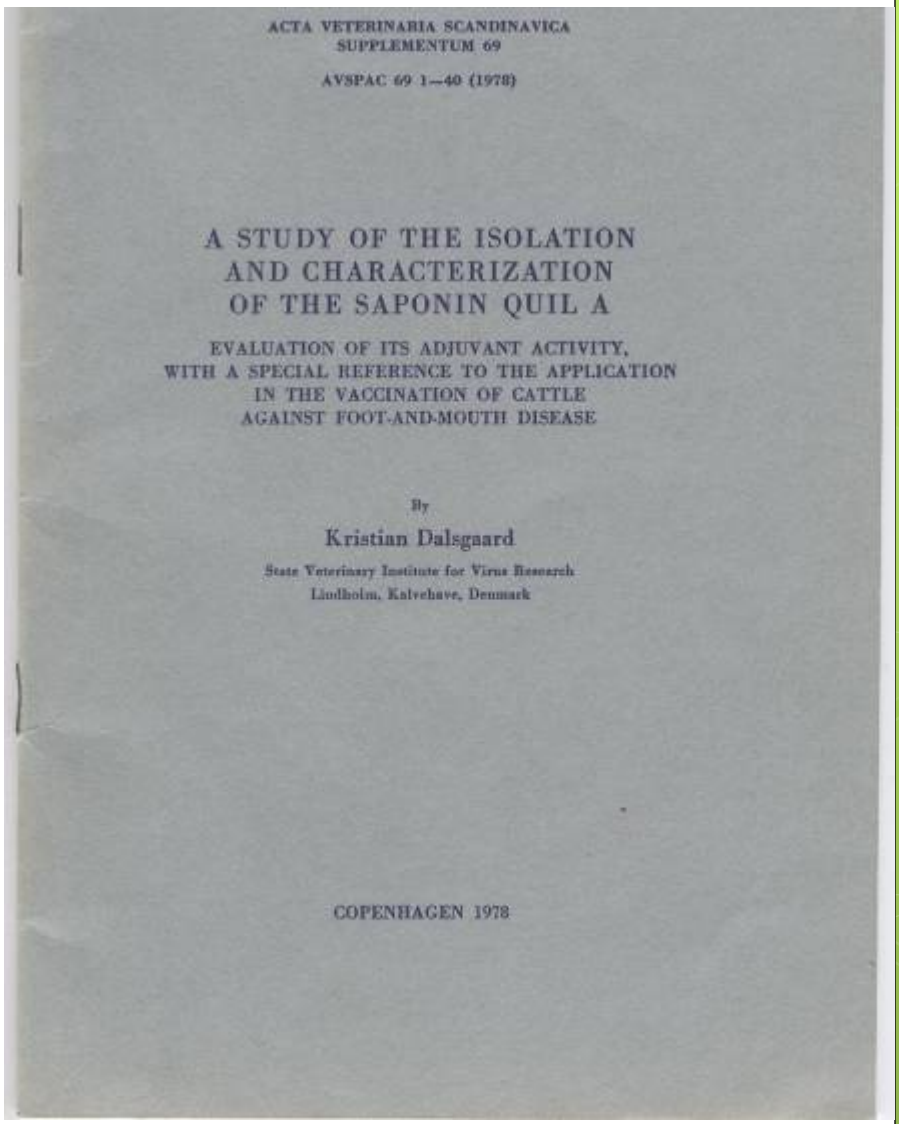
Saponins as adjuvants

Saponins as adjuvants

- Isolated from *Quillaja saponaria molina*
- Foaming agent
- Used in shampoos, drinks and drilling fluids
- Use as adjuvant as (semi-purified) saponins (Quil A, Q-vac, QS21)
- Use in defined structures
  - Iscom (with built in antigen)
  - (Empty) Iscomatrix / Iscom


Quillaic acid
Glucuronic acid
Acyl chain

The "Godfather" of saponin research



ACTA VETERINARIA SCANDINAVICA
SUPPLEMENTUM 69

AVSPAC 69 1–40 (1978)

A STUDY OF THE ISOLATION AND CHARACTERIZATION OF THE SAPONIN QUIL A

EVALUATION OF ITS ADJUVANT ACTIVITY, WITH A SPECIAL REFERENCE TO THE APPLICATION IN THE VACCINATION OF CATTLE AGAINST FOOT-AND-MOUTH DISEASE

By

Kristian Dalsgaard

State Veterinary Institute for Virus Research
Lindholm, Kalvehave, Denmark

COPENHAGEN 1978

Example of a non-licensed adjuvant : AS01 in a Malaria vaccine

EDITORIAL

A Vaccine for Malaria

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

First Results of Phase 3 Trial of RTS,S/AS01 Malaria Vaccine in African Children

The RTS,S Clinical Trials Partnership*

ABSTRACT

BACKGROUND

An ongoing phase 3 study of the efficacy, safety, and immunogenicity of candidate malaria vaccine RTS,S/AS01 is being conducted in seven African countries.

METHODS

From March 2009 through January 2011, we enrolled 15,460 children in two age categories — 6 to 12 weeks of age and 5 to 17 months of age — for vaccination with either RTS,S/AS01 or a non-malaria comparator vaccine. The primary end point of the analysis was vaccine efficacy against clinical malaria during the 12 months after vaccination in the first 6000 children 5 to 17 months of age at enrollment who received all three doses of vaccine according to protocol. After 250 children had an episode of severe malaria, we evaluated vaccine efficacy against severe malaria in both age categories.

RESULTS

In the 14 months after the first dose of vaccine, the incidence of first episodes of clinical malaria in the first 6000 children in the older age category was 0.32 episodes per person-year in the RTS,S/AS01 group and 0.55 episodes per person-year in the control group, for an efficacy of 50.4% (95% confidence interval [CI], 45.8 to 54.6) in the intention-to-treat population and 55.8% (97.5% CI, 50.6 to 60.4) in the per-protocol population. Vaccine efficacy against severe malaria was 45.1% (95% CI, 23.8 to 60.5) in the intention-to-treat population and 47.3% (95% CI, 22.4 to 64.2) in the per-protocol population. Vaccine efficacy against severe malaria in the combined age categories was 34.8% (95% CI, 16.2 to 49.2) in the per-protocol population during an average follow-up of 11 months. Serious adverse events occurred with a similar frequency in the two study groups. Among children in the older age category, the rate of generalized convulsive seizures after RTS,S/AS01 vaccination was 1.04 per 1000 doses (95% CI, 0.62 to 1.64).

CONCLUSIONS

The RTS,S/AS01 vaccine provided protection against both clinical and severe malaria in African children. (Funded by GlaxoSmithKline Biologicals and the PATH Malaria Vaccine Initiative; RTS,S ClinicalTrials.gov number, NCT00866619.)

The authors are listed in the Appendix. All the authors assume responsibility for the overall content and integrity of the article. Address reprint requests to Ms. Kelsey Mertes at PATH Malaria Vaccine Initiative, Communications and Advocacy Unit, 455 Massachusetts Ave. NW, Suite 1000, Washington, DC 20001-2621, or at kmertes@path.org.

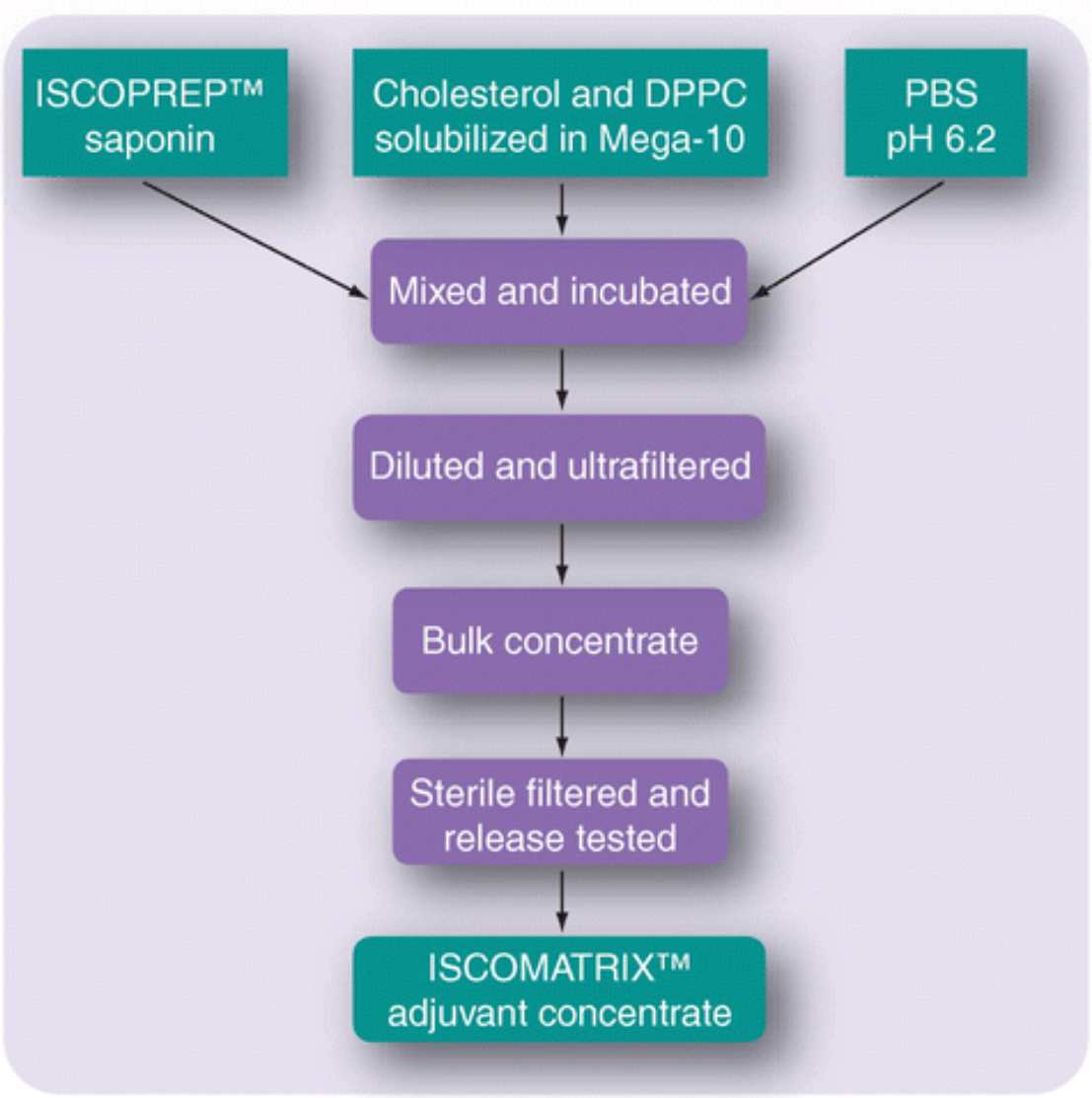
This article (10.1056/NEJMoa1102287) was published on October 18, 2011, at NEJM.org.

N Engl J Med 2011.

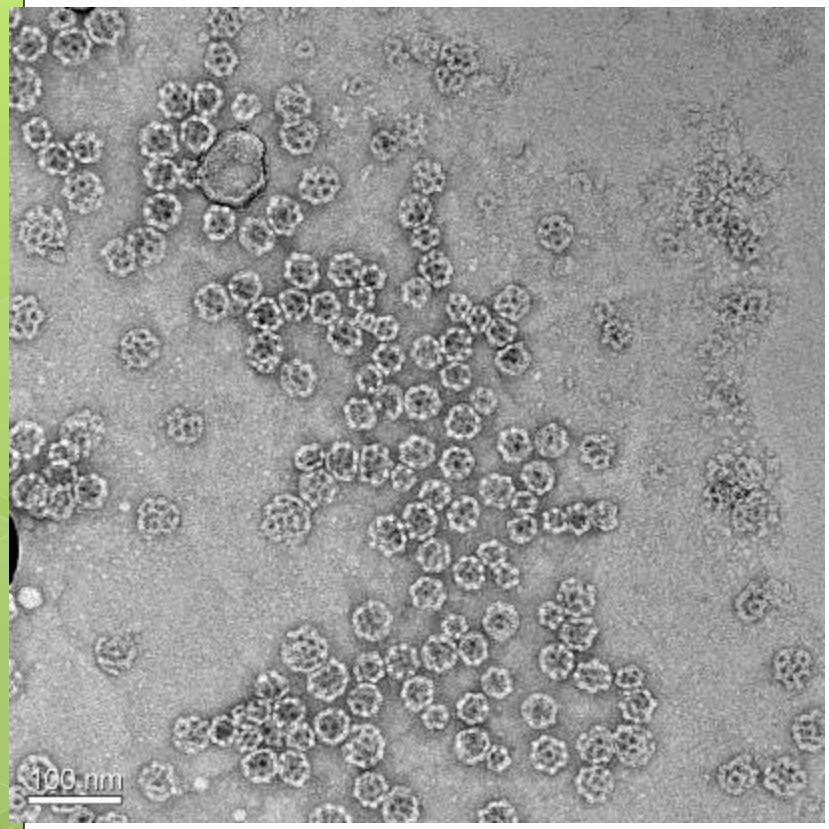
AS01= Liposomes+25µgQS21+25µgMPL

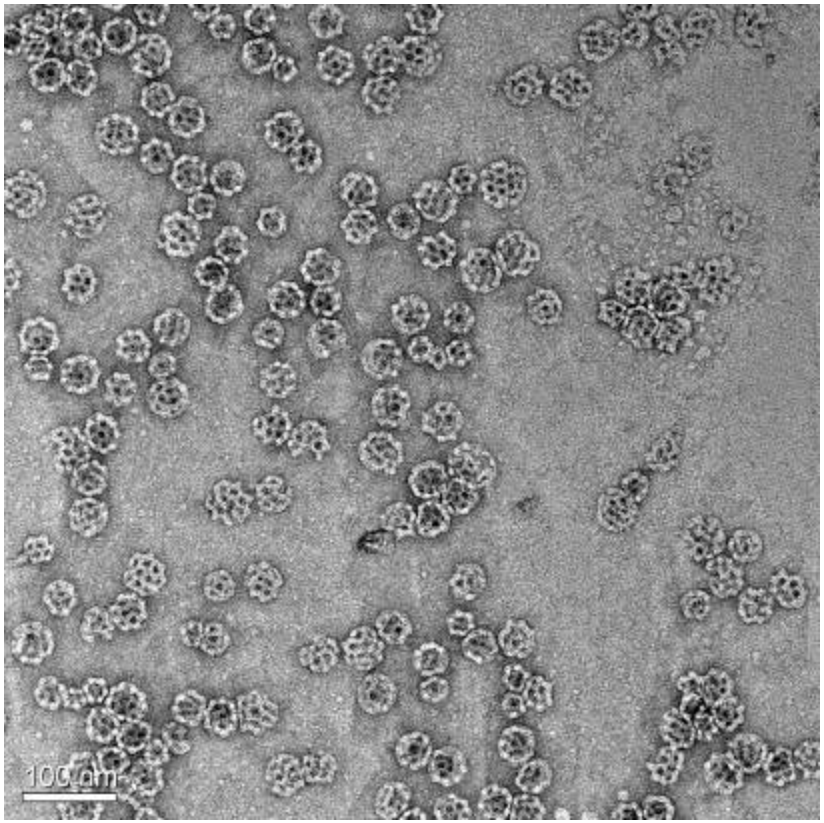
Saponin formulation in Iscom or Matrix

Formulation procedure ISCOMATRIX® by CSL

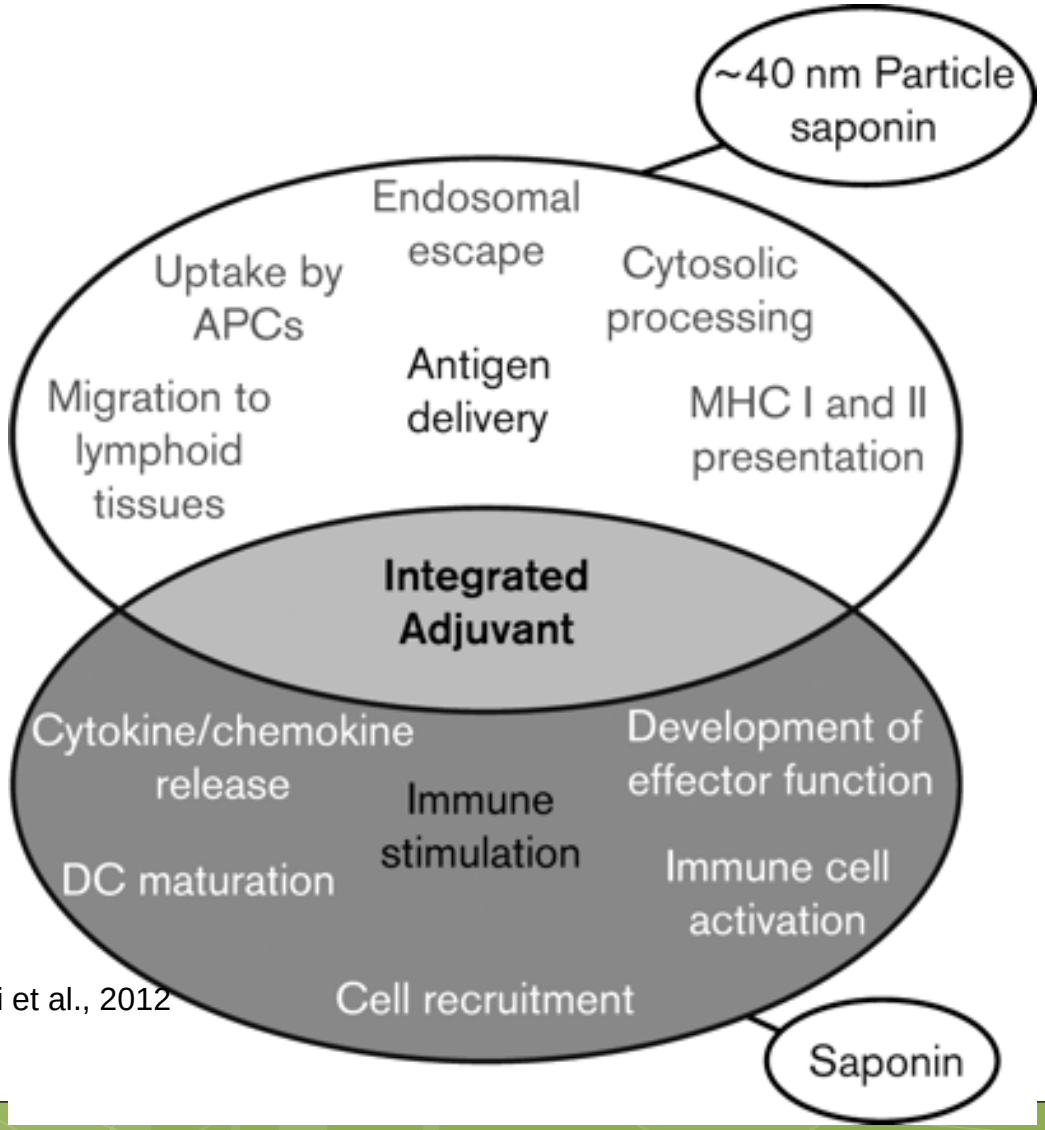


EM pictures Matrix formulations





Mode of action ISCOMS / ISCOMATRIX



From Baz-Morelli et al., 2012

Examples of adjuvants used in veterinary vaccines

Examples of adjuvants used in veterinary vaccines

Species	Adjuvant formulation
Cats	Carbigen® , Aluminium based, Saponin, Matrix C, Carbomer , Emulsigen®, EMA+Neocryl
Dogs	Quil A, Q-vac, Adjumer®, Carbigen, Polygen®, EMA+Neocryl, EMA, Aluminium based
Cattle	Alu-oil, $Al(OH)_3$ ± Saponin, Havlogen, Amphigen®, MO w/o, o/w and w/o/w emulsions, EMA+DDA
Sheep	DEAE-dextran, $Al(OH)_3$ ± Saponin, Alum, MO emulsions, Levamisol
Horses	Iscoms, Matrix C, Havlogen, Carbomers, Aluminium based, Saponin, Immunostim
Pigs	MO and NMO w/o o/w and w/o/w emulsions, Carbomers, Aluminium, Levamisol
Chickens	MO w/o emulsions, Aluminium based, Saponin
Fish	MO and NMO w/o and o/w emulsions

Adjuvants used in veterinary vaccines

- Final choices are influenced by:
  - Target animal species
  - Balance between safety and efficacy
  - Manufacturing issues: COG, use in *in vitro* potency tests
  - Marketing issues
  - Potential combination(s) with other (live) antigen components

Adjuvants for human vaccines

Adjuvants for human vaccines

Adjuvant name (year licensed)	Adjuvant class	Components	Vaccines (disease)
Adjuvants licensed for use in human vaccines			
Alum* (1924)	Mineral salts	Aluminium phosphate or aluminium hydroxide	Various
MF59 (Novartis; 1997)	Oil-in-water emulsion	Squalene, polysorbate 80 (Tween 80; ICI Americas), sorbitan trioleate (Span 85; Croda International)	Fluad (seasonal influenza), Focetria (pandemic influenza), Aflunov (pre-pandemic influenza)
AS03 (GlaxoSmithKline; 2009)	Oil-in-water emulsion	Squalene, Tween 80, α-tocopherol	Pandremix (pandemic influenza), Prepandrix (pre-pandemic influenza)
Virosomes (Berna Biotech; 2000)	Liposomes	Lipids, hemagglutinin	Inflexal (seasonal influenza), Epaxal (hepatitis A)
AS04* (GlaxoSmithKline; 2005)	Alum-absorbed TLR4 agonist	Aluminium hydroxide, MPL	Fendrix (hepatitis B), Cervarix (human papilloma virus)
Vaccine adjuvants tested in humans but not licensed for use			
CpG 7909, CpG 1018	TLR9 agonist	CpG oligonucleotides alone or combined with alum/emulsions	–
Imidazoquinolines	TLR7 and TLR8 agonists	Small molecules	–
Poly I:C	TLR3 agonist	Double-stranded RNA analogues	–
Pam3Cys	TLR2 agonist	Lipopeptide	–
Flagellin	TLR5 agonist	Bacterial protein linked to antigen	–
Iscomatrix	Combination	Saponin, cholesterol, dipalmitoylphosphatidylcholine	–
AS01	Combination	Liposome, MPL, saponin (QS21)	–
AS02	Combination	Oil-in-water emulsion, MPL, saponin (QS21)	–
AF03	Oil-in-water emulsion	Squalene, Montane 80, Eumulgin B1 PH	–
CAF01	Combination	Liposome, DDA, TDB	–
IC31	Combination	Oligonucleotide, cationic peptides	–

AF03, adjuvant formulation 03; CAF01, cationic adjuvant formulation 01; DDA, dimethyldioctadecylammonium; MPL, monophosphoryl lipid A; Pam3Cys, tripalmitoyl-S-glyceryl cysteine; Poly I:C, polyinosinic–polycytidylic acid; TDB, trehalose dibehenate; TLR, Toll-like receptor. *Adjuvants licensed in the United States.

Adjuvants for human vaccines

Adjuvant name (year licensed)	Adjuvant class	Components	Vaccines (disease)
Adjuvants licensed for use in human vaccines			
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Pam3Cys	TLR2 agonist	Lipopeptide	–
Flagellin	TLR5 agonist	Bacterial protein linked to antigen	–
Iscomatrix	Combination	Saponin, cholesterol, dipalmitoylphosphatidylcholine	–
AS01	Combination	Liposome, MPL, saponin (QS21)	–
AS02	Combination	Oil-in-water emulsion, MPL, saponin (QS21)	–
AF03	Oil-in-water emulsion	Squalene, Montane 80, Eumulgin B1 PH	–
CAF01	Combination	Liposome, DDA, TDB	–
IC31	Combination	Oligonucleotide, cationic peptides	–

AF03, adjuvant formulation 03; CAF01, cationic adjuvant formulation 01; DDA, dimethyldioctadecylammonium; MPL, monophosphoryl lipid A; Pam3Cys, tripalmitoyl-S-glyceryl cysteine; Poly I:C, polyinosinic–polycytidylic acid; TDB, trehalose dibehenate; TLR, Toll-like receptor. *Adjuvants licensed in the United States.

Why is there a difference between adjuvant use in licensed human and veterinary vaccines?

- **DIFFERENCES IN LEGISLATION**

Legislation of adjuvants

- **Veterinary vaccines**
  - Considered as excipient
  - Safety and efficacy documentation required of complete vaccine

- **Human vaccines[a]**
  - Safety and efficacy documentation required of vaccine ± antigen
  - Documentation of immunological mode of action
  - Full toxicological profile of all components required
  - Full details on adjuvant characteristics required

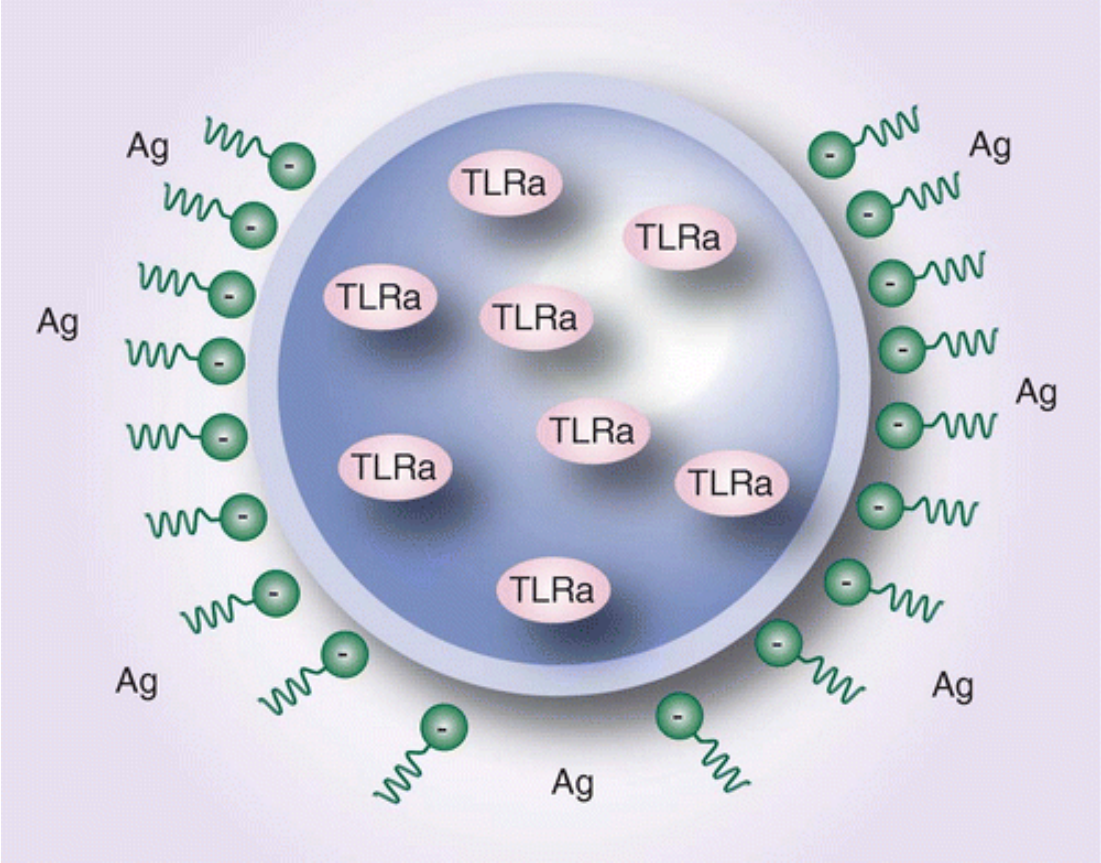
[a] Guideline on adjuvants in vaccines for human use EMEA 2005

Future perspectives

The ideal adjuvant candidate should be…..

- Safe, not associated with any long term effects
- Well tolerated
- Simple, synthetic pathway
- Simple and inexpensive component(s)
- Biodegradable
- Compatible with a wide variety of antigens
- Capable of co-delivery of antigen(s) and immunopotentiator(s)

Example of a new adjuvant formulation



PLG microparticles with TLR agonist encapsulated and antigen adsorbed

From Jain et al, Exp.Rev.Vaccines,10,1731-1742,2011

Some future outlooks

- There will still be a demand for (new) adjuvants
- Requirements for human and veterinary vaccines differ
- There will be a trend for using combinations of TLR and non-TLR agonists in suitable vehicles
- There will be a shift towards needle-less immunizations
- New adjuvant formulations will lead to new vaccines against:
  - Tumors
  - Fertility
  - Obesity
  - Allergy
  - High blood pressure
  - Addiction

Questions?