Innate immunity in livestock and adjuvants

Danny Goovaerts
DGVAC Consultancy

May 9th 2016
“Adjuvants are the immunologist’s dirty little secret”

(Prof. Dr. V. Schijns)
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 superseded 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
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

- Killed vaccines & VLPs
- Live attenuated vaccines & vectors
- Isolate, Inactivate, Inject the causative organism
- Subunit vaccines & recombinant & conjugate 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

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

<table>
<thead>
<tr>
<th>Vehicles</th>
<th>Immunostimulants</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adsorbants</strong>: Aluminium or calcium based</td>
<td><strong>TLR ligands</strong>: LPS, MPL</td>
</tr>
<tr>
<td><strong>Emulsions</strong>: Freund’s type, IFA, O/W, W/O, DOE</td>
<td><strong>Saponins</strong>: QuilA, Qvac</td>
</tr>
<tr>
<td><strong>Polymers</strong>: Carbopol</td>
<td><strong>Cytokines</strong>: GM-CSF</td>
</tr>
<tr>
<td><strong>Vesicles</strong>: Liposomes/Virosomes</td>
<td><strong>Bacterial toxins</strong>: CT and LT</td>
</tr>
<tr>
<td>Iscoms</td>
<td><strong>Drugs</strong>: Levamisole</td>
</tr>
<tr>
<td><strong>Microspheres or nanospheres</strong>: PLAGA</td>
<td></td>
</tr>
</tbody>
</table>
Some adjuvant structures

Oil-in-water

Water-in-oil

Liposomes

Iscoms
Adjuvants: a variety of structures

- EMA
- Carbomer
- Mineral oil
- Alhydrogel
More adjuvant structures

DDA

Vitamin E in AS03 and Diluvac Forte

Inulin (Advax)

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

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).
Makinon SR, Zhu Q, Davis H, L, Weeratna RD.

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

Development of CpG ODN Based Vaccine Adjuvant Formulations.
Gursel M, Gursel I.

Characterization of Immune Responses to an Inactivated Avian Influenza Virus Vaccine Adjuvanted with Nanoparticles Containing CpG ODN.
Singh SM, Alkile TN, Abdelaziz KT, Hodgins DC, Novy A, Nagy E, Sharif S.
MERICAN 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. 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.

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. 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. 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.

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

S. Martinez-Alonso\textsuperscript{a}, A. Martinez-Lopez\textsuperscript{b}, A. Estepa\textsuperscript{b}, A. Cuesta\textsuperscript{a}, C. Tafalla\textsuperscript{a,}\textsuperscript{*}

\textsuperscript{a} Centro de Investigación en Sanidad Animal (CISA-INIA), Carretera de Algete a El Casar km. 8.1, Valdeolmos 28130 (Madrid), Spain
\textsuperscript{b} IBMC, Miguel Hernández University, Elche 03202, Spain

\begin{tabular}{ll}
\textbf{ARTICLE INFO} & \textbf{ABSTRACT} \\
\hline
\end{tabular}

\begin{tabular}{l}
Article history: \\
Received 14 September 2010 \\
\end{tabular}

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

<table>
<thead>
<tr>
<th></th>
<th>W/O</th>
<th>O/W</th>
<th>W/O/W</th>
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<tbody>
<tr>
<td>Duration</td>
<td>Early onset possible</td>
<td>Low viscosity</td>
<td>Duration</td>
</tr>
<tr>
<td>Efficacious with most ags</td>
<td>Efficacious with most ags</td>
<td>Early onset</td>
<td>Early onset possible</td>
</tr>
<tr>
<td>Slow release of the waterphase</td>
<td>Fast release of the waterphase</td>
<td>Fast release of the waterphase</td>
<td>Efficacious with most ags</td>
</tr>
<tr>
<td>No systemic reactions</td>
<td>No residues</td>
<td>No residues</td>
<td>No residues</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>W/O</th>
<th>O/W</th>
<th>W/O/W</th>
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<tbody>
<tr>
<td><strong>Duration</strong></td>
<td>Efficacious with most ags</td>
<td>Low viscosity</td>
<td>Duration</td>
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<tr>
<td><strong>Viscosity</strong></td>
<td>Viscous</td>
<td>No duration</td>
<td>Not stable</td>
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<tr>
<td><strong>Early onset</strong></td>
<td>No early onset</td>
<td>Systemic reactions</td>
<td></td>
</tr>
<tr>
<td><strong>Release</strong></td>
<td>Slow release of the waterphase</td>
<td>Fast release of the waterphase</td>
<td></td>
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<tr>
<td><strong>Reactions</strong></td>
<td>No systemic reactions</td>
<td>Ag dependent efficacy</td>
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<tr>
<td><strong>Residues</strong></td>
<td>No residues</td>
<td></td>
<td></td>
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<tr>
<td><strong>Stability</strong></td>
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</table>
# Example FMDV Adjuvants

<table>
<thead>
<tr>
<th>Adjuvant</th>
<th>Cattle</th>
<th>Sheep</th>
<th>Pigs</th>
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<tbody>
<tr>
<td></td>
<td>Eff</td>
<td>Saf</td>
<td>Syr</td>
</tr>
<tr>
<td>O/W</td>
<td>+++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>W/O/W</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Gel</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

Eff, efficacy; Saf, safety; Syr, Syringability
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°C
- Drop dispersal test
- Stability at 4°C
- Accelerated stability at 37°C
Stability of an emulsion W/O

Stable Emulsion

Unstable Emulsion

Water droplets

Water layer

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

<table>
<thead>
<tr>
<th>Name</th>
<th>Chemical formula</th>
<th>Chemical name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alum</td>
<td>$\text{AlK(SO}_4\text{)}_2$</td>
<td>Aluminium potassium phosphate</td>
</tr>
<tr>
<td>Alhydrogel/Rehydragel</td>
<td>$\text{Al(OH)}_3$</td>
<td>Aluminiumhydroxide</td>
</tr>
<tr>
<td>Adjuphos/Rehydraphos</td>
<td>$\text{Al(PO}_4\text{)}_3$</td>
<td>Aluminiumphosphateylphosphatesulfate</td>
</tr>
<tr>
<td>Merck Aluminium Adjuvant</td>
<td></td>
<td>Aluminiumhydroxy-phosphatesulfate</td>
</tr>
<tr>
<td>Imject Alum</td>
<td>$\text{Al(OH)}_3 + \text{Mg(OH)}_2$</td>
<td>Aluminiumhydroxide + Magnesiumhydroxide</td>
</tr>
</tbody>
</table>
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 → loss 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 → Irf3 (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

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
Saponin basic structure

Quillaic acid

Glucuronic acid

Acyl chain
The “Godfather” of saponin research
Example of a non-licensed adjuvant: AS01 in a Malaria vaccine

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 15,461 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 endpoint of the analysis was vaccine efficacy against clinical malaria during the 12 months after vaccination in the first 6296 children 5 to 17 months of age at enrollment who received all three doses of vaccine according to protocol. After 236 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 0.83 episodes per person-year in the control group, for an efficacy of 59.4% (95% confidence interval [CI], 48.8 to 64.6) in the intention-to-treat population and 65.8% (97.5% CI, 50.6 to 79.4) in the per-protocol population. Vaccine efficacy against severe malaria was 45.8% (95% CI, 23.8 to 60.9) in the intention-to-treat population and 74.3% (95% CI, 22.4 to 84.2) in the per-protocol population. Vaccine efficacy against severe malaria in the combined age categories was 58.8% (95% CI, 36.2 to 74.1) 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, NCT00385619.)

AS01= Liposomes+25µgQS21+25µgMPL
Saponin formulation in Iscom or Matrix
Formulation procedure ISCOMATRIX® by CSL

1. ISCOPREP™ saponin
2. Cholesterol and DPPC solubilized in Mega-10
3. PBS pH 6.2

- Mixed and incubated
- Diluted and ultrafiltered
- Bulk concentrate
- Sterile filtered and release tested
- ISCOMATRIX™ adjuvant concentrate
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

<table>
<thead>
<tr>
<th>Species</th>
<th>Adjuvant formulation</th>
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<tbody>
<tr>
<td>Cats</td>
<td>Carbigen®, Aluminium based, Saponin, Matrix C, Carbomer, Emulsigen®, EMA+Neocryl</td>
</tr>
<tr>
<td>Dogs</td>
<td>Quil A, Q-vac, Adjumer®, Carbigem, Polygen®, EMA+Neocryl, EMA, Aluminium based</td>
</tr>
<tr>
<td>Cattle</td>
<td>Alu-oil, Al(OH)₃ ± Saponin, Havlogen, Amphigen®, MO w/o, o/w and w/o/w emulsions, EMA+DDA</td>
</tr>
<tr>
<td>Sheep</td>
<td>DEAE-dextran, Al(OH)₃ ± Saponin, Alum, MO emulsions, Levamisol</td>
</tr>
<tr>
<td>Horses</td>
<td>Iscoms, Matrix C, Havlogen, Carbomers, Aluminium based, Saponin, Immunostim</td>
</tr>
<tr>
<td>Pigs</td>
<td>MO and NMO w/o o/w and w/o/w emulsions, Carbomers, Aluminium, Levamisol</td>
</tr>
<tr>
<td>Chickens</td>
<td>MO w/o emulsions, Aluminium based, Saponin</td>
</tr>
<tr>
<td>Fish</td>
<td>MO and NMO w/o and o/w emulsions</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Adjuvant name (year licensed)</th>
<th>Adjuvant class</th>
<th>Components</th>
<th>Vaccines (disease)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adjuvants licensed for use in human vaccines</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alum* (1924)</td>
<td>Mineral salts</td>
<td>Aluminium phosphate or aluminium hydroxide</td>
<td>Various</td>
</tr>
<tr>
<td>MF59 (Novartis; 1997)</td>
<td>Oil-in-water emulsion</td>
<td>Squalene, polysorbate 80 (Tween 80; ICI Americas), sorbitan trioleate (Span 85; Croda International)</td>
<td>Fluad (seasonal influenza), Focetria (pandemic influenza), Aflunov (pre-pandemic influenza)</td>
</tr>
<tr>
<td>AS03 (GlaxoSmithKline; 2009)</td>
<td>Oil-in-water emulsion</td>
<td>Squalene, Tween 80, α-tocopherol</td>
<td>Pandremix (pandemic influenza), Prepandrix (pre-pandemic influenza)</td>
</tr>
<tr>
<td>Virosomes (Berna Biotech; 2000)</td>
<td>Liposomes</td>
<td>Lipids, hemagglutinin</td>
<td>Inflexal (seasonal influenza), Epaxal (hepatitis A)</td>
</tr>
<tr>
<td>AS04* (GlaxoSmithKline; 2005)</td>
<td>Alum-absorbed TLR4 agonist</td>
<td>Aluminium hydroxide, MPL</td>
<td>Fendrix (hepatitis B), Cervarix (human papilloma virus)</td>
</tr>
</tbody>
</table>

### Vaccine adjuvants tested in humans but not licensed for use

<table>
<thead>
<tr>
<th>Adjuvant name</th>
<th>Adjuvant class</th>
<th>Components</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CpG 7909, CpG 1018</td>
<td>TLR9 agonist</td>
<td>CpG oligonucleotides alone or combined with alum/emulsions</td>
<td>–</td>
</tr>
<tr>
<td>Imidazoquinolines</td>
<td>TLR7 and TLR8 agonists</td>
<td>Small molecules</td>
<td>–</td>
</tr>
<tr>
<td>Poly: C</td>
<td>TLR3 agonist</td>
<td>Double-stranded RNA analogues</td>
<td>–</td>
</tr>
<tr>
<td>Pam3Cys</td>
<td>TLR2 agonist</td>
<td>Lipopeptide</td>
<td>–</td>
</tr>
<tr>
<td>Flagellin</td>
<td>TLR5 agonist</td>
<td>Bacterial protein linked to antigen</td>
<td>–</td>
</tr>
<tr>
<td>Iscomatrix</td>
<td>Combination</td>
<td>Saponin, cholesterol, dipalmitoylphosphatidylcholine</td>
<td>–</td>
</tr>
<tr>
<td>AS01</td>
<td>Combination</td>
<td>Liposome, MPL, saponin (QS21)</td>
<td>–</td>
</tr>
<tr>
<td>AS02</td>
<td>Combination</td>
<td>Oil-in-water emulsion, MPL, saponin (QS21)</td>
<td>–</td>
</tr>
<tr>
<td>AF03</td>
<td>Oil-in-water emulsion</td>
<td>Squalene, Montane 80, Eumulgin B1 PH</td>
<td>–</td>
</tr>
<tr>
<td>CAF01</td>
<td>Combination</td>
<td>Liposome, DDA, TDB</td>
<td>–</td>
</tr>
<tr>
<td>IC31</td>
<td>Combination</td>
<td>Oligonucleotide, cationic peptides</td>
<td>–</td>
</tr>
</tbody>
</table>
## Adjuvants for human vaccines

<table>
<thead>
<tr>
<th>Adjuvants licensed for use in human vaccines</th>
<th>Components</th>
<th>Vaccines (disease)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alum</strong> (1924)</td>
<td>Aluminium phosphate or aluminium hydroxide</td>
<td>Various</td>
</tr>
<tr>
<td><strong>MF59</strong> (Novartis; 1997)</td>
<td>Squalene, polysorbate 80 (Tween 80; ICI Americas), sorbitan trioleate (Span 85; Croda International)</td>
<td>Fluad (seasonal influenza), Focetria (pandemic influenza), Aflunov (pre-pandemic influenza)</td>
</tr>
<tr>
<td><strong>AS03</strong> (GlaxoSmithKline; 2009)</td>
<td>Squalene, Tween 80, α-tocopherol</td>
<td>Pandremix (pandemic influenza), Prepandrix (pre-pandemic influenza)</td>
</tr>
<tr>
<td><strong>Virosomes</strong> (Berna Biotech; 2000)</td>
<td>Liposomes</td>
<td>Lipids, hemagglutinin</td>
</tr>
<tr>
<td><strong>AS04</strong> (GlaxoSmithKline; 2005)</td>
<td>Alum-absorbed TLR4 agonist</td>
<td>Fendrix (hepatitis B), Cervarix (human papilloma virus)</td>
</tr>
</tbody>
</table>

### Vaccine adjuvants tested in humans but not licensed for use

<table>
<thead>
<tr>
<th>Vaccine adjuvants tested in humans but not licensed for use</th>
<th>Components</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CpG 7909, CpG 1018</strong></td>
<td>TLR9 agonist</td>
<td>CpG oligonucleotides alone or combined with alum/emulsions</td>
</tr>
<tr>
<td><strong>Imidazoquinolines</strong></td>
<td>TLR7 and TLR8 agonists</td>
<td>Small molecules</td>
</tr>
<tr>
<td><strong>PolyI:C</strong></td>
<td>TLR3 agonist</td>
<td>Double-stranded RNA analogues</td>
</tr>
<tr>
<td><strong>Pam3Cys</strong></td>
<td>TLR2 agonist</td>
<td>Lipopeptide</td>
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AF03, adjuvant formulation 03; CAF01, cationic adjuvant formulation 01; DDA, dimethyldioctadecylammonium; MPL, monophosphoryl lipid A; Pam3Cys, tripalmitoyl-S-glyceryl cysteine; PolyI: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**
  - 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

- **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?