# Adjuvants for Vaccines:

# A pragmatic approach

***Pragmatic:*** *more concerned with practical results than with theories and principles*


## Martin Friede Ph.D.

## Initiative for Vaccine Research

World Health Organization

# Adjuvants

- Added to a vaccine to improve the immune response
  - Increase antibody titers
  - Provide appropriate bias (Th1 / Th2)
  - Induce cell-mediated immunity
  - Reduce antigen dose, number of doses
  - Enhance breadth of response
  - Enable immunization in weakened immune system (eg geriatric)

- For subunit/recombinant vaccines considered critical enabling component

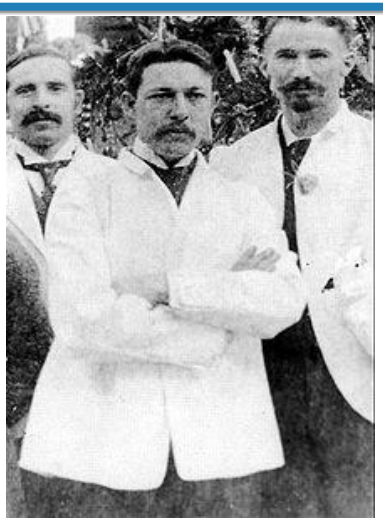
# Alchemy

- The blind testing of anything and everything to turn lead to gold

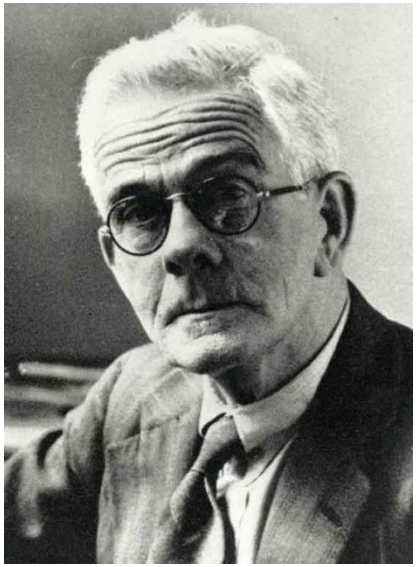

- Vaccinologists testing of anything and everything to convert a poor immunogen into a vaccine

# Alchemy and adjuvants

- 1893: W. Coley
  - Killed bacteria



- 1925: G. Ramon
  - Starch, tapioca, agar,
  - Fish oil,
  - Bark extracts,..



1926: A. Glenny
  - Aluminum hydroxide gel

# Modern adjuvants

- 1893: W. Coley
  - Killed bacteria
- 1925: G. Ramon
  - Starch, tapioca,
  - Fish oil,
  - Bark extracts,..

1926: A. Glenny
  - Aluminum hydroxide

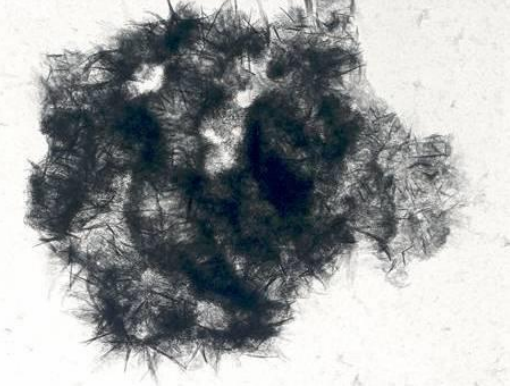
- MPL: AS04, AS01
- CpG
- Inulin
- Squalene: MF59, AS03
- QS21: AS01
- Used ever since…

# Basic mechanisms of adjuvants

- Promote antigen uptake by APCs
- Stimulation of APC
  - Upregulation of cytokines, MHC, co-stimulatory molecules
- Migration of APC to T-cell area of lymph nodes
- Modification of intracellular trafficking ?

*Most adjuvants probably act through several of these mechanisms.*

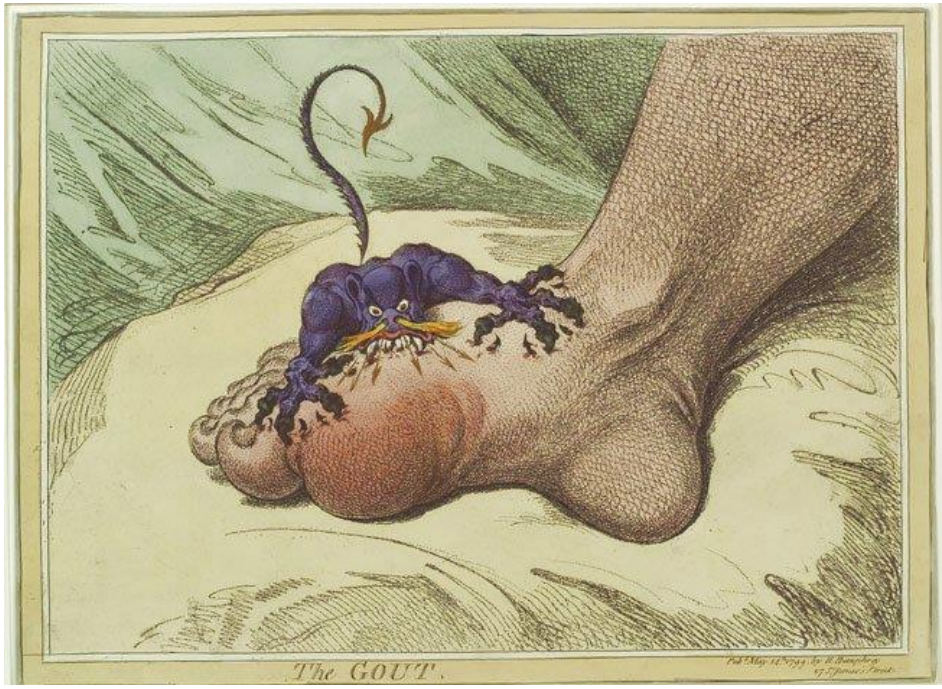
# Aluminium salts (Alum)



- Originally, and still, used for water purification..
- Highly charged, large surface area, adsorbs antigen
- Used since 1920s in many vaccines:
  - DT, TT, Pertussis, HepB, HepA, S. pneu, Meningitis, JE,…
- Different forms available
  - Aluminium hydroxide gel (aluminium oxyhydroxide), phosphate gel, hydroxysulphophosphate,..
  - Different physical characteristics and adjuvant properties !!

# Alum: Modes of action

- Maintain antigen at site (depot effect)
  - Differences observed if antigen adsorbed, not adsorbed
- Local recruitment of APCs and migration to lymph nodes
- NOD-like receptor (cf uric acid for gout)
  - inflammasome complex
  - Proinflammatory cytokine

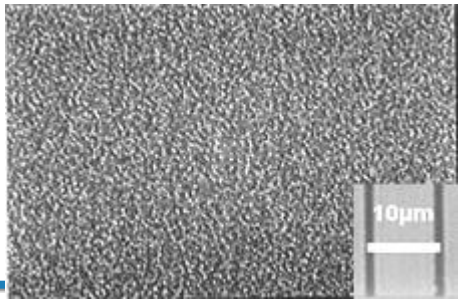



**De Gregorio et al 2008, Eur. J. Immunol**

# The challenges to aluminium salts

- For most antigens adjuvant effect best if adsorbed to alum
  - Buffer effects !
- For some antigens (eg HiB) binding inhibits response.
  - Combo vaccines complex !
- Freezing destroys alum-containing vaccines
  - Accidental freezing is VERY frequent in cold-chain
- Alum salts generate minimal CMI alone, limits usefulness.
  - Add other adjuvants

# Water-in-oil (w/o) emulsions




- Droplets of aqueous phase with surfactant in oil phase

  (mayonnaise)

- 1960s: flu vaccine in UK based on water-in-mineral oil.
  - 60 cases of severe local reaction out of 1,000,000 doses given
- Seppic ISA 720: 30% water 70% squalene
  - >70 completed clinical trials (mainly cancer vaccines, HIV,etc)
  - No product on market

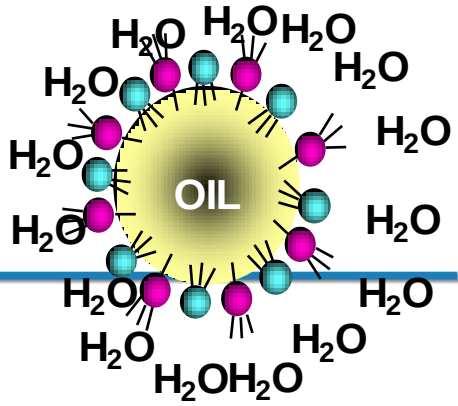
# Mode(s) of action of w/o emulsions

- Not well established
- Depot effect probably critical:
  - slow release of antigen at injection site, enhanced antigen delivery to APC.
  - Size of aqueous droplets also affects response
- Involvement of Nalp / inflammasome ?
  - Nature of oil has effect so local necrosis may also play role

# The difficulties to w/o emulsions

- Frequent local reactogenicity (abscess)
  - Possibly acceptable for therapy, not prophylaxis
- Formulation and scale up: not easy
  - Point-of-use formulation not really feasible !
- Antigen instability
- Role of antigen nature

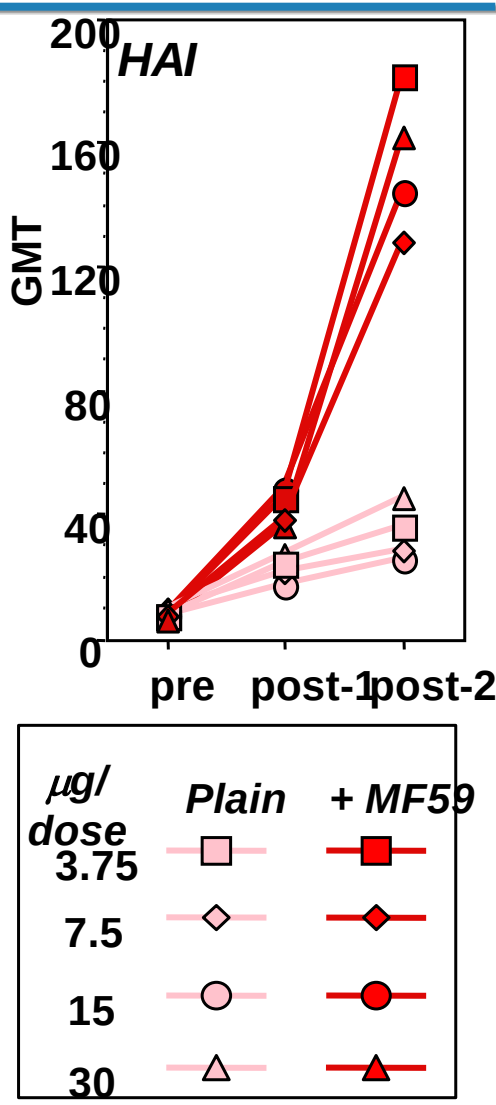
# Oil-in-water emulsions




- Droplets of oil, in water, stabilised with surfactant
  - Squalene, squalane, olive oil, sunflower oil,…
  - Egg-yolk lecithin, tween 80,.. (Salad dressing)
- MF59™ (Novartis): squalene in water with Tween + Span
  - Component of Fluad™ influenza vaccine for elderly
    - Approved in some EU countries since 1996
- AS02 (GSK): squalene + tocopherol + MPL + QS21
  - Developed for malaria vaccines
- AS03 (GSK): squalene+tocopherol
- AF03 (Sanofi), SE (IDRI),… others

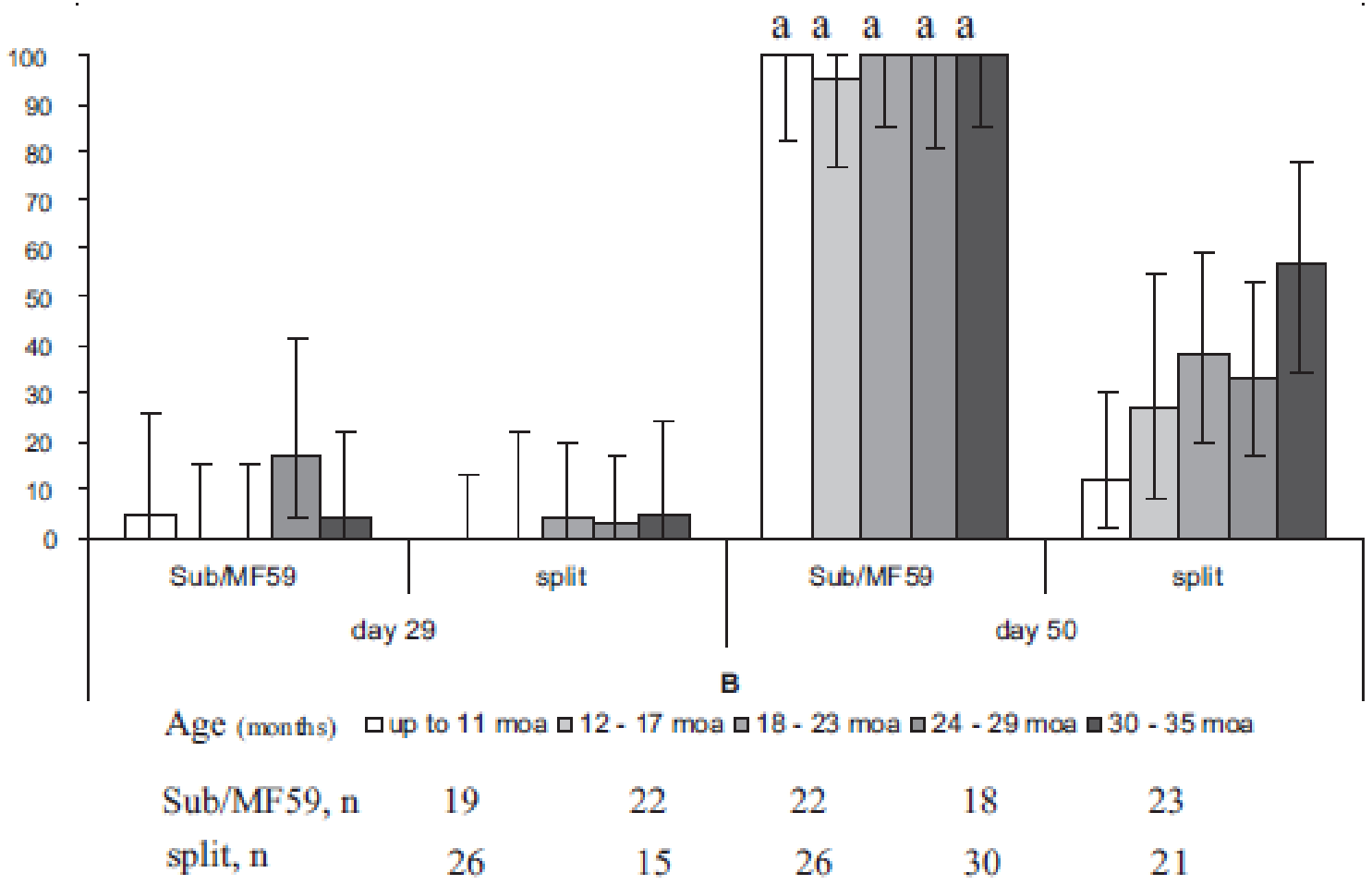
# o/w emulsions and pandemic influenza

- Dose-sparing of antigen seen with o/w
  - 8-10 fold dose reduction for H5N1
    - MF59 (squalene), Novartis
    - AS03 (squalene + tocopherol), GSK
    - AF3 (squalene), Sanofi Pasteur
    - SE (squalene), IDRI
- Incorporated in 2009 H1N1 pandemic vaccine
  - 2-4 fold dose sparing
  - Increase global vaccine production capacity
  - >200 million doses distributed for all ages



# Effect on immune response to inactivated influenza vaccine in infants

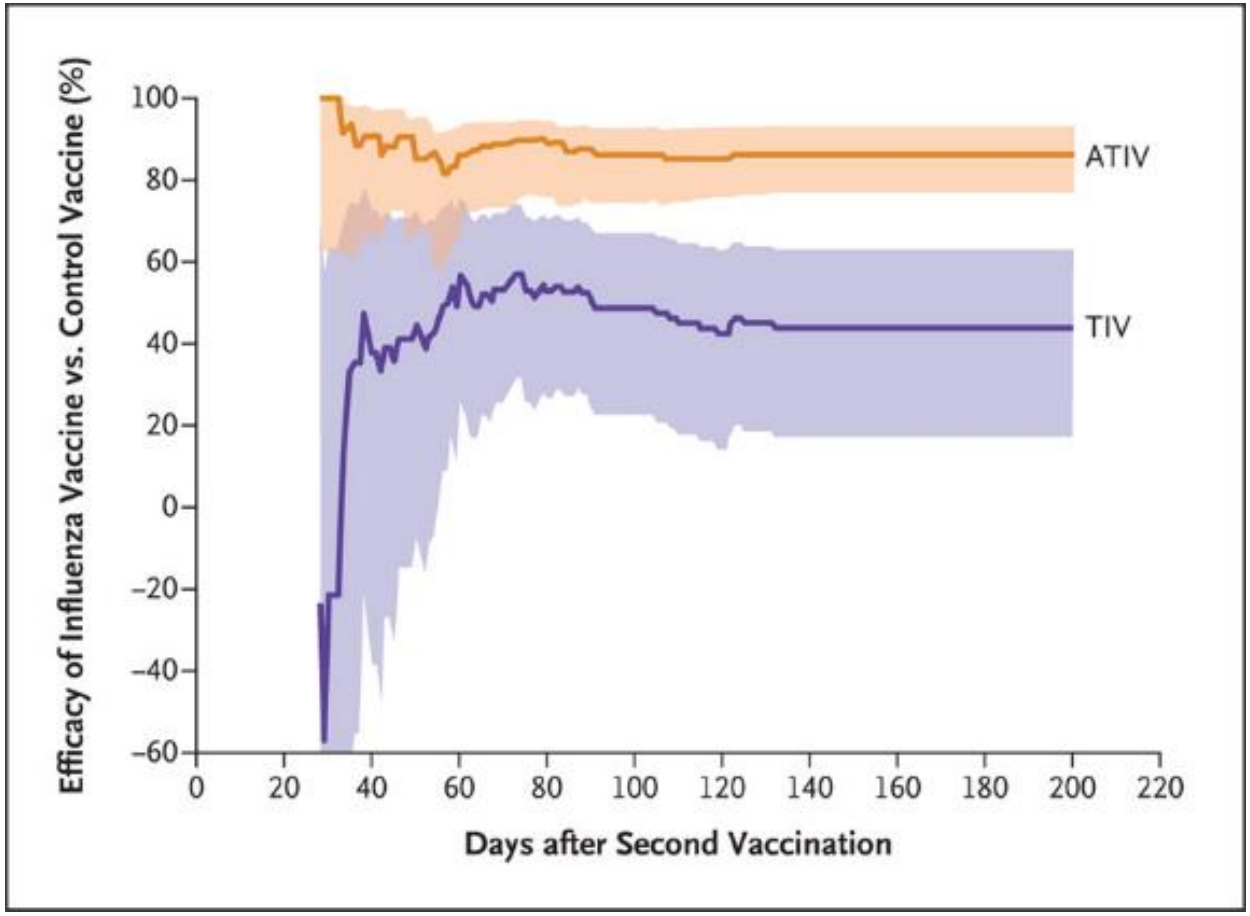
## % HI>40




| | | | | | |
|---|---|---|---|---|---|
| Sub/MF59, n | 19 | 22 | 22 | 18 | 23 |
| split, n | 26 | 15 | 26 | 30 | 21 |

From Vesikari T et al., 2009

# And this translates into efficacy..




Vesikari et al. NEJM October 2011.

- Efficacy of adjuvanted or non-adjuvanted trivalent vaccine

6-72month old infants

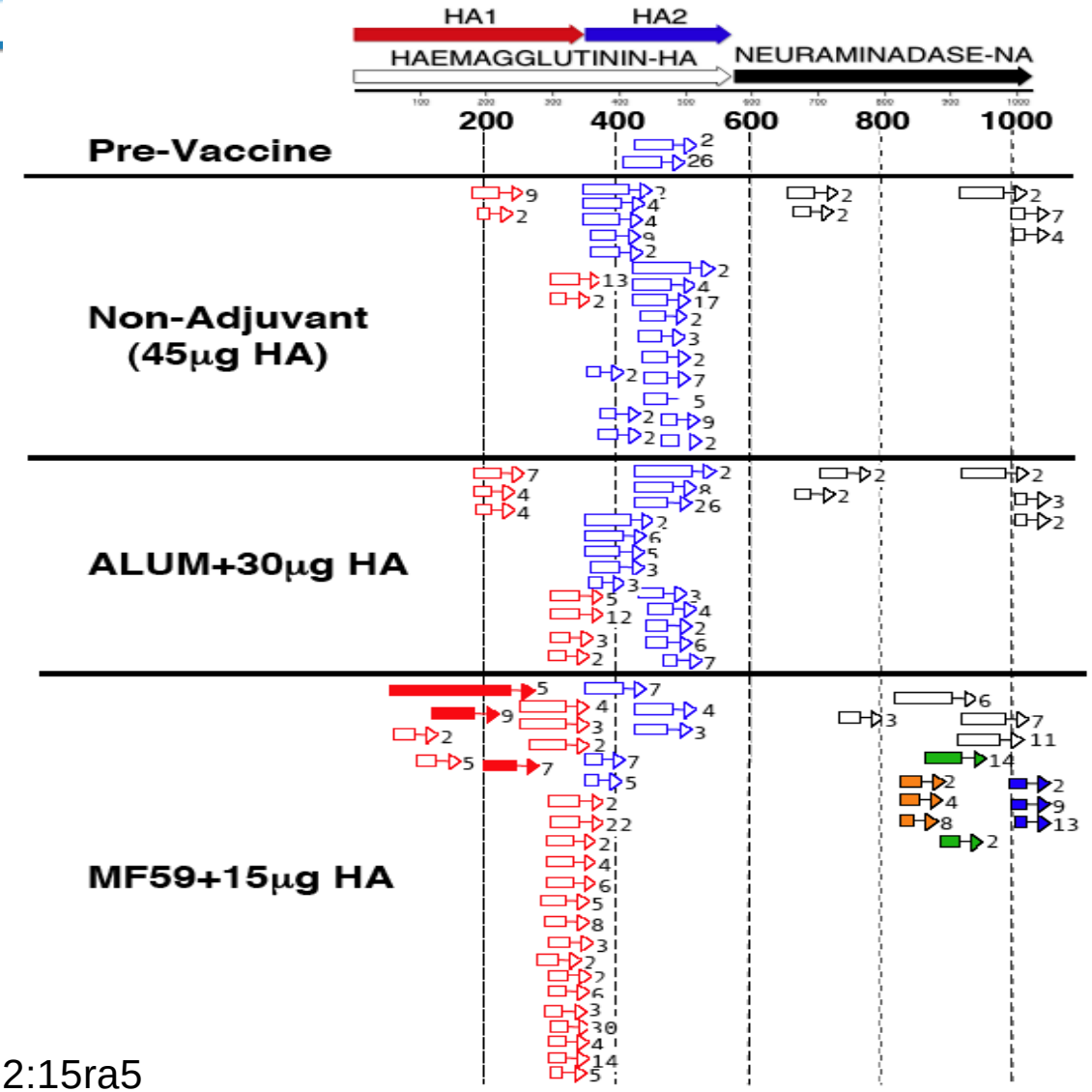
86 vs 45%

6-24 month

75 vs 2 % !!

# O/W adjuvants affect breadth of immune response

- Induction of antibodies against regions of HA (and NA) not normally seen by immune system with unadjuvanted vaccine
  - Common epitopes
  - Neutralising epitopes




Khurana S, et al.(2010) Science Transl Med 20 2010 2:15ra5

# Mode(s) of action of o/w emulsions

- Not fully understood…
  - Direct immune potentiation[1]
    - Local expression of Ptx3 in muscle fibers
    - Local immunocompetent environment (TNFa, IL1b, CCLs)
  - Sustained antigen presentation[1]
    - recruitment of CD11b monocytes, differentiation into DCs expressing high MHCII
- But… how does it achieve this…?
  - Something unique to squalene – not other oils.



1) Mosca et al. *Proc. Natl. Acad. Sci USA*, 2008

# Squalene and adjuvant fears…

UGESKRIFT FOR LÆGER

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Home > Previous issues > 2009 > 43 > Dangerous adjuvant in swine influenza vaccine

SummaryPlus

- Climate change and health
- Convalescence and sick leave
- Hypertension II
- In Hypertension
- Quality in health care II
- Quality in health care in
- Highlights
- Pharmacological treatment

All Databases 2009; 171 (43): 3122

## Harmful adjuvant in swine influenza vaccine

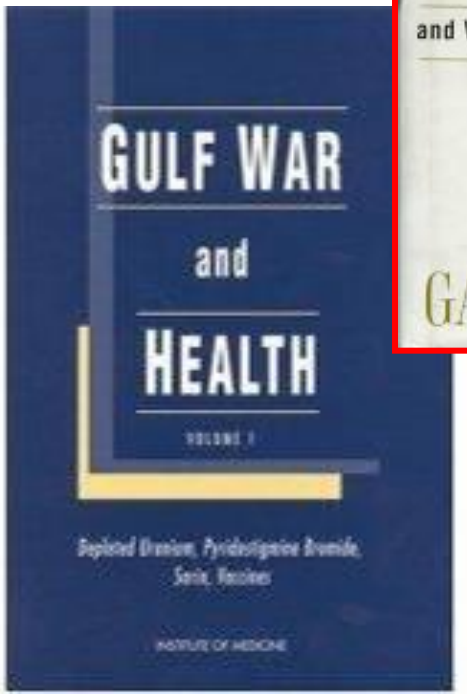
DISCUSSION

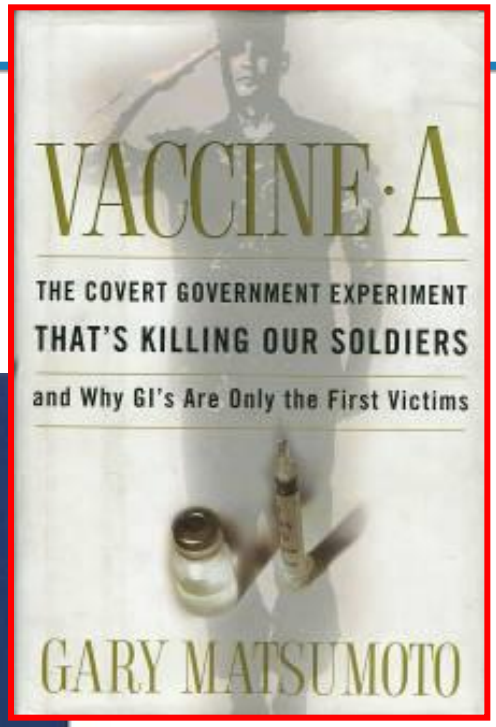
Retired Dr. Anders Brown Laursen, Flensburg. E-mail: brown-laursen@privat.dk

Re. Klaus Larsen interview with Dr. Karl Mølbak in Ugeskrift for Doctors (2009, 171:2616). In the interview mentioned peripherally, that 'the GSK vaccine, Denmark has commissioned, has an adjuvant. The EMEA says that danger can only be determined during and after mass vaccination!

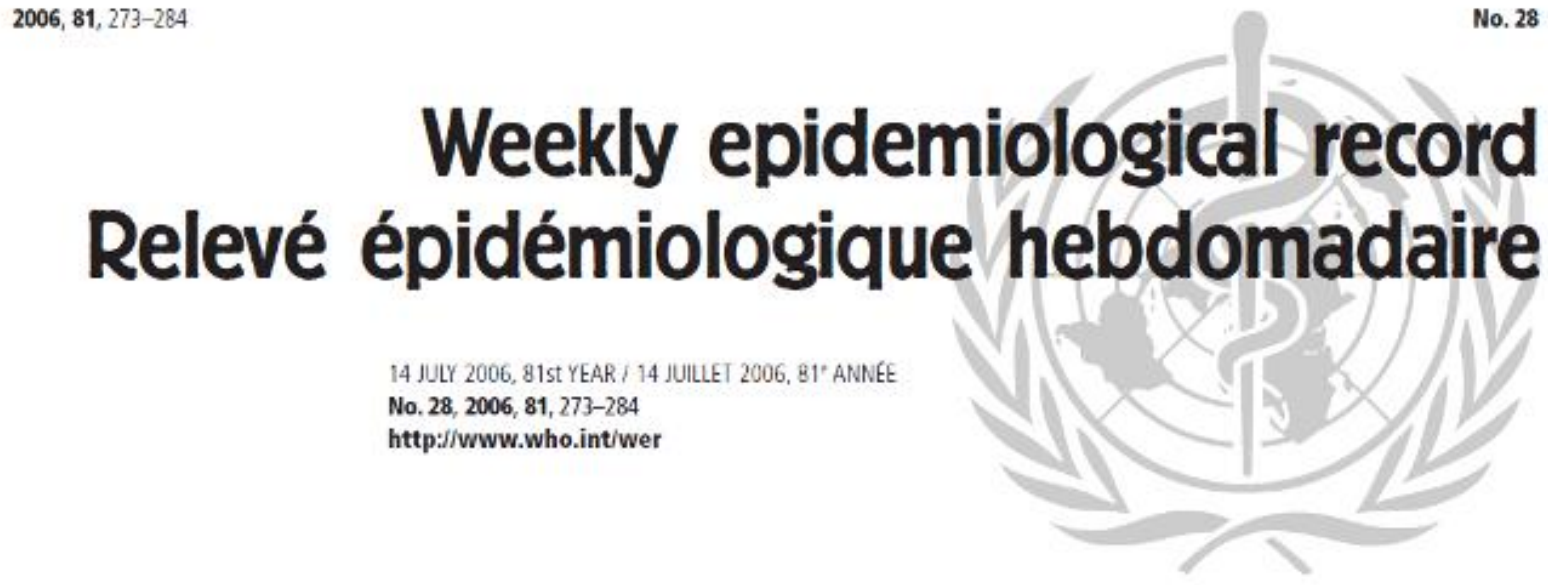
Adjuvants are squalene, hajolie. There are very strong indications that Gulf W syndrome from the first Gulf War (and later cases) just became - and remains caused by squalene in anthrax vaccine for both deployed and hjemmestationeret U.S. military personnel.

Both groups - which excludes radiation as the cause - got the syndrome: inability to concentrate, allergies, polyartritis, lymphomas, elevated ESR, feve lupus erythematosus disseminatus and MS, including In a few studies [1, 2] h the sick elevated antibody titres against squalene - which was not the case with the healthy. Professor R.F. Garry commented thereon to the U.S. Congress. U.S. Defense Department will not make a lot of study of squalenets role. Animal studies confirm squalene as trigger of autoimmune diseases.



# WHO Global Advisory Committee on Vaccine Safety (GACVS)



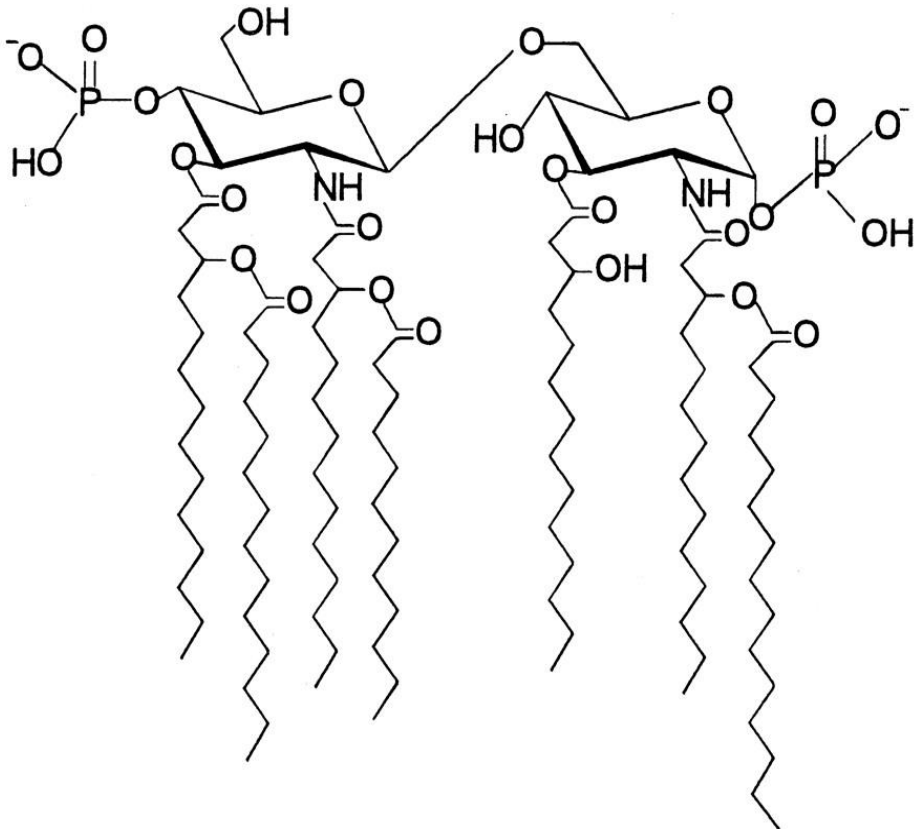

The Committee concurred that fears of squalene in vaccine inducing pathological anti-squalene antibodies are unfounded...

# Narcolepsy…….zzzzzzz

- Finland: 2009-2010 pandemic H1N1 influenza vaccination campaign
  - vaccine with adjuvant (squalene/tocopherol)
- risk in 4-19 yr old 9X higher than that among no vaccinated
  - 1 per 12,000
  - Only found in (HLA) DQB1*0602 genotype
- Proposed mechanism:
  - H1N1 antigen formed shape resembling epitope resembling epitope on neural cell
  - Adjuvant enhanced immune response to this epitope (antibody or CD4)

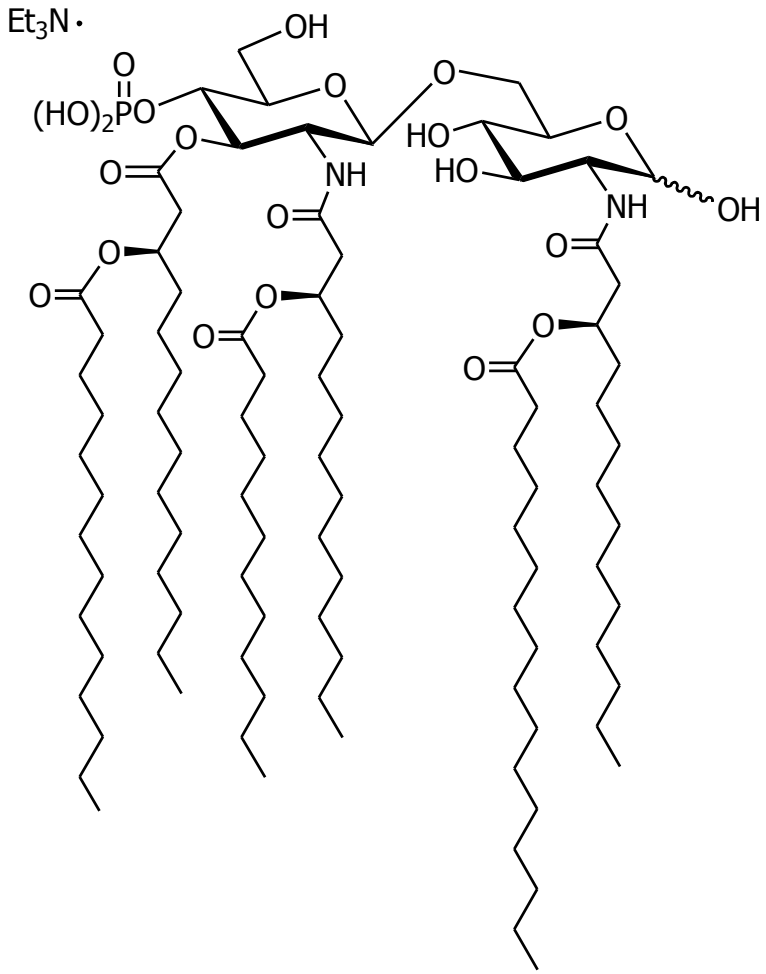
# LPS-derived adjuvants

- Lipid-A
  - Toxic, pyrogenic
- Incorporation into liposomes reduces toxicity
  - Experimental adjuvant for malaria (1990).



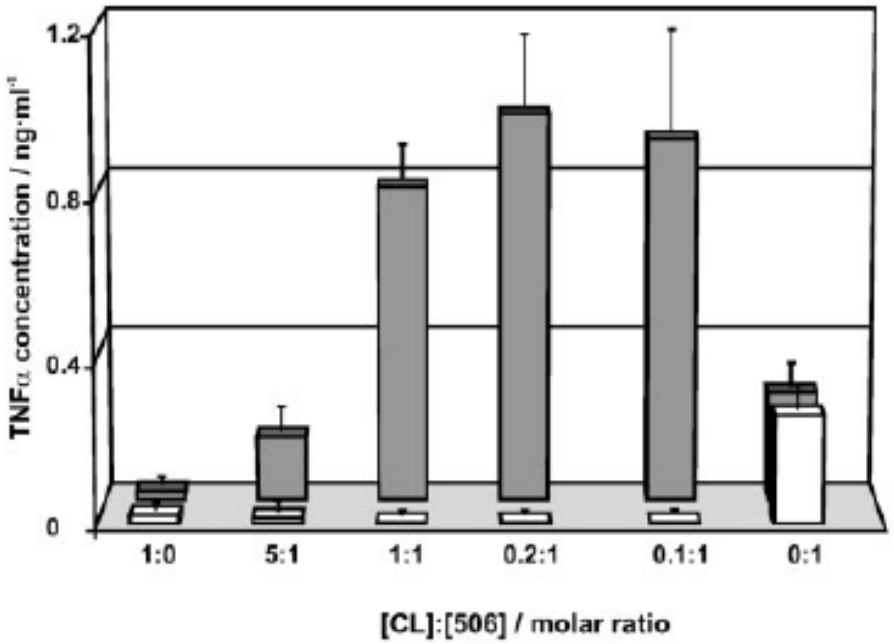
# LPS-derived adjuvants

- MPL
  - Ribi, 1979, shows that partial hydrolysis eliminates toxicity but adjuvant effect retained
    - Monophosphoryl lipid A, MPL, MPLA, 3D-MPL
- Derived from Salmonella
  - Heterogenous
  - 4, 5, 6 acyl chains
- Combined with Alum = AS04 (GSK)
- Approved in
  - Cervarix (HPV vaccine),
  - Fendrix (HBV vaccine)



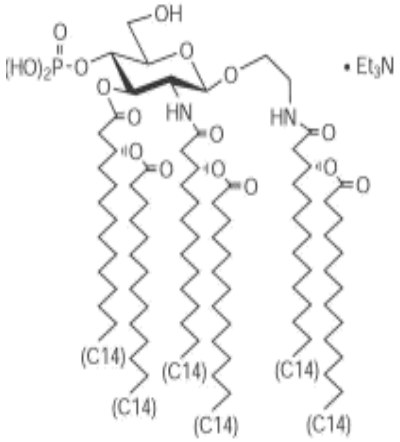
# Challenges to the using LPS derived adjuvants

- Act via TLR4/ND2 activate MyD88 and TRIF
- But….
  - Mouse vs human receptors (humans only recognise hexa form)
  - Minor change to structure has major effect on activity
  - Insoluble: Formulation effect critical but not fully understood

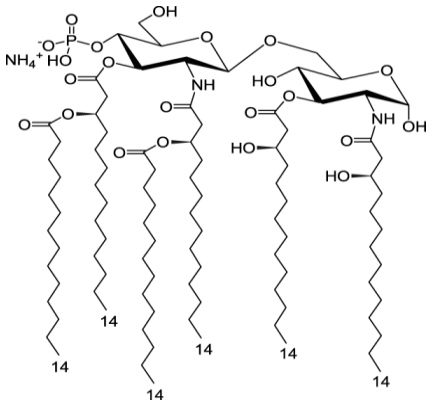


# Other TLR-4 agonists

- RC-529



GLA



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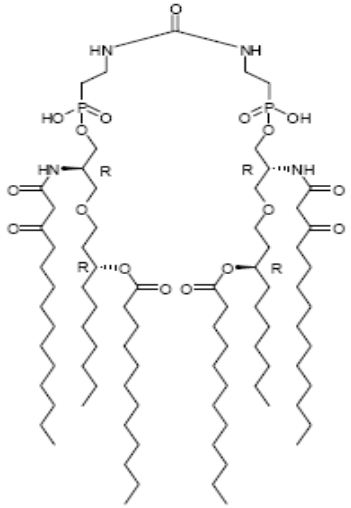

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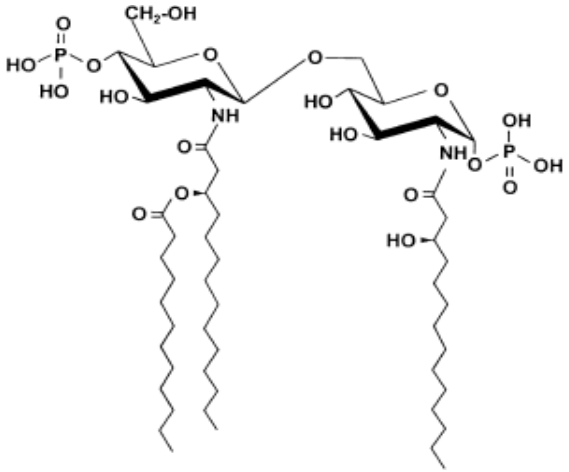


# Immunostimulatory oligonucleotides

- 1894 tumorigenic effect of dead bacteria (Coley)
- 1983 active component is bacterial DNA (Tokumaya)
- 1995 non-methylated CpG sequence shown to be active part (Krieg)
  - Development of CpG adjuvants for vaccines
  - With phosphothioate linkage to enhance stability
  - Demonstration of strong CMI adjuvant effect

5'd(TpCpCpApTpGpApCpGpTpTpCpCpTpGpApCpGpTpT)-3'

# Immunostimulatory oligonucleotides

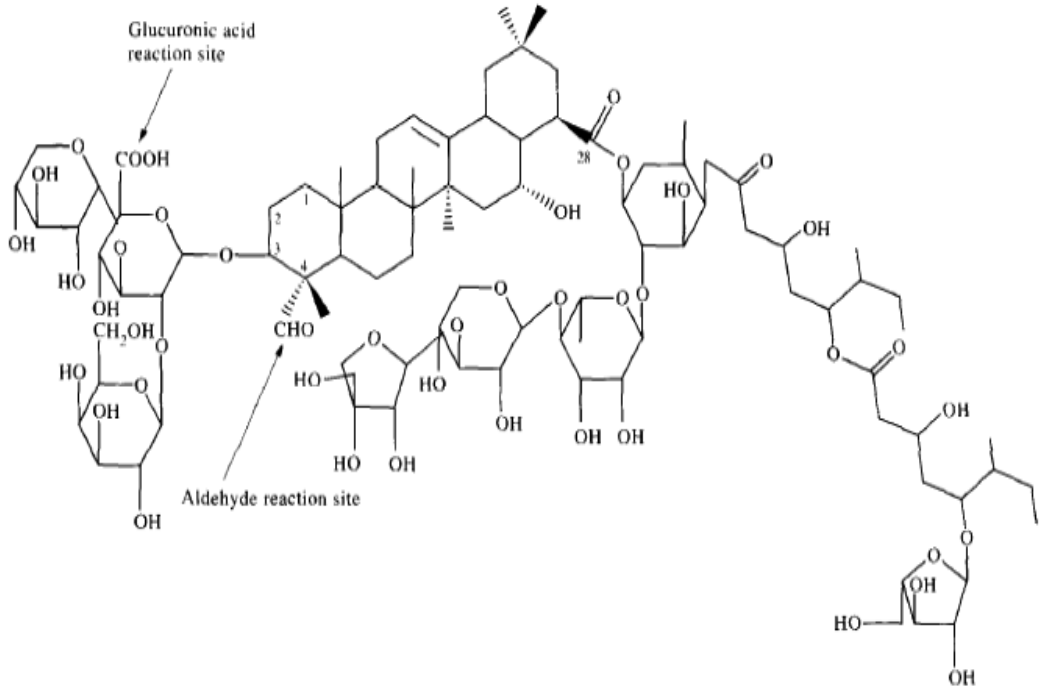
- Identification of intracellular TLR-9 as receptor for CpG
  - Different TLR9 expression in different cells in different species
  - Different sequence specificities across species
- Complex development pathway (relevance of preclin model ?)
- Issues: perceived safety
- Competing approaches – IC31 (poly dI:dC + cation),..

# Other TLR agonists as adjuvants

- TLR-3: recognizes double-stranded RNA
  - Poly I:C, Poly A:U
  - Challenge to manufacture reproducibly. Some candidates.
- TLR 5: recognizes bacterial flagellin
  - Recombinant influenza HA- flagellin candidate under development
- TLR 7,8: recognise G- or U-rich single-stranded RNA
  - Imidazoquinolines or guanosine analogues act as agonists
  - Imiquimod, R848, loxoribine,…
  - Formulation critical for effect – under development

# Quilaria saponin adjuvants

- Saponin extracted from Quillaria saponaria.
- Highly purified component (QS21) used in AS01 (GSK)
- Strong CMI induction
- Mode(s) of action largely unknown
  - Inflammasome involved, but aldehyde critical…
- Unstable ! Reactogenic !



# Conclusion 1

- Don't use adjuvants !

- Make the antigen immunogenic !
  - VLPs, conjugates, polymeric forms, particulate, etc…

# Conclusion 2

- If you HAVE to use an adjuvant – use one that is already in an approved vaccine that has wide clinical use with good safety history.
- If not available or not suitable…

# Conclusion 3

- Use an adjuvant that is in late clinical development
  - With no known vaccine-related SAEs
  - If that is not available, not suitable…

# Conclusion 4

- Use an adjuvant with extensive preclinical and early clinical testing
  - Consider cost, GMP availability, mechanism of action etc.
  - If that is not available or not suitable…

# Conclusion 5

- Try a different antigen
- Try a different disease target

- If that doesn't work…

# Conclusion 6

- Try a different profession