Saponins:

Their use as vaccine adjuvants

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Contents

- Properties of saponins
- Saponin use as adjuvants in vaccines
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- Saponin availability
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- Formulation of Iscoms and Iscomatrix
- Mode of action
- Examples of saponins as adjuvants in vaccines
- Future perspectives

Some properties of saponins

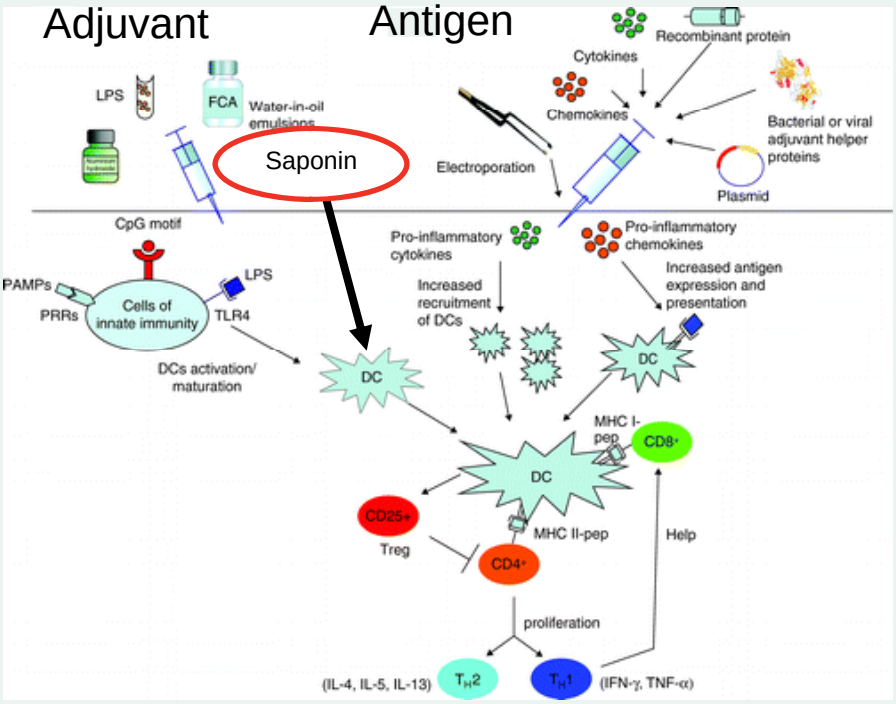
- Adrenocorticotrophic
- Analgesic
- Antiamnestic
- Antibacterial
- Antidepressant
- Antidiabetic
- Antifungal
- Antihypertensive
- Anti-inflammatory
- Antinarcotic
- Antioxidative
- Antispermal/ contraceptive
- Antistress
- Antiviral
- Psychotropic
- Vasodilatory
- Diuretic
- Effects on enzyme activity
- **Foaming agent**
- **Hemolytic**
- **Permeation enhancer**
- **Surfactant / detergent micelles**
- **Bind cholesterol**
- Induce apoptosis in tumor cells
- Growth promoter
- Binds ammonia

Adapted from Brambell, 1995

Effect of saponins on the immune system:

- As immunostimulant
- As adjuvant in vaccines
  - in combination with antigen(s)

Vaccines, adjuvants and immune response



From Chiarella et al. Expert Opin. Biol. Ther. 2007

Saponins as adjuvants- historical highlights

- ??- Mapuches (Chile) identified foaming properties of Quillaja bark
- 1782- *Quillaja saponaria* trees described by Molina
- 1936- First use in anti-toxin vaccines by Thibault et al
- 1951- Use in FMDV vaccines by Espinet et al
- 1970- Purification of saponins leads to QuilA product by Dalsgaard
- 1984- Iscoms described by Morein et al
- 1991- Iscom structure proposed by Kersten et al
- 1991- QS-21 (Stimulon) by Kensil et al
- 1995- Empty Iscoms (Matrix) by Rimmelzwaan et al
- 1999- GPI-0100 by Marciani et al

Isolation of saponins

Quillaja trees

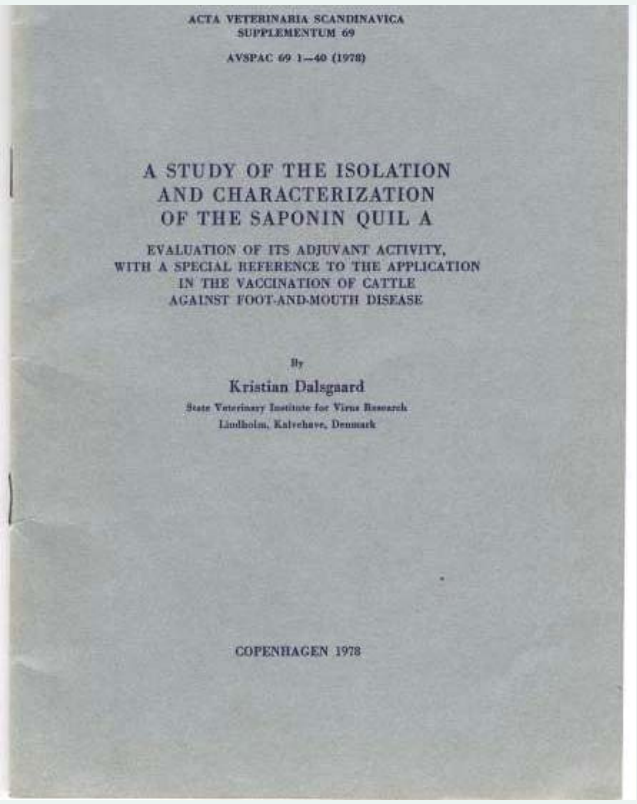
Wood & Bark

Water extraction

As such (stabilized) or spray-dried

Ultrafiltration

Column purification(s)



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

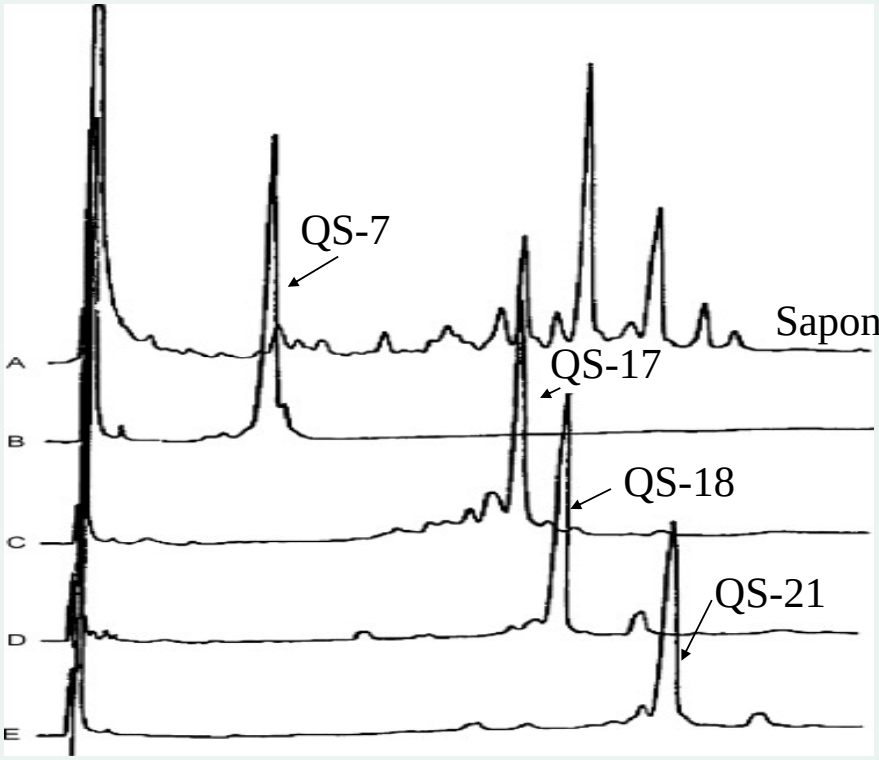
State Veterinary Institute for Virus Research
Lindholm, Kalvehave, Denmark

COPENHAGEN 1978

Need for more refined Saponin purifications

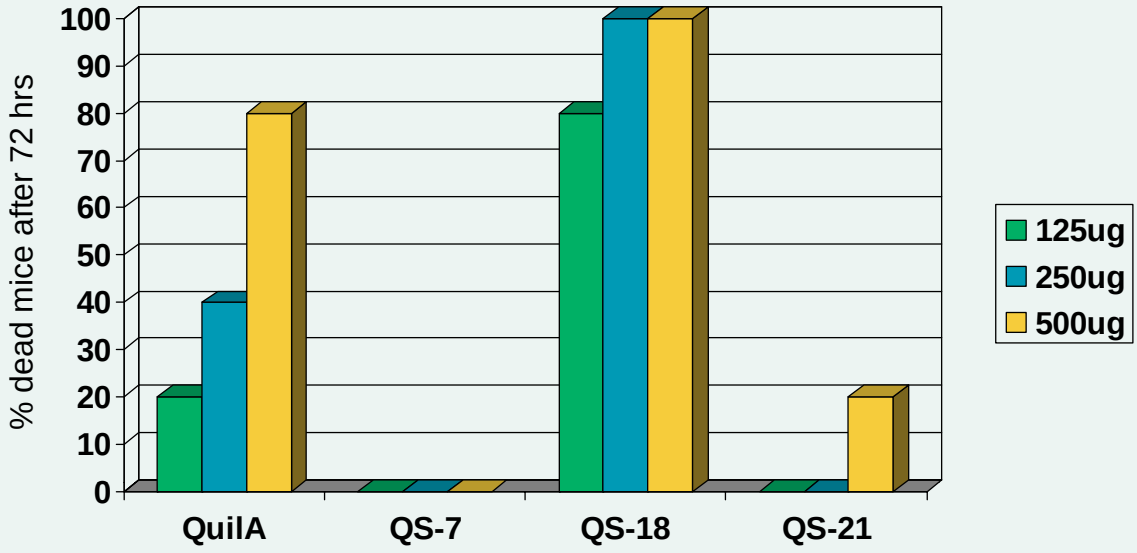
- Less toxic components
- Consistent production
- More stable

Saponin profiles RP-HPLC Vydac column



From Kensil, 1991

Toxicity of saponins in CD-1 mice after intradermal injection

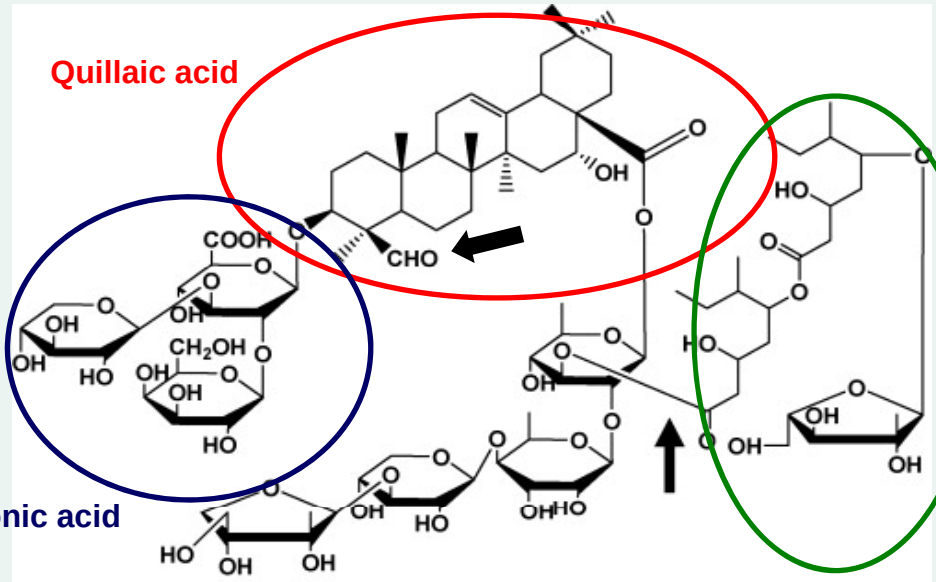


From Kensil et al. J. Immunol. 146, 431-437, 1991

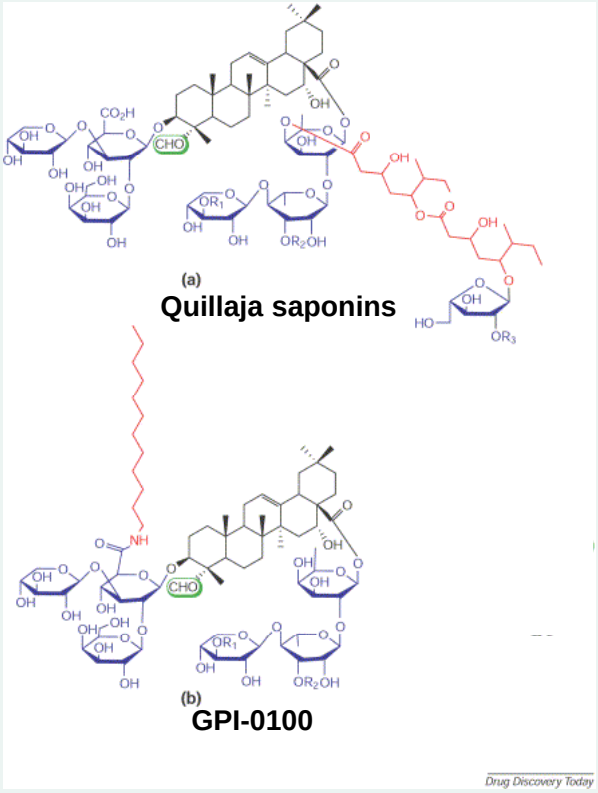
Some available Quillaja saponins as adjuvants

Company	Product(s)
Brenntag	QuilA
Biolang	Qvac
Berghausen	BioQ
Quest	Saponin 5012
Desert King / Natural Response	QL 1000 (.9%), QP 1000 (.22%) QL Ultra (13%)(QP UF 1000 (>70%), Vax Sap (90%), Super Sap
QSM	QSM VCN P200, VCN P700, VCN P900 1000 Ultra VCN
Schmittmann	Saponins from Quillaja
Fisher	Quillaja derived
Sigma	Quillaja derived
Antigenics	QS-21 (Stimulon)
Hawaii Biotech	GPI-0100
Isconova	AbISCO 100 (Matrix M), AbISCO 300 (Matrix Q) and MatrixC
CSL	Iscomatrix

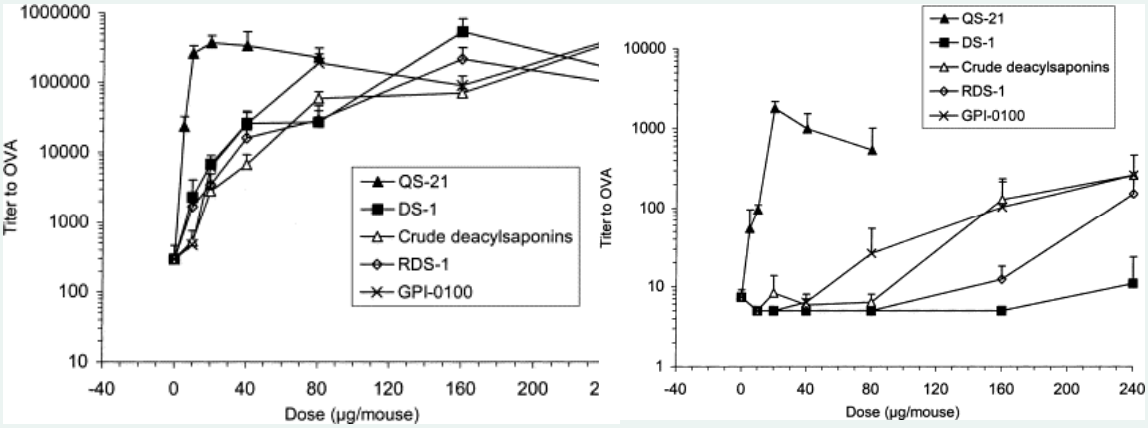
Saponin structure 1



Saponin structure 2



Effect of saponins on OVA antibody responses in mice



IgG1 IgG2a

From Liu et al, Vaccine, 20,2808-2815 (2002)

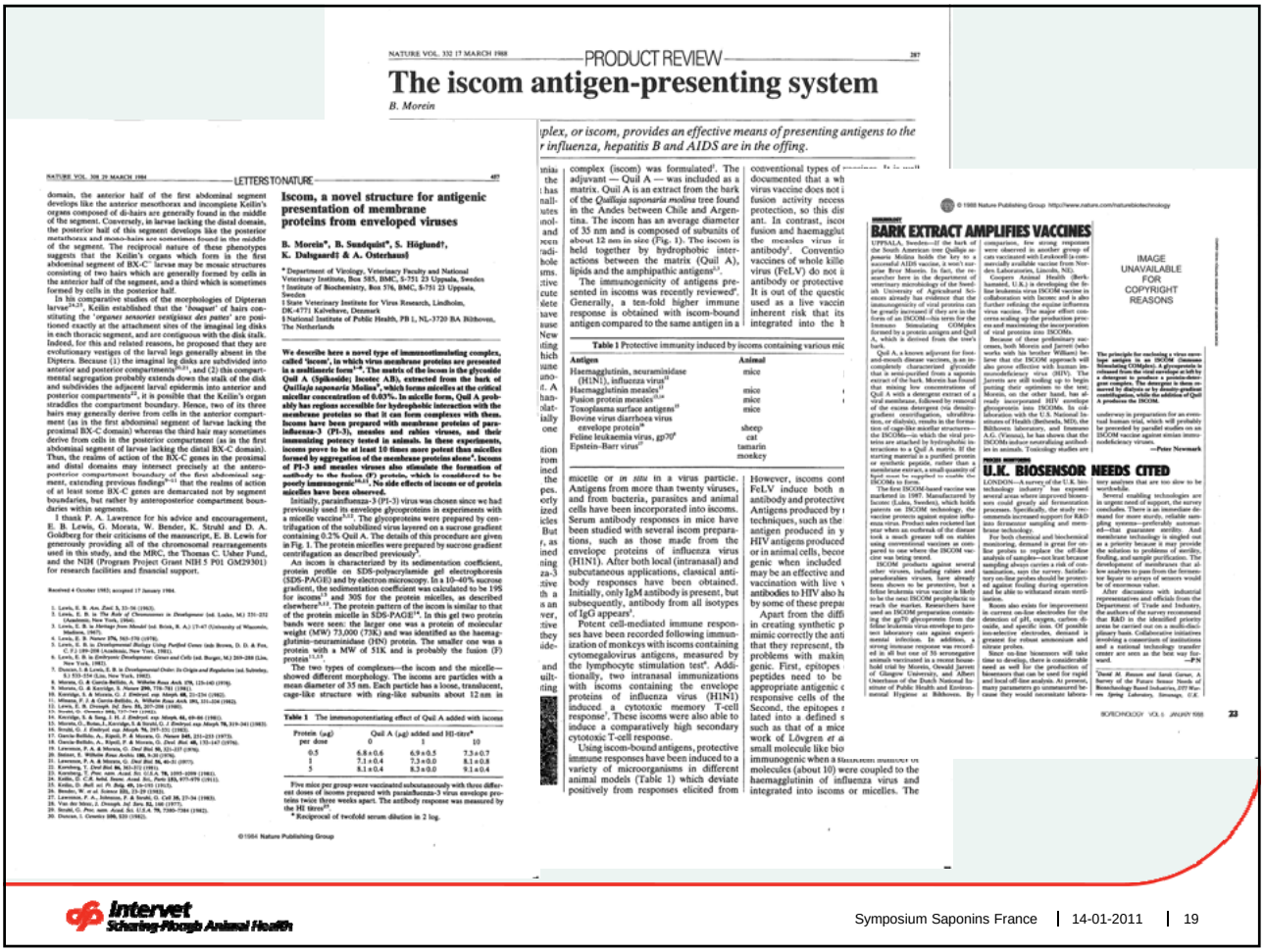
Options for saponin formulation in vaccines

- Liquid form as micelles or as free molecules
- Liposomes
- Microspheres
- Implants
- Combination with other adjuvants (e.g. Aluminiumhydroxide)
- Iscoms or Iscomatrix
- Posintro
- Pluscoms

Application of saponins in vaccines

- Route of vaccination
  - Parenteral (sc, im, ip), intranasal, oral, immersion
- Species
  - mice, rats, rabbits, birds, dogs, cats, pigs, cattle, sheep, horse, monkeys, humans, fish and shrimp
- Dose
  - Depending on species and antigen (1-1000 µg)
- Antigen specificity
  - Membrane bound antigens, virus, parasites, proteins

Formulation as Iscom or Matrix



NATURE VOL. 332 17 MARCH 1988 — PRODUCT REVIEW — 287

The iscom antigen-presenting system

B. Morein

...plex, or iscom, provides an effective means of presenting antigens to the ...r influenza, hepatitis B and AIDS are in the offing.

NATURE VOL. 308 29 MARCH 1984 — LETTERS TO NATURE — 457

Iscom, a novel structure for antigenic presentation of membrane proteins from enveloped viruses

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We describe here a novel type of immunostimulating complex, called 'iscom', in which virus membrane proteins are presented in a multimeric form[1–3]. The matrix of the iscom is the glycoside Quil A (Spikosides; Isconet AB), extracted from the bark of *Quillaja saponaria Molina*[4], which forms micelles at the critical micellar concentration of 0.03%. In micelle form, Quil A probably has regions accessible for hydrophobic interaction with the membrane proteins so that it can form complexes with them. Iscoms have been prepared with membrane proteins of parainfluenza-3 (PI-3), measles and rabies viruses, and their immunizing potency tested in animals. In these experiments, iscoms prove to be at least 10 times more potent than micelles formed by aggregation of the membrane proteins alone[3]. Iscoms of PI-3 and measles viruses also stimulate the formation of antibody to the fusion (F) protein, which is considered to be poorly immunogenic[5,6,11]. No side effects of iscoms or of protein micelles have been observed.

Initially, parainfluenza-3 (PI-3) virus was chosen since we had previously used its envelope glycoproteins in experiments with a micelle vaccine[7–12]. The glycoproteins were prepared by centrifugation of the solubilized virus layered on a sucrose gradient containing 0.2% Quil A. The details of this procedure are given in Fig. 1. The protein micelles were prepared by sucrose gradient centrifugation as described previously[9].

An iscom is characterized by its sedimentation coefficient, protein profile on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and by electron microscopy. In a 10–40% sucrose gradient, the sedimentation coefficient was calculated to be 19S for iscoms[1,3] and 30S for the protein micelles, as described elsewhere[9,13]. The protein pattern of the iscom is similar to that of the protein micelle in SDS-PAGE[3,9]. In this gel two protein bands were seen: the larger one was a protein of molecular weight (MW) 73,000 (73K) and was identified as the haemagglutinin–neuraminidase (HN) protein. The smaller one was a protein with a MW of 51K and is probably the fusion (F) protein[11,12].

The two types of complexes—the iscom and the micelle—showed different morphology. The iscoms are particles with a mean diameter of 35 nm. Each particle has a loose, translucent, cage-like structure with ring-like subunits about 12 nm in

Table 1 The immunopotentiating effect of Quil A added with iscoms

Protein (μg) per dose	Quil A (μg) added and HI-titre*		
	0	1	10
0.5	6.8±0.6	6.9±0.5	7.3±0.7
1	7.1±0.4	7.2±0.0	8.1±0.8
5	8.1±0.4	8.3±0.0	9.1±0.4

Five mice per group were vaccinated subcutaneously with three different doses of iscoms prepared with parainfluenza-3 virus envelope proteins twice three weeks apart. The antibody response was measured by the HI titres[27].
* Reciprocal of twofold serum dilution in 2 log.

...complex (iscom) was formulated[1]. The adjuvant — Quil A — was included as a matrix. Quil A is an extract from the bark of the *Quillaja saponaria molina* tree found in the Andes between Chile and Argentina. The iscom has an average diameter of 35 nm and is composed of subunits of about 12 nm in size (Fig. 1). The iscom is held together by hydrophobic interactions between the matrix (Quil A), lipids and the amphipathic antigens[2,3].

The immunogenicity of antigens presented in iscoms was recently reviewed[4]. Generally, a ten-fold higher immune response is obtained with iscom-bound antigen compared to the same antigen in a

Table 1 Protective immunity induced by iscoms containing various ...

Antigen	Animal
Haemagglutinin, neuraminidase (H1N1), influenza virus[11]	mice
Haemagglutinin measles[11]	mice
Fusion protein measles[13,14]	mice
Toxoplasma surface antigens[15]	mice
Bovine virus diarrhoea virus envelope protein[16]	sheep
Feline leukaemia virus, gp70[9]	cat
Epstein–Barr virus[17]	tamarin monkey

micelle or *in situ* in a virus particle. Antigens from more than twenty viruses, and from bacteria, parasites and animal cells have been incorporated into iscoms. Serum antibody responses in mice have been studied with several iscom preparations, such as those made from the envelope proteins of influenza virus (H1N1). After both local (intranasal) and subcutaneous applications, classical antibody responses have been obtained. Initially, only IgM antibody is present, but subsequently, antibody from all isotypes of IgG appears[3].

Potent cell-mediated immune responses have been recorded following immunization of monkeys with iscoms containing cytomegalovirus antigens, measured by the lymphocyte stimulation test[6]. Additionally, two intranasal immunizations with iscoms containing the envelope proteins of influenza virus (H1N1) induced a cytotoxic memory T-cell response[7]. These iscoms were also able to induce a comparatively high secondary cytotoxic T-cell response.

Using iscom-bound antigens, protective immune responses have been induced to a variety of microorganisms in different animal models (Table 1) which deviate positively from responses elicited from conventional types of ... documented that a wh... virus vaccine does not i... fusion activity necess... protection, so this dis... ant. In contrast, iscoms... fusion and haemagglut... the measles virus is... antibody[7]. Conventio... vaccines of whole kille... virus (FeLV) do not i... antibody or protective... It is out of the questio... used as a live vaccin... inherent risk that its... integrated into the h...

However, iscoms cont... FeLV induce both n... antibody and protective... Antigens produced by ... techniques, such as the... antigen produced in y... HIV antigens produced... or in animal cells, becoe... genic when included ... may be an effective and... vaccination with live ... antibodies to HIV also h... by some of these prepa...

Apart from the diffi... in creating synthetic p... mimic correctly the anti... that they represent, th... problems with makin... genic. First, epitopes ... peptides need to be ... appropriate antigenic ... responsive cells of the... Second, the epitopes ... lated into a defined s... small molecule like bio... immunogenic when a ... molecules (about 10) were coupled to the haemagglutinin of influenza virus and integrated into iscoms or micelles. The

NEWS AND VIEWS

BARK EXTRACT AMPLIFIES VACCINES

UPPSALA, Sweden—If the bark of the South American tree *Quillaja saponaria* Molina holds the key to a successful AIDS vaccine, it won't surprise Bror Morein. In fact, the researcher here in the department of veterinary microbiology of the Swedish University of Agricultural Sciences already has evidence that the immunogenicity of viral proteins can be greatly increased if they are in the form of an ISCOM—his term for the Immune Stimulating COMplex formed by a protein antigen and Quil A, which is derived from the tree's bark.

Quil A, a known adjuvant for foot-and-mouth disease vaccines, is an incompletely characterized glycoside that is semi-purified from a saponin extract of the bark. Morein has found that mixing low concentrations of Quil A with a detergent extract of a viral membrane, followed by removal of the excess detergent (via density-gradient centrifugation, ultrafiltration, or dialysis), results in the formation of cage-like micellar structures—the ISCOMs—in which the viral proteins are attached by hydrophobic interactions to a Quil A matrix. If the starting material is a purified protein or synthetic peptide, rather than a membrane extract, a small quantity of lipid must be supplied to enable the ISCOMs to form.

The first ISCOM-based vaccine was marketed in 1987. Manufactured by Iscotec (Lidea, Sweden), which holds patents on ISCOM technology, the vaccine protects against equine influenza virus. Product sales rocketed last year when an outbreak of the disease took a much greater toll on stables using conventional vaccines as compared to one where the ISCOM vaccine was being tested.

ISCOM products against several other viruses, including rabies and parainfluenza viruses, have already been shown to be protective, but a feline leukemia virus vaccine is likely to be the next ISCOM prophylactic to reach the market. Researchers have used an ISCOM preparation containing the gp70 glycoprotein from the feline leukemia virus envelope to protect laboratory cats against experimental infection. In addition, a strong immune response was recorded in all but one of 30 seronegative animals vaccinated in a recent household trial by Morein, Oswald Jarrett of Glasgow University, and Albert Osterhaus of the Dutch National Institute of Public Health and Environmental Hygiene at Bilthoven. By comparison, few strong responses were observed in another group of cats vaccinated with Leukocell (a commercially available vaccine from Norden Laboratories, Lincoln, NE).

Coopers Animal Health (Berkhamsted, U.K.) is developing the feline leukemia virus ISCOM vaccine in collaboration with Iscotec and is also further refining the equine influenza virus vaccine. The major effort concerns scaling up the production process and maximizing the incorporation of viral proteins into ISCOMs.

Because of these preliminary successes, both Morein and Jarrett (who works with his brother William) believe that the ISCOM approach will also prove effective with human immunodeficiency virus (HIV). The Jarretts are still tooling up to begin putting their optimism to the test; Morein, on the other hand, has already incorporated HIV envelope glycoprotein into ISCOMs. In collaboration with the U.S. National Institutes of Health (Bethesda, MD), the Bilthoven laboratory, and Immunos A.G. (Vienna), he has shown that the ISCOMs induce neutralizing antibodies in animals. Toxicology studies are

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

The principle for enclosing a virus membrane antigen in an ISCOM (Immune Stimulating COMplex). A glycoprotein is released from the viral envelope at left by a detergent to produce a protein micelle. Incorporation of the detergent is observed by dialysis or by density-gradient centrifugation, while the addition of Quil A produces the ISCOM.

underway in preparation for an eventual human trial, which will probably be preceded by parallel studies on an ISCOM vaccine against simian immunodeficiency viruses.

—Peter Newmark

NEWS AND VIEWS

U.K. BIOSENSOR NEEDS CITED

LONDON—A survey of the U.K. biotechnology industry[1] has exposed several areas where improved biosensors could greatly aid fermentation processes. Specifically, the study recommends increased support for R&D into fermentor sampling and membrane technology.

For both chemical and biochemical monitoring, demand is great for online probes to replace the off-line analysis of samples—no least because sampling always carries a risk of contamination, says the survey. Satisfactory on-line probes should be protected against fouling during operation and be able to withstand steam sterilization.

Room also exists for improvement in current on-line electrodes for the detection of pH, oxygen, carbon dioxide, and specific ions. Of possible non-selective electrodes, demand is greatest for robust ammonium and nitrate probes.

Since on-line biosensors will take time to develop, there is considerable need as well for the production of biosensors that can be used for rapid and local off-line analysis. At present, many parameters go unmeasured because they would necessitate laboratory analyses that are too slow to be worthwhile.

Several enabling technologies are in urgent need of support, the survey concludes. There is an immediate demand for more sturdy, reliable sampling systems—preferably automated—that guarantee sterility. And membrane technology is singled out as a priority because it may provide the solution to problems of sterility, fouling, and sample purification. The development of membranes that allow analytes to pass from the fermentor liquor to arrays of sensors would be of enormous value.

After discussions with industrial representatives and officials from the Department of Trade and Industry, the authors of the survey recommend that R&D in the identified priority areas be carried out on a multi-disciplinary basis. Collaborative initiatives involving a consortium of institutions and a national technology transfer center are seen as the best way forward.

—PN

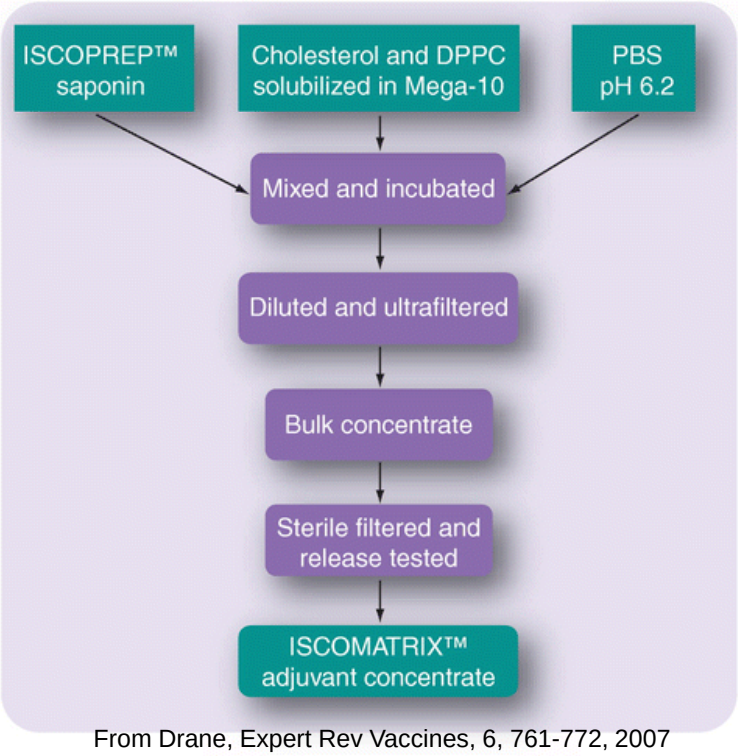
[1] *David M. Rissom and Sarah Garner, A Survey of the Future Sensor Needs of Biotechnology Based Industries, 277 Wavers Spring Laboratory, Stevenage, U.K.*

BIOTECHNOLOGY VOL 6 JANUARY 1988 — 23

Iscom or Iscomatrix formulation methods

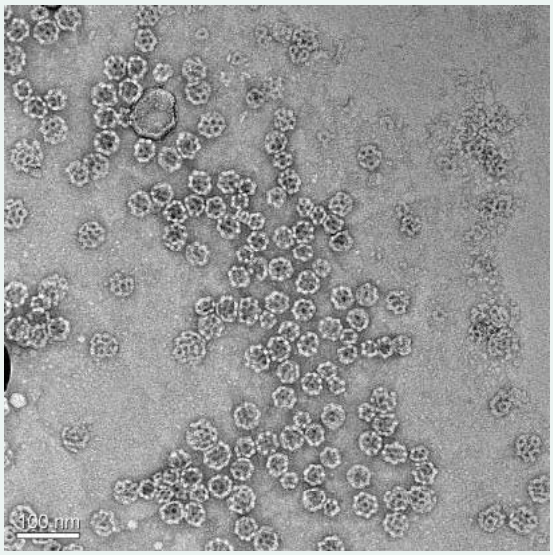
- Centrifugation
- Dialysis or ultrafiltration
- Lipid film hydration
- Ethanol injection
- Chloroform / ether injection techniques

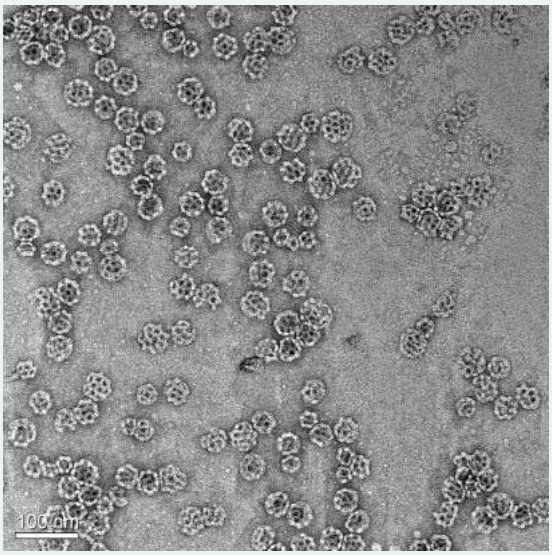
CSL Iscomatrix preparation



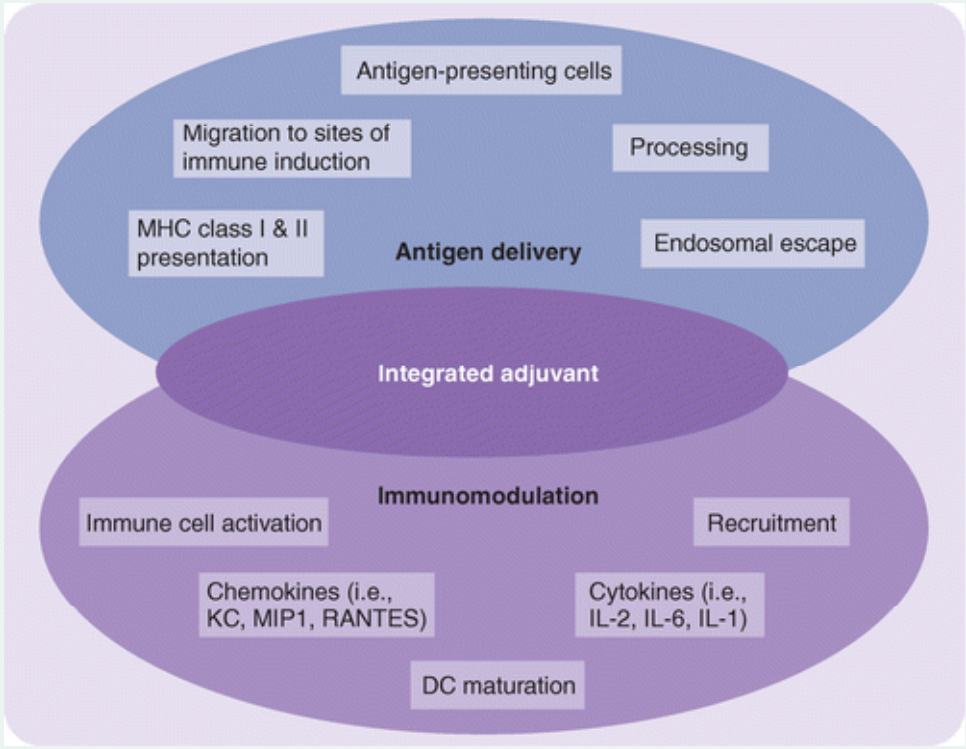
From Drane, Expert Rev Vaccines, 6, 761-772, 2007

EM pictures Matrix formulations





Mode of action Iscoms / Iscomatrix

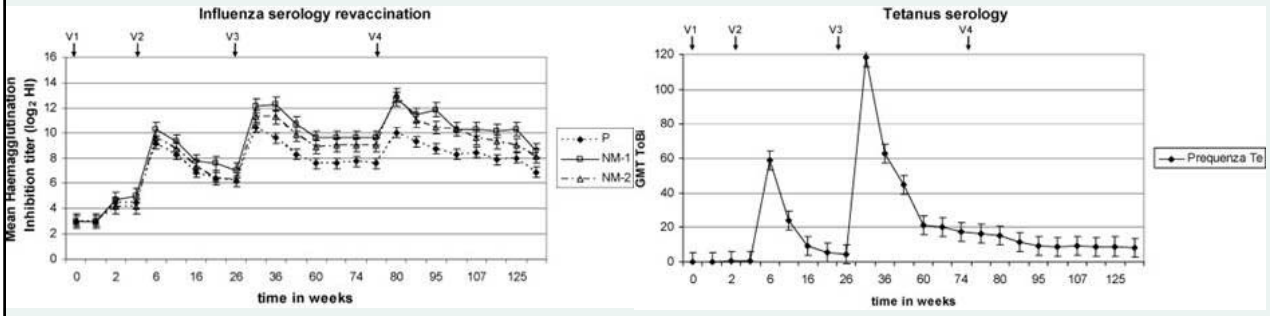


From Drane, Expert Rev Vaccines, 6, 761-772, 2007

Iscom vaccine for horses based on HA and N:

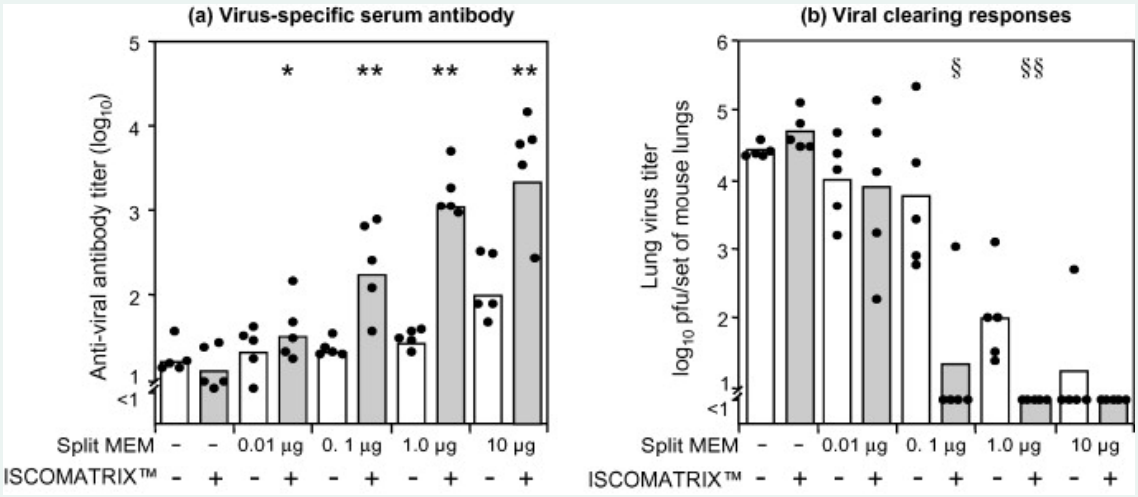
PrequenzaTe™-Trivalent Influenza + Tetanus

Immune response



From Heldens et al. Vaccine, 28, 6989-6996, 2010

Intranasal immunization of mice with an Iscomatrix vaccine based on a split Influenza antigen: dose finding



From Sanders et al. Vaccine, 27, 2475-2482, 2009

Experiences with saponins in human vaccines
(Hepatitis B surface antigen)

QS-21 use in Glaxo Smith Kline Adjuvant Systems:

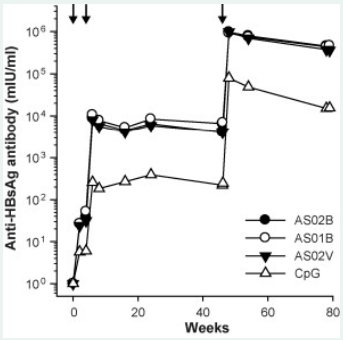
- AS01B = Liposomes + 50µg QS 21 + 50µg MPL
- AS02B = o/w Tocopherol/squalene + 100µg MPL + 100µg QS 21
- AS02V = low volume o/w emulsion + 50µg QS 21 + 50µg MPL

- As control 500 µg CpG 7909 class B oligonucleotide

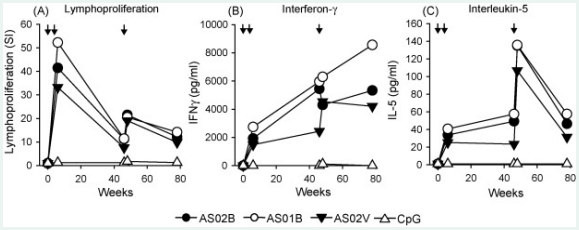
Data from Vandepapelière et al. Vaccine, 26, 1375-1386, 2008

Effect of QS-21- containing vaccines on human immune responses

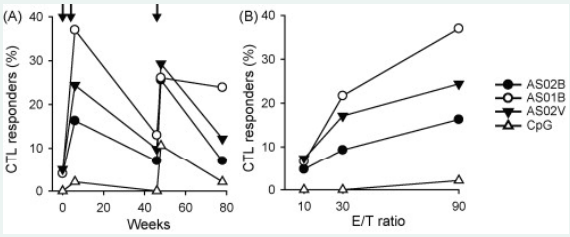
Antibodies



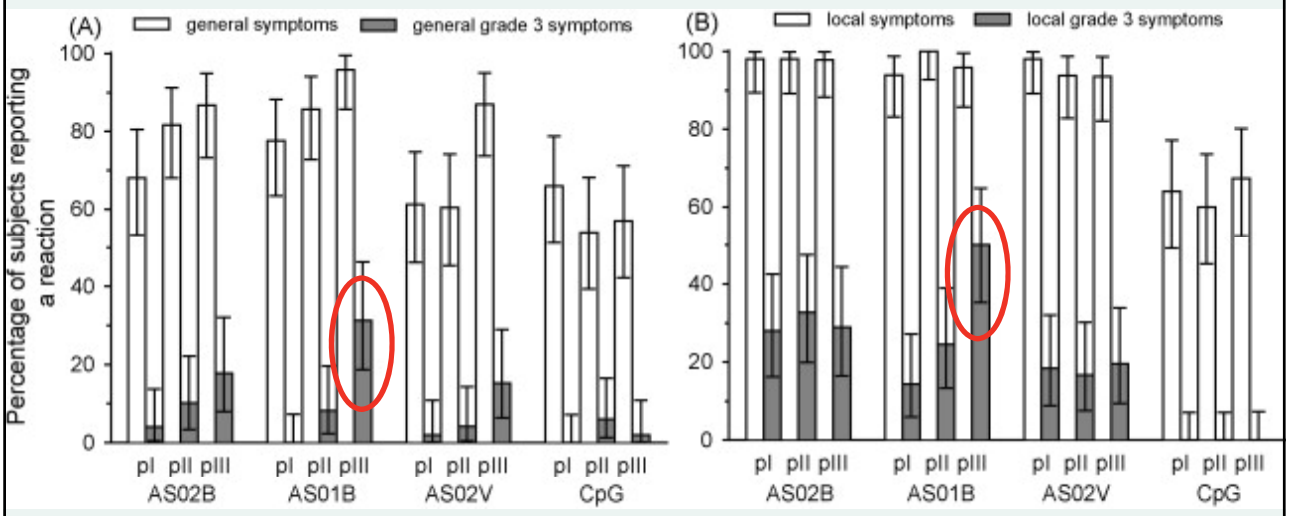
Cytokine profile



Cytotoxic T cells



Safety aspects of QS-21 in humans



➔ AS01B was selected for further studies

What can be expected for the future?

- Other sources for saponins will be identified
- Further purification and stabilization
- Complete synthesis e.g. QS-21
- Different formulation methods
- Combinations with other adjuvants or immunostimulants
- Possible use in human therapeutic or preventive vaccines


Questions?