Vaccine 27 (2009) 1787–1796

Contents lists available at ScienceDirect

Vaccine

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

Review

Advances in saponin-based adjuvants

Hong-Xiang Sun [a,*], Yong Xie [a,b], Yi-Ping Ye [c]

[a] *Key Laboratory of Animal Epidemic Etiology & Immunological Prevention of Ministry of Agriculture, College of Animal Sciences, Zhejiang University, Kaixuan Road 268, Hangzhou 310029, PR China*
[b] *Department of Pharmacy, Fujian University of Traditional Chinese Medicine, Fuzhou 350108, PR China*
[c] *Institute of Materia Medica, Zhejiang Academy of Medical Sciences, Hangzhou 310013, PR China*

ARTICLE INFO

Article history:
Received 11 July 2008
Received in revised form 18 January 2009
Accepted 19 January 2009
Available online 7 February 2009

Keywords:
Vaccine
Adjuvant
Saponin
Structure–activity relationship

ABSTRACT

Saponins are natural glycosides of steroid or triterpene which exhibited many different biological and pharmacological activities. Notably, saponins can also activate the mammalian immune system, which have led to significant interest in their potential as vaccine adjuvants. The most widely used saponin-based adjuvants are Quil A and its derivatives QS-21, isolated from the bark of *Quillaja saponaria* Molina, which have been evaluated in numerous clinical trials. Their unique capacity to stimulate both the Th1 immune response and the production of cytotoxic T-lymphocytes (CTLs) against exogenous antigens makes them ideal for use in subunit vaccines and vaccines directed against intracellular pathogens as well as for therapeutic cancer vaccines. However, *Quillaja* saponins have serious drawbacks such as high toxicity, undesirable haemolytic effect and instability in aqueous phase, which limits their use as adjuvant in vaccination. It has driven much research for saponin-based adjuvant from other kinds of natural products. This review will summarize the current advances concerning adjuvant effects of different kinds of saponins. The structure–activity relationship of saponin adjuvants will also be discussed in the light of recent findings. It is hoped that the information collated here will provide the reader with information regarding the adjuvant potential applications of saponins and stimulate further research into these compounds.

Contents

* Corresponding author. Tel.: +86 571 8697 1091; fax: +86 571 8697 1091.
E-mail address: sunhx@zju.edu.cn (H.-X. Sun).

0264-410X/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2009.01.091

1. Introduction

Vaccination remains the most cost-effective biomedical approach for the control and prevention of infectious diseases. New generations of vaccines, particularly those based on purified recombinant proteins, synthetic peptides and plasmid DNA, are likely to be less reactogenic and immunogenic than traditional vaccines [1]. Therefore, there is an urgent need for the development of a new and improved vaccine adjuvant [2–4].

Immunological adjuvants were originally described by Ramon [5] as "substances used in combination with a specific antigen that produce more immunity than the antigen alone". Nowadays, the increase of knowledge in the immunology field is leading to a more rational vaccine design aiming to elicit a specific, protective, and long-lasting immunity after vaccination. A rational selection of adjuvants can be driven by the nature of the immune response required (Th1, Th2, antibodies, and CTLs) [6]. In case of toxins, a good humoral immune response is required; however, in case of intracellular bacteria the cell mediated response, mainly cytotoxic T cells and Th1 cells, is the most important. In case of viral infection both humoral and cellular response are fundamental to control the infection. After this, a strategy to elicit the suitable type of immunity should be planned [1].

Adjuvants have significant effects on the nature of the immune responses, and can tilt the immune system in favor to Th1 or Th2 type response [7]. The versatile adjuvant that can induce the appropriate type of immune response to antigens for producing optimal protection against each type of infection would be highly desirable in the vaccine industry [8]. Thus, one of the main challenges for the development of adjuvants is to learn how to selectively induce the appropriate type of immune response against each type of infection. On the other hand, suitable adjuvant should be low toxicity and side effects allowing their license to be used in human or veterinary vaccine formations [9].

While several hundred different adjuvants including mineral salts, microorganism-derived adjuvants, emulsions, cytokines, polysaccharides, nucleic acid-based adjuvants have been tested for the research or usage in novel vaccine design over the last few decades, the vast majority have not been successful in being approved for human use, with limitations including lack of efficacy, unacceptable local or systemic toxicity, difficulty of manufacture, poor stability, and prohibitive cost [9–11]. Meanwhile, exacerbating sub-clinical autoimmune diseases in addition to fever and erosion at the local injected lesion induced by nature of adjuvants has limited their clinical use [12]. For example, freund's complete adjuvant (FCA) causes inflammation, induration or necrosis with disseminated granulomas being reported in the lungs, liver, kidneys, heart, lymph nodes and skeletal muscles of rabbits or rats [13]. For this reason, until recently, only aluminum-based mineral salts (alum) remain the most widely used adjuvant in human vaccines [14]. Alum was the first adjuvants discovered in 1926 [15] and has a good safety record. However, alum is a weak adjuvant for antibody induction to protein subunits and a poor adjuvant for cell-mediated immunity [16]. Moreover, alum can induce immunoglobulin E antibody responses, which is associated with some allergic reactions in human subjects [17]. In addition, alum mainly induces the increases of IgG1, instead of IgG2a and IgG2b, indicating mainly Th2 immunity induced in mouse medol [18]. MF59, consisting of emulsified squalene, was the only adjuvant licensed for human use in addition to alum [19–21]. Similarly, MF59 was also reported to favor Th2 immune response [22].

Saponins are natural glycosides of steroid or triterpene which exhibited many different biological and pharmacological actions such as immunomodulatory, antitumor, antiinflammatory, molluscicidal, antiviral, antifungal, hypoglycemic, hypocholesterolemic [23,24]. Saponins have a diverse range of properties, which include sweetness, bitterness [25–27], foaming, emulsifying [28], and haemolytic properties [23,29]. Saponins have wide applications in beverages and confectionery, as well as in cosmetics [30,31] and pharmaceutical products [23]. They are believed to form the main constituents of many plant drugs and folk medicines, and are considered responsible for numerous pharmacological properties [32]. Notably, saponins can activate the mammalian immune system, which has led to significant interest in their potential as vaccine adjuvants [33]. The lead candidate saponin adjuvants are Quil A and its derivatives QS-21 [34], which have been included as adjuvant in vaccine formulations against HIV in guinea pig and human [35–37], cancer in human [7,38], malaria in Aotus monkeys and mice [39], respiratory syncytial virus [40], cytomegalovirus [41], *Toxoplasma gondii* [42], and visceral leishmaniasis in mice [43–45]. The unique capacity of Quil A and QS-21 to stimulate both the Th1 immune response and the production of CTLs against exogenous antigens makes them ideal for use in subunit vaccines and vaccines directed against intracellular pathogens as well as for therapeutic cancer vaccines [46,47]. However, *Quillaja* saponins have serious drawbacks such as high toxicity, undesirable haemolytic effect and unstability in aqueous phase, which limits their use as adjuvant in human vaccination [48–52]. Meanwhile, the overexploitation of the *Q. saponaria* bark has caused important ecological damage and a considerable shortage of the available supplies [53]. Therefore, many saponins from other kinds of natural product have been screened and shown to possess the adjuvant activities during the last decade. There have been several reviews in recent years of published reports about saponin-based adjuvants [54,55]. Most of them, however, deal with the chemical structure and adjuvant activities of *Quillaja* saponins. The purpose of the present review was to summarize adjuvant effects of different kinds of saponins and their structure–function relationship and try to understand the molecular mechanism of their activity, as far as the available literature permits. It is hoped that the information collated here will provide the reader with information regarding the adjuvant potential applications of saponins and stimulate further research into these compounds.

2. Saponins with the adjuvant properties

2.1. Quil A and its purified saponins

The literature dealing with the adjuvant properties of saponins almost exclusively focuses on the extracts of *Quillaja saponaria* Molina [54,55]. *Q. saponaria* extract as adjuvants was first described in the 1930s, and later used to improve a foot-and-mouth disease vaccine. In 1978, Dalsgaard first obtained an enriched mixture of saponins (Quil A) from this extract, and found Quil A stimulated both humoral and cellular immunity as well as to induce differential antibody isotypes [56]. Quil A had been used commercially in a veterinary foot-and-mouth disease vaccine as well as in some experimental vaccines [57–59]. However, its toxicity precludes expanded use in human vaccines.

Since Quil A was a heterogenous mixture of saponins when analyzed using RP-HPLC (Fig. 1) [60], it was possible that the various components may produce different levels of adjuvanticity and toxicity that could be exploited to produce useful adjuvants for human vaccines. The purification and structure–function relationships of adjuvant-active saponins have been the subject of interest. The first detailed immunological study of saponin fractions isolated by RP-HPLC using bovine serum albumin as the antigen showed that 10 of the fractions including the major peaks QS-7, QS-17, QS-18, and QS-21 had adjuvant activity [61]. While the adjuvant and physical properties of these saponins are similar, their toxicity varies considerably. QS-18 is lethal in mice at doses as low as 25 μg, while QS-21 shows only some lethality at 500 μg [60]. These results and others

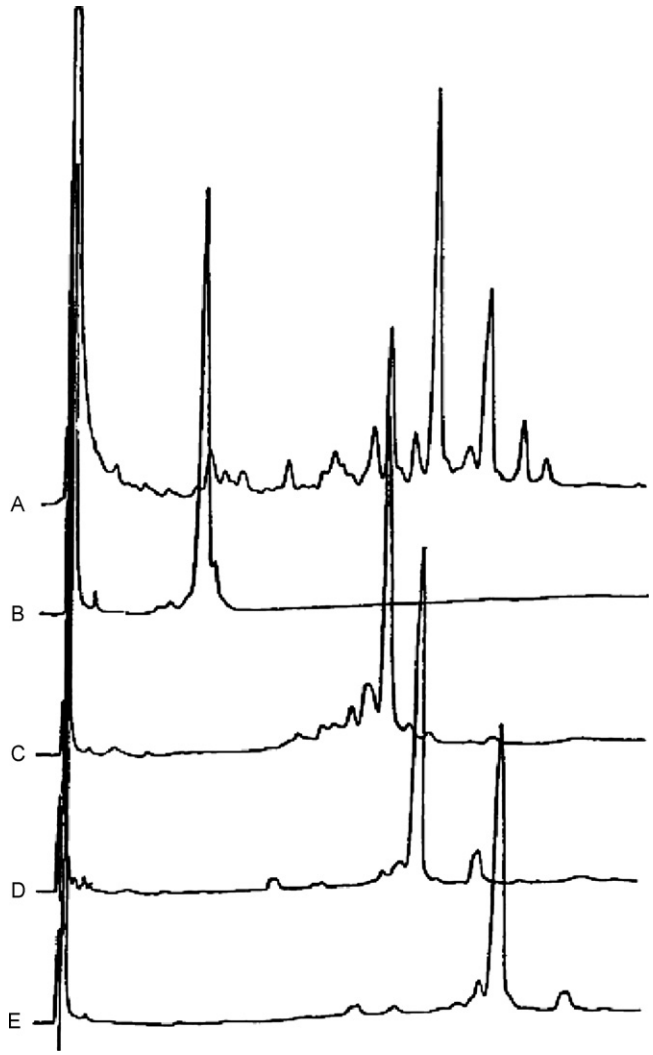

Fig. 1. HPLC chromatograms of an aqueous extract of *Q. saponaria* bark treated by ultrafiltration (A), saponin QS-7 (B), saponin QS-17 (C), saponin QS-18 (D) and saponin QS-21 (E). Gradient was 30–40% 0.1% TFA/acetonitrile/30 min, 40%/15 min at a flow rate of 1 ml/min. A total of 100 μg of purified saponin or 200 μg bark extract (dry weight) was used per injection [60].

led to the development of QS-21 as an effective adjuvant with a recombinant subunit vaccine against feline leukemia virus (FeLV) which is commercially available [60,62]. The addition of QS-21 to a denatured recombinant FeLV-A envelope glycoprotein expressed in *Escherichia coli* resulted in the protection of cats against a challenge with infectious FeLV. In effect, several authors have shown that *Quillaja* saponins (including QS-21) stimulated the production of CTLs and induces Th1 cytokines (IL-2 and IFN-γ) and antibodies of the IgG2a isotype to protein antigens [46,63,64]. In addition, QS-21 can be applied in subunit vaccines to produce variant-specific antibody responses, inducing CTL also to an additional unidentified epitope outside antigen-specific region [65]. QS-21 has also been claimed to perform as an adjuvant for DNA vaccines, following both systemic and mucosal administration [66].

The high toxicity and undesirable haemolytic effects of Quil A and QS-21 have been pointed out as the main restriction to their use in human vaccination [50]. The hemolytic activity of Quil A and QS-21 was shown to relate to the presence of side chains bearing aglycone (sugar chains) [67], acyl residues or the epoxy-framework system [28]. QS-21 consists of quillaic acid with one branched trisaccharide and one unbranched tetrasaccharide attached, and a dimeric fatty acyl group attached to the first sugar of the tetrasaccharide by an ester linkage (Fig. 2). The presence of fatty acids could favor interactions between the saponin and membrane cholesterol promoting the haemolysis [68], because deacylsaponins showed no toxicity [45]. Their lipophilic acyl side chain was showed to be responsible for the remarkable stimuli for CTL production against exogenous proteins and instability under physiological conditions [51]. Spontaneous deacylation would occur in aqueous solution [69], leading to the production of the deacylated saponins which are significantly less toxic and capable of eliciting a Th2 response while fail to stimulate either a lymphoproliferative response or the formation of CTL [51].

Adjuvant activities of Quil A and its purified saponins have been proved in the research of veterinary and human vaccination. In veterinary vaccine research, QS-21 was found to improve immune response of both particulate and soluble antigens of Aujeszky's disease virus (ADV) by promoting the production of IgG1 and IgG2a antibodies in a large extent [70]. The advantages of Quil A over leading to Th1 immune response can be demonstrated by their comparison with other adjuvants in combination with the fucose–mannose ligand of *Leishmania donovani* (FML). It was showed that the QS-21/FML and Quil A/FML groups achieved the highest IgG2a response, while Quil A/FML developed the strongest delayed type of hypersensitivity (DTH) and QS-21/FML animals showed the highest serum IFN-γ concentrations among five kinds of adjuvants including Riedel de Haen (R), Quil A, QS-21 saponins, IL-12 and BCG in vaccination of an outbred murine against visceral leishmaniasis [71]. Moreover, vaccination research against *Plasmodium falciparum* [72], purified opacity protein from the major outer membrane proteins of the pathogenic *Neisseria* [73], native *Ostertagia ostertagi* polyprotein allergen in cattle [74], foot-and-mouth disease [75] and *Measles virus* in mice [76] all showed that Quil A or QS-21 can elicit serum antigen-specific Th1-associated immunoglobulin level in the vaccinated animals. While being suitable for veterinary applications, Quil A was less satisfactory for human applications as it causes the local reactions, typically consisting of transient mild or moderate pain, tenderness, and induration. In some human vaccine design, QS-21 has been evaluated for their adjuvant activities. Improving immunogenicity of a synthetic malaria peptide vaccine SPf66 was achieved by using SPf66/QS-21 vaccines. It reported that vaccine formulations containing QS-21 induced a 45- to over 200-fold increase in anti-SPf66 IgG titers over the alum formulation after the second and third doses, respectively in healthy adults. And antibody responses generated by the QS-21 formulations were of longer duration compared to those evoked by the alum formulation, demonstrating that the use of QS-21 can substantially enhance the immunogenicity of peptide vaccines [77]. However, no clear immune response advantage was identified among healthy adults for QS-21 adjuvanted influenza vaccine while vaccination site pain and post vaccination myalgias were greater in the QS-21 group [78], indicating that adjuvant activities of QS-21 in human vaccine should be further confirmed in the future studies.

Quil A can also be applied in the novel design of immunotherapeutic or tumor vaccine. Leishmune vaccine, formulated with an increased adjuvant from Quil A, decreased average of the clinical scores, but increased average of $CD4^+$ Leishmania-specific lymphocytes for the immunotherapy-treated dogs, indicating the immunotherapeutic potential of this vaccination on canine visceral leishmaniasis [79]. Induction of an immune response against cancer antigens is generally difficult because these antigens are autoantigens. To get maximal benefit from the adjuvant component of cancer vaccines, researches had tested effects of combinations of optimal adjuvants on improving immune response against vaccines containing two cancer antigens, GD3 ganglioside and MUC1 peptide, covalently attached to keyhole limpet hemocyanin (KLH) [80–82]. It showed that twelve different adjuvant combinations and

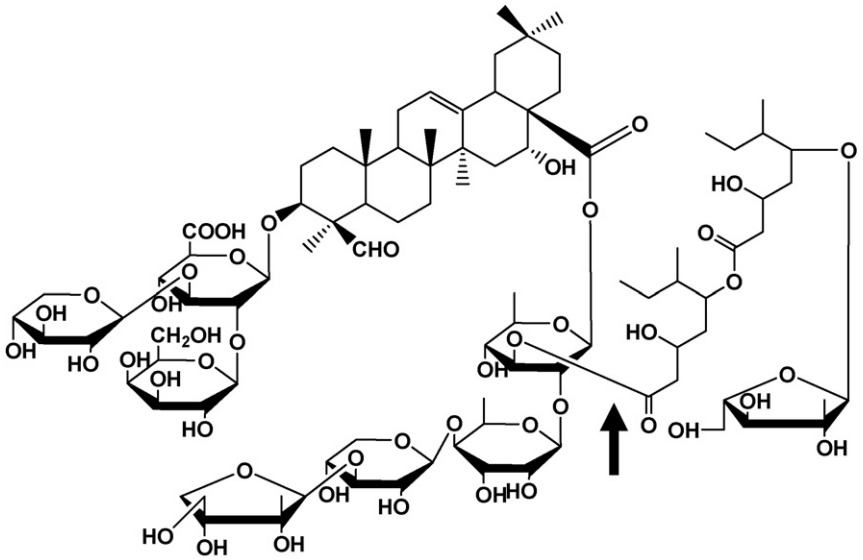

Fig. 2. Chemical structure of QS-21 from *Quillaja saponaria*. The arrows point the local of the acyl side chain responsible for Th1 type responses, CTL stimulation and toxicity.

GPI-0100 (semi-synthetic saponin from Quil A) were superior to QS-21 alone for induction of IgM and IgG antibodies against MUC1 and/or GD3 and their corresponding IFN-γ release and DTH against KLH.

2.2. Ginseng saponins

Ginseng saponins (ginsenosides) are believed to be the active substances in the root of *Panax ginseng*. Ginseng extract significantly increased the blood polymorphonuclear leukocyte phagocytosis and intracellular killing [83], and lymphocyte proliferation [84], and IFN-γ and TNF production [85]. Ginseng extract could also enhance specific antibody response against diphtheric toxoids in mice [86] and increase IgG and IgM antibody responses in mice immunized with sheep red blood cells (SRBC) [87]. Rivera et al. [88] reported that ginseng extract potentiated the antibody response to porcine parvovirus (PPV) in guinea pigs. Adjuvant effect of ginsenoside on bacterial antigens can be obtained by evaluating the enhancing effect on vaccinating pigs against *Erysipelothrix rhusiopathiae* infections [89]. Ginsenosides may induce Th1 and Th2 immune isotype, varying according to the antigen and the species [90]. Ginsenoside Rb_1 induced balanced Th1 or Th2 type of immunity of PPV vaccines [91], while ginsenoside Rg_1 enhanced Th2 lineage development from the naive $CD4^+$ T cell both by increasing Th2 specific cytokine secretion and by repressing Th1 specific cytokine production [92].

2.3. Panax notoginseng saponins

The roots of *P. notoginseng* (Burk.) F. H. Chen has been used in traditional Chinese medicine for treatment of cardiovascular diseases, inflammation, different body pains, trauma, and internal and external bleeding due to injury [93]. Over 50 different saponins isolated from *P. notoginseng* belong to the dammarane-type saponins, which are the main bioactive principles in this drug and account for 12% of the total root [94]. These saponins include ginsenosides, notoginsenosides and gypenosides and are composed of a protopanaxadiol and of protopanaxatriol glycosides [95,96]. Although some of its chemical constituents were similar to those present in two other well-known species in the same plant genus—*P. ginseng* and *P. quinquefolium*, notoginsenosides are the inherent constituents in *P. notoginseng*. *P. notoginseng* saponins were shown to display a slight haemolytic effect and enhance significantly a specific antibody and cellular immune response against OVA in mice [97]. From this extract, Sun et al. isolated eleven immunological adjuvant-active saponins, notoginsenosides K, R_1, R_2, R_4 and U, as well as ginsenosides Rb_1, Rd, Re, Rg_1, Rh_1, Rh_4 [98–101]. Yoshikawa et al. also examined the adjuvant effect of eleven notoginsenosides (A, C, D, G–N), two ginsenosides (Rb_1, Rg_1), and five quinquenosides (I–V) from *P. notoginseng* and *P. ginseng*, and found that notoginsenosides D, G, H and K could increase the sera IgG level in OVA-immunized mice, and notoginsenosides A, C, I, L, and N and quiquenosides III–V tended to show this activity [102]. In order to further elucidate the mechanism responsible for adjuvant activity of *P. notoginseng* saponin, ginsenoside Rd was evaluated for inducing Th1 or Th2 immune responses in mice against OVA, and was proved to increase a antigen-specific antibody and cellular response and elicit a Th1 and Th2 immune response by regulating production and gene expression of Th1 cytokines and Th2 cytokines [103].

2.4. Platycodon grandiflorum saponins

The saponins from the root of *P. grandiflorum* increased a specific antibody and cellular response against OVA in mice, and could be a promising balanced Th1 and Th2 directing immunological adjuvants [104]. The further purification of this extract afforded four adjuvant-active saponins, platycodin D, D2, D3, and platycoside E. These four saponins all significantly enhanced the Con A-, LPS-, and OVA-induced splenocyte proliferation, serum OVA-specific IgG, IgG1, IgG2a, and IgG2b antibody titers in the immunized mice. Platycodin D and D2 were found to promote the mRNA expression of cytokines IL-2, IFN-γ, IL-4, and IL-10 and transcription factors T-bet and GATA-3 in Con A-stimulated mice splenocytes, suggesting that these saponins could simultaneously elicited a Th1 and Th2 immune response by regulating gene expression of Th1/Th2 cytokines and transcription factors [105,106]. Platycodin D and D2 have recently proved to possess the adjuvant activities on recombinant hepatitis B surface antigen (rHBsAg), Newcastle disease virus-based live attenuated vaccine, and fowlpox virus expressing the avian influenza virus H5 gene [107–109].

2.5. Polygala saponins

The earliest report on adjuvant activity of saponins from *Polygala* was given by Mita et al. [110] in 1979. In his study, onjisaponin B was reported to increase antigen-specific antibody producing cells in the serum in SRBC-immunized mice. Two saponin fractions

from the root of *P. senega* increased specific antibody levels in mice immunized with ovalbumin and hens immunized with rotavirus. In mice, there was a preferential increase of the IgG2a subclass, high IL-2 and IFN-γ production. These two fractions were less toxic than Quil A at the same dose [111]. Katselis et al. [112] evaluated the immunological activity of eight pure saponins from the root of *P. senega* in mouse models with OVA. Among eight saponins, PS1, onjisaponin A and onjisaponin B significantly increased the IgG2a subclass antibody and IL-2 production. Among the hot water extracts from 267 different types of Chinese and Japanese medicinal plants screened for the adjuvant activity, the root of *Polygala tenuifolia* contained the most potent adjuvants when combined with nasal influenza or diphtheria–pertussis–tetanus (DPT) vaccine, and its active substances were identified as onjisaponins A, E–G. These four onjisaponins provided safe and potent adjuvants for intranasal inoculation of influenza HA and DPT vaccines [113]. We have recently isolated six adjuvant-active saponins, onjisaponins A, B, polygalasaponin XXVII, XXXII, and tenuifolisaponin A, B from the root of *P. tenuifolia* [114]. Tenuifolisaponin A and B significantly enhanced ORF2-specific IgG, IgG1 and IgG2b antibody titers in the mice immunized with porcine circovirus type 2 ORF2-based DNA vaccine by up-regulating expressions of cytokine IL-2, 4, 10 and INF-γ mRNA [115].

2.6. Others

The other adjuvant-active saponins isolated in recent years were shown in Table 1.

3. Structure–activity relationship of saponins with the adjuvant properties

Saponins are present in a wide range of plant species and in some marine organisms [147]. Saponins are complex molecules consisting of non-sugar aglycone coupled to sugar chain units [148]. Saponins are often subdivided into two main classes, the triterpenoid and the steroid saponins [149], which are both derived from the 30 carbon atoms containing precursor oxidosqualene [150]. The difference between the two classes lies in the fact that the steroid saponins have three methyl groups removed (i.e. they are molecules with 27 C-atoms), whereas in the triterpenoid saponins all 30 C-atoms are retained. Saponins have one or more linear or branched sugar chains containing glucose, galactose, glucuronic acid, xylose, rhamnose or methylpentose, attached to the aglycone via a glycosidic ether or ester link. In some saponins, the presence of acylated sugars has also been detected. According to the number of sugar chains attached to the aglycone, the saponins can be monodesmosidic saponins (with a single sugar chain), or bidesmosidic saponins (with two sugar chains). In the monodesmosidic saponins, the sugar chain is typically attached by a glycosidic ether linkage at the C-3 of the aglycone. In addition to the C-3 linked sugar chain, bidesmosidic saponins have a second sugar chain bound at C-28 (triterpene saponins) or at C-26 (steroid saponins) by an ester linkage. Because of the typical lability of esters, bidesmosidic saponins are readily converted into their monodesmosidic forms by mild hydrolysis. The bidesmosidic saponins may have potent biological and pharmacological activities in animals [151].

3.1. Structure–activity relationship of the adjuvant activities of saponins

The adjuvanticity of saponin depends on its structure comprised of hydrophilic sugar side chains and hydrophobic aglycone backbone [29]. The adjuvant activity of saponins was also thought to be related to branched sugar chains or aldehyde groups [144] or to an acyl residue bearing the aglycone [63].

3.1.1. Effect of some particular functional groups on the adjuvant activities of saponins

The adjuvant activity of saponins was thought to be related to aldehyde groups in the aglycones [47,144]. QS-21 derivatives that were modified at the carboxyl group on an anionic sugar, glucuronic

Table 1
Specification of the other adjuvant-active saponins isolated in recent years.

Species	Saponin type[a]	Features	Ref.
Achyranthes bidentata saponins	3-MD, 3,28-BD	Slight haemolytic; promote OVA-specific splenocyte proliferation, and IgG, IgG1 and IgG2b antibody titers.	[116]
Anemone raddeana saponins	3-MD, 3,28-BD		[117]
Astragalus membranaceus	3-MD, 3,6 (25)-BD, 3,6,25-TD	Slight hemolytic; enhance OVA-specific splenocyte proliferation, and IgG, IgG1 and IgG2b antibody titers; promote the peripheral lymphocyte proliferation and serum antibody titer in chicken vaccinated with Newcastle disease vaccine; activate macrophages.	[118–120]
Chenopodium quinoa saponins	3-MD, 3,28-BD	Mucosal adjuvant; potentiate specific IgG and IgA antibody responses to cholera toxin and OVA; increase mucosal permeability.	[121,122]
Escins	3-MD	Low toxicity; induce lower antibody responses to OVA than QS-21.	[29]
Glycyrrhiza saponins	3-MD	Slight haemolytic; enhance OVA-specific splenocyte proliferation, IgG, IgG1 and IgG2b antibody titers, and IL-12 production from lymphocytes and macrophages.	[123,124]
Gypenosides	3-MD, 3,21-BD	Slight haemolytic; increase OVA-specific splenocyte proliferation, IgG, IgG1 and IgG2b antibody levels, release of IL-2 from splenocyte and IL-1 from macrophages.	[125–127]
Jujubosides	3-MD	Less or no haemolytic; increase OVA-specific antibody response.	[128]
Kinmoonosides	3,28-BD	Activate T and B cells; enhance OVA-specific IgG, IgG1 IgG2a and IgG2b antibody levels.	[129,130]
Lablabosides	3,28-BD	Induce the production of large IgG1 and little IgG2a antibody response to ADV antigen.	[131,132]
Periandradulcins	3-MD	Slight haemolytic; increase IgG, IgG1, IgG2a and IgG2b response to FML antigen.	[67]
Pulcherrimasaponin (CP05)	3,28-BD	Induced an equally potent DTH to FML and IgG2b response, and a slight lower IgG, IgG2a and IgG3 titers compared with QS-21.	[133,134]
Quillaja brasiliensis saponins	3-MD	Low toxicity; enhance bovine herpesvirus type 1 specific IgG, IgG1 and IgG2a antibody levels.	[135]
Saikosaponins	3-MD	Slight haemolytic; enhance OVA-specific splenocyte proliferation, and serum IgG, IgG1 and IgG2b antibody levels; increase level of IL-1 and cellular lysosomal enzyme, induce cytostatic activity and expression of Fc receptor and Ia antigen of macrophages.	[136–142]
Soyasaponins	3-MD, 3,22-BD	Little haemolytic; induce a stronger antibody response to OVA than QS-21, predominantly the IgG1 isotype but little IgG2a.	[143–145]
Taurosides	3-MD	Induce strong humoral immune responses to HIV-1 envelope glycoproteins rgp160 and rgp120.	[146]
Trigoneosides	3,26-BD	No haemolytic; increase OVA-specific antibody response.	[29].

[a] Described on the basis of the number and position of sugar chain(s). MD: monodesmoside; BM: bisdesmoside; TD: tridesmoside.

acid, by reacting the glucuronic acid carboxyl group of QS-21 with free amino groups, retained adjuvant activity for antibody stimulation, in contrast, QS-21 derivatives modified at an aldehyde on the triterpene did not show adjuvant activity for antibody stimulation or for induction of cytotoxic T-lymphocytes [47]. These results stress the pivotal role that the aldehyde group plays in the adjuvant properties of *Quillaja* saponins. One possible mechanism involving the aldehyde might be the formation of a Schiff base with a free amino group on the surface of an immune cell target [152]. Palatnik de Sousa et al. [153] suggested that the proportion of conformational isomers of the triterpen-aldehyde is crucial for the integrity of the Th1 adjuvant response and it seems that axial aldehyde are more important in humoral immune response while equatorial aldehyde are more relevant to the cellular protective immune response. Although the similarities in the potency of the humoral response induced by the QS-21 and CP05–FML formulations are related to their similarities in composition and structure, the presence of the aldehyde group in QS-21 but not in CP05 could then explain the stronger induction of the typical Th1 IgG2a subtype in QS-21 [44]. The study on the saponins from the root of *P. tenuifolia* showed that a carboxyl function at position 23 instead of an aldehyde group can be just as effective for inducing adjuvant activity [113].

It was reported that the adjuvant activity of saponins also relates to the acyl residue bearing the aglycone [63]. In contrast to the majority of saponins from other species, *Quillaja* saponins are acylated. The three most predominant saponins (QS-17, QS-18 and QS-21) are acylated at the 4-hydroxyl position of fucose with two linked 3,5-dihydroxy-6-methyloctanoic acids containing a glycosylation site at the 5-OH position of one of the acyl chains. It was proved that the remarkable property of *Quillaja* saponins to stimulate CTL production against exogenous proteins appears to depend on their lipophilic acyl side chain [51]. Deacylated QS-18 and QS-21 induced a lower total-IgG response to bovine serum albumin than that induced by the native acylated forms [61], and the deacylsaponin of QS-21 (termed DS-1) did not stimulate a strong level of antibody or OVA-specific CTL responses [154]. DS-1 (deacylated QS-21) and RDS-1 (reacylated DS-1 with dodecylamine at a different site than QS-21) induced IgG1 responses at higher doses compared with that induced by QS-21. However, DS-1 was inactive for inducing IgG2a or CTL responses at any doses, while RDS-1 showed moderate IgG2a response, but did not show CTL response at any dose evaluated [155]. Oliveira-Freitas et al. [45] reported that the QS-21 exhibited mild toxicity, significant adjuvant effect on the anti-FML humoral response before and after *L. donovani* infection, and decrease in liver relative weight, while the deacylsaponins showed no toxicity, less haemolysis, antibody and DTH responses increased mainly after infection. This suggests that acylation is highly critical to Th1 type responses (CTL, IgG2a), but less critical to Th2 type responses (IgG1). However, studies from another kind of saponin CP05 from *Calliandra pulcherrima* gave the different results [134]. Balb/c mice were immunized either intact saponin (CP05), the monoterpene-deprived (BS), the C-28 carbohydrate-deprived (HS) or the sapogenin fraction in formulation with the FML antigen of *L. donovani* and challenged with *L. chagasi*. The results showed that the monoterpene acylated moiety is not necessary for the induction of a protective global Th1 response, because BS vaccine showed powerful protective effect and anti-FML antibody or IFN-γ secretion [134].

Although aldehyde, carboxyl or some acylate groups in molecular structure play the pivotal role in the adjuvant properties of *Quillaja* saponins, many saponins lacking the aldehyde group and acyl residues such as soyasaponins and lablabosides also showed strong adjuvant activity. On the other hand, the most of the escins that have acyl residues did not show adjuvant activity. Thus, the aldehyde group and the acyl residue in a saponin molecule are not considered to be essential for adjuvant activity [29].

3.1.2. Influence of the sugar side chain on the adjuvant activities of saponins

It was recognized that the sugar side chain in saponins may be essential to their adjuvanticity [61]. Soyasaponins bearing sugar chain(s) showed adjuvanticity stimulating anti-OVA total-IgG and IgG1 antibody responses while their corresponding aglycones soyasapogenols A and B, did not. Among bisdesmosidic soyasaponins, soyasaponin A1 with a long sugar side chain induced stronger total-IgG and IgG1 antibody responses than soyasaponin A2. For monodesmosidic soyasaponins, the ranking in terms of antibody response was soyasaponin I > soyasaponin II > soyasaponin III. Thus, Oda et al. concluded that the adjuvant activity tended to increase with the sugar side chain length and the HLB value [145]. The importance of glycidic chain was also demonstrated in other saponins with triterpenoid aglycone such as CP05 [134]. The HS saponin of CP05, which is C-28 carbohydrate-deprived, showed diminished responses in FML vaccine, except for IFN-γ secretion, indicating that the integrity of the carbohydrate moiety attached to C-28 is mandatory for the these functions. In a study of relationship between haemolytic and adjuvant activity and structure of protopanaxadiol- and protopanaxatriol-type saponins from the roots of *P. notoginseng*, it was found that the number, length and position of sugar side chains, and the type and the linkage of glucosyl group in the structure of these saponins could not only affect the adjuvant potentials, but have significant effects on the nature of the immune responses [156,157]. It has recently been reported that the number of sugar residues in the glycidic chains attached to C-3 of aglycone could affect the adjuvant activities of platycodigenin-type saponins, and the adjuvant activity decreased with the increased number of monosaccharide of the glycidic moieties at the C-3 of the aglycone [105].

A lot of adjuvant components have an amphipathic structure. Saponins also have those features. Especially the overall conformation harmoniously constructed by both hydrophilic and hydrophobic functional groups, rather than each individual functional group itself, is the most essential element for the consideration of adjuvant activity.

3.2. Structure–activity relationship of the haemolytic activities of saponins

It was claimed in the past that saponins should not be used as adjuvant due to their intrinsic haemolytic properties. Nevertheless, there are saponins that show low or no haemolytic effect. The haemolytic activity of saponins is considered to be influenced by the affinity of the aglycone to cholesterol in cell membranes [158]. The degree of activity or affinity depends on the type of aglycone itself [159,160], or the presence of side chains bearing aglycone; i.e. sugar chains [67,161–163], acyl residues [164], or the epoxy-framework system [165]. Takechi and Tanaka analyzed the haemolytic activity of 27 saponins out of 75 synthetic steroid and triterpene glycosides and found that the haemolytic activity of the steroids were greater than that of the triterpenoids and the haemolytic rates of the steroids were faster than those of the triterpenoids [160]. Santos et al. [67] also reported that saponins of *Agave sisalana* and *Smilax officinalis* shared a steroid aglycone moiety were the most haemolytic, while saponins obtained from *Bredemeyera floribunda* and *Periandra mediterranea* with a triterpenoid aglycone moiety the less haemolytic. QS-21, however, although a triterpenoidal saponin is still highly haemolytic [45,153]. The fatty acid moiety in QS-21 was reported to be the responsible for the toxicity evident by hemolytic activity [71], mice lethality [69] and loss of hair [71]. Deacylsaponins from QS-21 or CP05 were reported to show no toxicity, less haemolysis [45,134]. Ronnberg et al. [166] also demonstrated that reduction of toxicity of *Quillaja* saponins is obtained after the carbohydrate moiety degradation with IO_4Na treatment.

Another treatment of Riedel de Haen saponin (R) and Quil A with H_2SO_4 gave rise to their sapogenin fractions, which showed much slighter in vivo toxicity and reduced hemolytic potential without affecting their aldehyde and Th1 cellular immune response [153]. Thus, the presence of a monoterpene hydrophobic moiety could favor interactions between the saponin and membrane cholesterol promoting the haemolysis. On the other hand, the size of the attached glicidic chains also modulates the hemolytic activity of saponins. The saponin from *P. mediterranea*, for instance, has a single sugar chain which is composed of two residues of glucuronic acid attached to carbon C-3 via oxygen. The hemolytic effect of this triterpenoid saponin was further reduced by removal of the glycosidic moiety [67]. The saponin and sapogenin fractions isolated from *B. floribunda* and Riedel De Haen or Quil A [153] also showed that the removal of the glicidic moiety abolished the undesirable hemolytic activity but still maintaining the adjuvant potential. Sun et al. reported that the number, the length and the location of sugar side chains, and the type of sugar in sugar moiety all could affect the haemolytic activity of protopanaxadiol-type and protopanaxatriol-type saponins [156,157]. The hemolytic potentials of platycodigenin-type saponins could decrease with the increased number of monosaccharide of the glycidic moieties at the C-3 of the aglycone [105]. Therefore, it is considered that not only the functional groups and the glycidic moieties themselves, but their overall conformation affects haemolytic activity of saponins.

4. Conclusion and perspective

This review has summarized the current development of saponin-based adjuvant for potential use in human or veterinary vaccines. More and more researches have focused on improved saponin-based adjuvant which may increase the effectiveness of current vaccines. There is, however, no universal ideal adjuvant for each vaccine and they should be adapted according to specific criteria to have the best balance between safety and efficacy. Until recently, Quil A remains most widely used in research or production for novel vaccines, and most studies on the mechanisms or structure–activity relationship of saponins are focused on Quil A or its purified compounds. However, several drawbacks of Quil A and its purified saponins have limited their clinical use in vaccine designs. The researches have paid more and more attentions to other kinds of saponins extracted from natural products or traditional Chinese medicines.

Several saponins have been showed to possess excellent adjuvant effect with relatively lower haemolysis, making them ideal adjuvant candidate for future use. Consequently, these kinds of adjuvants also need elaborated research to show their detailed mechanism of protection against different diseases and their specific enhancement of humoral or cellular immune response should be further confirmed in various novel vaccines for human or veterinary use. ISCOM™ and ISCOMATRIX™ combine the advantages of a particulate carrier system with the presence of an in-built adjuvant (Quil A) and consequently have been found to be more immunogenic than other colloidal systems such as liposomes and protein micelles [167]. Critically, formulation of ISCOM™ and ISCOMATRIX™ vaccines retained the adjuvant activity of the saponin, while removing its haemolytic activity, producing no toxicity. They also required substantially less antigen and adjuvant to induce immunity in the host than vaccination with simple mixtures of free antigen and saponins [33]. Many studies have demonstrated the ability of ISCOM™ and ISCOMATRIX™ vaccines to induce strong antigen-specific antibody and cell-mediated immune responses to a wide range of antigens in a number of animal models [168–170]. As such, the adjuvant properties of these other saponins deserve further investigations when incorporated into ISCOM™ and ISCOMATRIX™.

Although some saponins have a strong adjuvant activity when administered parenterally, in general, they have a low or no activity when delivered orally. This low oral activity may be due to (i) the relatively low doses of saponin delivered to the gastrointestinal tract, and (ii) the saponin's breakdown to non-absorbable byproducts by gastric and intestinal secretions and the intestinal flora [55]. Nevertheless, some ingested saponins (i.e. licorice) show significant pharmacological activity that indicates some gastrointestinal absorption occurs. Thus, how or what the routes of injection influence the adjuvant effect of saponin remains to be resolved. Other remaining questions include the levels of IgE generated with these saponin-based vaccines and the crucial question of longevity of the immune response that is generated.

Acknowledgements

This work was supported by Grant-in-Aid from the National Natural Science Foundation of China (No. 30871888), the Zhejiang Provincial Natural Science Foundation of China (No. R3080027) and the Administration of Traditional Chinese Medicine of Zhejiang Province (No. A2006Z017).

References

[1] Lima KM, dos Santos SA, Rodrigues Jr JM, Silva CL. Vaccine adjuvant: it makes the difference. Vaccine 2004;22:2374–9.
[2] O'Hagan DT, Mackichan ML, Singh M. Recent developments in adjuvants for vaccines against infectious diseases. Biomol Eng 2001;18(3):69–85.
[3] Marciani DJ. Vaccine adjuvants: role and mechanisms of action in vaccine immunogenicity. Drug Discov Today 2003;8:934–43.
[4] Rock KL, Hearn A, Chen CJ, Shi Y. Natural endogenous adjuvants. Springer Semin Immunopathol 2005;26(3):231–46.
[5] Ramon G. Sur la toxine et sur l'anatoxine diphtheriques. Ann Inst Pasteur 1924;38:1–10.
[6] Leclerc C. New approaches in vaccine development. Comp Immunol Microbiol Infect Dis 2003;26:329–41.
[7] Livingston PO, Adluri S, Helling F, Yao TJ, Kensil CR, Newman MJ, et al. Phase I trial of immunological adjuvant QS-21 with a GM2 ganglioside-keyhole limpet haemocyanin conjugate vaccine in patients with malignant melanoma. Vaccine 1994;12(14):1275–80.
[8] Mcneela EA, Mills KH. Manipulating the immune system: humoral versus cell-mediated immunity. Adv Drug Deliv Rev 2001;51(1-3):43–54.
[9] Aguilar JC, Rodríguez EG. Vaccine adjuvants revisited. Vaccine 2007;25(19):3752–62.
[10] McCluskie MJ, Weeratna RD. Novel adjuvant systems. Curr Drug Targets Infect Disord 2001;1(3):263–71.
[11] Spickler AR, Roth JA. Adjuvants in veterinary vaccines: modes of action and adverse effects. J Vet Intern Med 2003;17(3):273–81.
[12] Azuma I, Seya T. Development of immunoadjuvants for immunotherapy of cancer. Int Immunopharmacol 2006;1(7):1249–59.
[13] Holmdahl R, Lorentzen JC, Lu S, Olofsson P, Wester L, Holmberg J, et al. Arthritis induced in rats with nonimmunogenic adjuvants as models for rheumatoid arthritis. Immunol Rev 2001;184:184–202.
[14] Pascual DM, Morales RD, Gil ED, Muñoz LM, López JE, Casanueva OLJ. Adjuvants: present regulatory challenges. Vaccine 2006;24(S2):88–9.
[15] Glenny AT, Pope CG, Waddington H, Wallace U. The antigenic value of toxoid precipitated by potassium alum. J Pathol Bacteriol 1926;29:38–9.
[16] Gupta RK. Aluminum compounds as vaccine. Adv Drug Deliv Rev 1998;32:155–72.
[17] Relyveld EH, Bizzini B, Gupta RK. Rational approaches to reduce adverse reactions in man to vaccines containing tetanus and diphtheria toxoids. Vaccine 1998;16:1016–23.
[18] Marshall DJ, Rudnick KA, McCarthy SG, Mateo LR, Harris MC, McCauley C, et al. Interleukin-18 enhances Th1 immunity and tumor protection of a DNA vaccine. Vaccine 2006;24(3):244–53.
[19] Friel H, Lederman H. A nutritional supplement formula for influenza A (H5N1) infection in humans. Med Hypotheses 2006;67(3):578–87.
[20] de Roux A, Marx A, Burkhardt O, Schweiger B, Borkowski A, Banzhoff A, et al. Impact of corticosteroids on the immune response to a MF59-adjuvanted influenza vaccine in elderly COPD-patients. Vaccine 2006;24(10):1537–42.
[21] Schultze V, D'Agosto V, Wack A, Novicki D, Zorn J, Hennig R. Safety of MF59™ adjuvant. Vaccine 2008;26(26):3209–22.
[22] Wack A, Baudner BC, Hilbert AK, Manini I, Nuti S, Tavarini S. Combination adjuvants for the induction of potent, long-lasting antibody and T-cell responses to influenza vaccine in mice. Vaccine 2008;26(24):552–61.
[23] Sparg SG, Light ME, van Staden J. Biological activities and distribution of plant saponins. J Ethnopharmacol 2004;94(2–3):219–43.

[24] Lacaille-Dubois MA. Bioactive saponins with cancer related and immunomodulatory activity: recent developments. Stud Nat Prod Chem 2005;32(12):209–46.
[25] Grenby TH. Intense sweeteners for the food industry: an overview. Trends Food Sci Technol 1991;2:2–6.
[26] Kitagawa I. Licorice root. A natural sweetener and an important ingredient in Chinese medicine. Pure Appl Chem 2002;74:1189–98.
[27] Heng L, Vincken JP, van Koningsveld GA, Legger L, Gruppen H, van Boekel MAJS, et al. Bitterness of saponins and their content in dry peas. J Sci Food Agric 2006;86:1225–31.
[28] Price KR, Johnson IT, Fenwick GR. The chemistry and biological significance of saponins in foods and feedstuffs. Crit Rev Food Sci Nutr 1987;26:27–135.
[29] Oda K, Matsuda H, Murakami T, Katayama S, Ohgitani T, Yoshikawa M. Adjuvant and haemolytic activities of 47 saponins derived from medicinal and food plants. Biol Chem 2000;381(1):67–74.
[30] Petit PR, Sauvaire YD, Hillaire-Buys DM, Leconte OM, Baissac YG, Posin GR, et al. Steroid saponins from fenugreek seeds: extraction, purification, and pharmacological investigation on feeding behaviour and plasma cholesterol. Steroids 1995;60:674–80.
[31] Uematsu Y, Hirata K, Saito K. Spectrophotometric determination of saponin in Yucca extract used as food additive. J AOAC Int 2000;83:1451–4.
[32] Liu J, Henkel T. Traditional Chinese medicine (TCM): are polyphenols and saponins the key ingredients triggering biological activities? Curr Med Chem 2002;9:1483–5.
[33] Skene CD, Sutton P. Saponin-adjuvanted particulate vaccines for clinical use. Methods 2006;40(1):53–9.
[34] Kensil CR, Kammer R. QS-21: a water-soluble triterpene glycoside adjuvant. Exp Opin Invest Drugs 1998;7(9):1475–82.
[35] Bomford R, Stapleton M, Winsor S, Mc Night A, Andronova T. The control of the antibody isotype response to recombinant immunodeficiency virus gp120 antigen by adjuvants. AIDS Res Hum Retrovir 1992;8:1765–71.
[36] Sjolander S, Hansen JE, Lovgren-Bengtsson K, Akerblom L, Morein B. Induction of homologous virus neutralizing antibodies in guinea-pigs immunized with two human immunodeficiency virus type 1 glycoprotein gp120-iscom preparations: a comparison with other adjuvant systems. Vaccine 1996;14:344–52.
[37] Evans TG, McElrath MJ, Matthews T, Maontefiori D, Weinhold K, WolffM, et al. QS-21 promotes an adjuvant effect allowing for reduced antigen dose during HIV-1 envelope subunit immunization in humans. Vaccine 2001;19:2080–91.
[38] Chapman PB, Morrissey DM, Panageas KS, Hamilton WB, Zhan C, Destro AN, et al. Induction of antibodies against GM2 ganglioside by immunizing melanoma patients using GM2-keyhole limpet hemocyanin + QS-21 vaccine: a dose–response study. Clin Cancer Res 2000;6:874–9.
[39] Moreno CA, Rodriguez R, Oliveira GA, Ferreira V, Nussenzweig RS, Castro ZRM, et al. Preclinical evaluation of a synthetic *Plasmodium falciparum* MAP malaria vaccine in Aotus monkeys and mice. Vaccine 2000;18:89–99.
[40] Hancock GE, Speeelman DJ, Frenchick PJ, Mineo-Kuhn MM, Baggs RB, Hahn DJ. Formulation of the purified fusion protein of respiratory syncytial virus with the saponin QS-21 induces protective immune response in Balb/c mice that are similar to those generated by experimental infection. Vaccine 1995;13:391–400.
[41] Britt W, Fay J, Seals J, Kensil C. Formulation of an immunogenic human cytomegalovirus vaccine: response in mice. J Infect Dis 1995;171:18–25.
[42] Khan IA, Ely KH, Kasper LH. A purified parasite antigen (p30) mediates $CD8^+$ T cell immunity against fatal *Toxoplasm gondii* infection in mice. J Immunol 1991;147:3501–6.
[43] Borja-Cabrera GP, Cruz Mendes A, Paraguai de Souza E, Hashimoto Okada LY, de A, Trivellato FA, et al. Effective immunotherapy against canine visceral leishmaniasis with the FML-vaccine. Vaccine 2004;22(17–18):2234–43.
[44] da Silva BP, Correa Soares JBR, Paraguai de Souza E, Palatnik M, Palatnik de Sousa CB, Parente JP. Pulcherrimasaponin, from the leaves of *Calliandra pulcherrima*, as adjuvant for immunization in the murine model of visceral leishmaniasis. Vaccine 2005;23(8):1061–71.
[45] Oliveira-Freitas E, Casas CP, Borja-Cabrera GP, Santos FN, Nico D, Souza LO, et al. Acylated and deacylated saponins of *Quillaja saponaria* mixture as adjuvants for the FML-vaccine against visceral leishmaniasis. Vaccine 2006;24(18):3909–20.
[46] Takahashi H, Takeshita T, Morein B, Putney S, Germain RN, Berzofsky JA. Induction of $CD8^+$ cytotoxic T cells by immunization with purified HIV-1 envelope proteins in ISCOMS. Nature 1990;344(6269):873–5.
[47] Soltysik S, Wu JY, Recchia J, Wheeler DA, Newman MJ, Coughlin RT, et al. Structure/function studies of QS-21 adjuvant: assessment of triterpene aldehyde and glucuronic acid roles in adjuvant function. Vaccine 1995;13(15):1403–10.
[48] Schetters TP, Kleuskens J, Scholtes NC, Bos HJ. Vaccination of dogs against *Babesia canis* infection using parasite antigens from in vitro culture. Parasite Immunol 1992;14(3):295–305.
[49] Cox SJ, Barnett PV, Dani P, Salt JS. Emergency vaccination of sheep against foot-and-mouth disease: protection against disease and reduction in contact transmission. Vaccine 1999;17(15–16):1858–68.
[50] Waite DC, Jacobson EW, Ennis FA, Edelman R, White B, Kammer R, et al. Three double-blind, randomized trials evaluating the safety and tolerance of different formulations of the saponin adjuvant QS-21. Vaccine 2001;19(28–29):3957–67.
[51] Marciani DJ, Press JB, Reynolds RC, Pathak AK, Pathak V, Gundy LE, et al. Development of semisynthetic triterpenoid saponin derivatives with immune stimulating activity. Vaccine 2000;18(27):3141–51.
[52] Marciani DJ, Reynolds RC, Pathak AK, Finley-Woodman K, May RD. Fractionation, structural studies, and immunological characterization of the semisynthetic quillaja saponins derivative GPI-0100. Vaccine 2003;21(25–26):3961–71.
[53] San Martín R, Briones R. Industrial uses and sustainable supply of *Quillaja saponaria* (Rosaceae) saponins. Econ Bot 1999;53(3):302–11.
[54] Barr IG, Sjolander A, Cox JC. ISCOMs and other saponin based adjuvants. Adv Drug Deliv Rev 1998;32(3):247–71.
[55] Press JB, Reynolds RC, May RD, Marciani DJ. Structure/function relationships of immunostimulating saponins. Stud Nat Prod Chem 2000;24(5):131–74.
[56] Campbell JB, Peerbaye YA. Saponin. Res Immunol 1992;143(5):526–30.
[57] Dalsgaard K. Saponin adjuvants. 3. Isolation of a substance from *Quillaja saponaria* Molina with adjuvant activity in food-and-mouth disease vaccines. Arch Gesamte Virusforsch 1974;44(3):243–54.
[58] Dalsgaard K, Jensen MH, Sorensen KJ. Saponin adjuvants. IV. Evaluation of the adjuvant quil A in the vaccination of cattle against foot-and-mouth disease. Acta Vet Scand 1977;18(3):349–60.
[59] Dalsgaard K. 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. Acta Vet Scand Suppl 1978;(69):7–40.
[60] Kensil CR, Patel U, Lennick M, Marciani D. Separation and characterization of saponins with adjuvant activity from *Quillaja saponaria* Molina cortex. J Immunol 1991;146(2):431–7.
[61] Kensil CR, Soltysik S, Patel U, Marciani D. Structure/function relationship on adjuvants from *Quillaja saponaria* Molina. In: Brown F, Chanock RM, Ginsberg HS, Lerner RA, editors. Vaccines Vol. 92. New York: Cold Spring Harbor Laboratory Press; 1992. p. 35–40.
[62] Marciani DJ, Kensjl CR, Beltz GA, Hung CH, Cronier J, Aubert A. Genetically-engineered subunit vaccine against feline leukaemia virus: protective immune response in cats. Vaccine 1991;9(2):89–96.
[63] Kensil CR. Saponins as vaccine adjuvants. Crit Rev Ther Drug Carrier Syst 1996;13(1–2):1–55.
[64] Kensil CR, Wu JY, Soltysik S. Structural and immunological characterization of the vaccine adjuvant QS-21. Pharm Biotechnol 1995;6(1):525–41.
[65] Newman MJ, Wu JY, Gardner BH, Anderson CA, Kensil CR, Recchia J, et al. Induction of cross-reactive cytotoxic T-lymphocyte responses specific for HIV-1 gp120 using saponin adjuvant (QS-21) supplemented subunit vaccine formulations. Vaccine 1997;15(9):1001–7.
[66] Sasaki S, Sumino K, Hamajima K, Fukushima J, Ishii N, Kawamoto S, et al. Induction of systemic and mucosal immune responses to human immunodeficiency virus type 1 by a DNA vaccine formulated with QS-21 saponin adjuvant via intramuscular and intranasal routes. J Virol 1998;72(6):4931–9.
[67] Santos WR, Bernardo RR, Peçanha LT, Palatnik M, Parente JP, Palatnik de Sousa CB. Haemolytic activities of plant saponins and adjuvants. Effect of saponin on the humoral response to the FML antigen of *Leishmania donovani*. Vaccine 1997;15(9):1024–9.
[68] Jacobsen NE, Fairbrother WJ, Kensil CR, Lim A, Wheeler DA, Powell MF. Structure of the saponin adjuvant QS-21 and its base-catalyzed isomerization product by ^1H and natural abundance ^{13}C NMR spectroscopy. Carbohydr Res 1996;280(1):1–14.
[69] Marciani DJ, Pathak AK, Reynolds RC, Seitz L, May RD. Altered immunomodulating and toxicological properties of degraded *Quillaja saponaria* Molina saponins. Int Immunopharmacol 2001;1(4):813–8.
[70] Katayama S, Oda K, Ohgitani T, Hirahara T, Shimizu Y. Influence of antigenic forms and adjuvants on the IgG subclass antibody response to Aujeszky's. Vaccine 1999;17(20–21):2733–9.
[71] Santos WR, de Lima VMF, Paraguai de Souza E, Bernardo RR, Palatnik M, Palatnik de Sousa CB. Saponins, IL12 and BCG adjuvant in the FML-vaccine formulation against murine visceral leishmaniasis. Vaccine 2002;21(1–2):30–43.
[72] Rafi-Janajreh A, Tongren JE, Kensil C, Hackett C, Candal F, Lala A, et al. Influence of adjuvants in inducing immune responses to different epitopes included in a multiepitope, multivalent, multistage *Plasmodium falciparum* candidate vaccine (FALVAC-1) in outbred mice. Exp Parasitol 2002;101(1):3–12.
[73] de Jonge ME, Vidarsson G, van Dijken HH, Hoogerhout P, Alphen V, Dankert J, et al. Functional activity of antibodies against the recombinant OopaJ protein from *Neisseria meningitides*. Infect Immun 2003;71(5):2331–40.
[74] Vercauteren I, Geldhof P, Vercruysse J, Peelaers I, Broeck WVD, Gevaert K, et al. Vaccination with an *Ostertagia ostertagi* polyprotein allergen protects calves against homologous challenge infection. Infect Immun 2004;72(5):2995–3001.
[75] Xiao CW, Rajput ZI, Hu SH. Improvement of a commercial foot-and-mouth vaccine by supplement of Quil A. Vaccine 2007;25(25):4795–800.
[76] Pickering RJ, Smith SD, Strugnell RA, Wesselingh SL, Webster DE. Crude saponins improve the immune response to an oral plant-made measles vaccine. Vaccine 2006;24(2):144–50.
[77] Kashala O, Amador R, Valero MV, Moreno A, Barbosa A, Nickel B, et al. Safety, tolerability and immunogenicity of new formulations of the *Plasmodium falciparum* malaria peptide vaccine SPf66 combined with the immunological adjuvant QS-21. Vaccine 2002;20(17–18):2263–77.
[78] Mbawuike I, Zang Y, Couch RB. Humoral and cell-mediated immune responses of humans to inactivated influenza vaccine with or without QS21 adjuvant. Vaccine 2007;25(17):3263–9.
[79] Santos FN, Borja-Cabrera GP, Miyashiro LM, Grechi J, Reis AB, Moreira MA, et al. Immunotherapy against experimental canine visceral leishmaniasis

with the saponin enriched-Leishmune vaccine. Vaccine 2007;25(33):6176–90.
[80] Kim SK, Ragupathi G, Cappello S, Kagan E, Livingston PO. Effect of immunological adjuvant combinations on the antibody and T-cell response to vaccination with MUC1-KLH and GD3-KLH conjugates. Vaccine 2000;19(4–5):530–7.
[81] Livingston PO, Hood C, Ragupathi G. Botanicals as immunological adjuvants and immunomodulators. In: AACR meeting abstracts 2006; 2006. p. 1306–7.
[82] Ragupathi G, Gathuru J, Livingston P. Antibody inducing polyvalent cancer vaccines. Cancer Treat Res 2005;123(1):157–80.
[83] Scaglione F, Ferrara F, Dugnani S, Falchi M, Santoro G, Fraschini F. Immunomodulatory effects of two extracts of *Panax ginseng* C.A. Meyer. Drugs Exp Clin Res 1990;16(10):537–42.
[84] Wu S, Hua ZJ, Xio YL, Wang Y. Effect of Ginsengopolypeptide on the ^{3}H-TdR integration of human blood lymphocyte. Chin Med J 1991;104(5):399–401.
[85] Smolina TP, Solovéva TF, Besednova NN. Immunotropic activity of panaxans-bioglycans isolated from ginseng. Antibiot Khimioter 2001;46(7):19–22.
[86] Yang GZ, Bao T, Fu N, Gen PL. A preliminary study on the immunomodulatory effects of ginseng saponins in vitro and in vivo. J Norman Bethune Univ Med Sci 1983;9:1–7.
[87] Jie YH, Cammisuli S, Baggliolini M. Immunomodulatory effects of *Panax ginseng* CA Meyer in the mouse. Agents Actions 1984;15(3–4):386–91.
[88] Rivera E, Hu S, Concha C. Gingseng and aluminum hydroxide act synergistically as vaccine adjuvants. Vaccine 2003;21(11–12):1149–57.
[89] Rivera E, Daggfeldt A, Hu S. Ginseng extract in aluminium hydroxide adjuvanted vaccines improves the antibody response of pigs to porcine parvovirus and *Erysipelothrix rhusiopathiae*. Vet Immunol Immunopathol 2003;91(1):19–27.
[90] Tiong GKL, Gill HS, Lofthouse S, Puri NK. Comparison of conventional adjuvants and adjuvant-free monoclonal antibody targeting for stimulating antibody responses against a conjugate of luteinizing hormone releasing hormone and avidin. Vaccine 1993;11(4):425–30.
[91] Rivera E, Pettersson FE, Inganas M, Paulie S, Gronvik KO. The Rb1 fraction of ginseng elicits a balanced Th1 and Th2 immune response. Vaccine 2005;23(46–47):5411–9.
[92] Lee EJ, Ko EJ, Lee JW, Rho SW, Ko SG, Shin MK, et al. Ginsenoside Rg1 enhances CD4^{+} T-cell activities and modulates Th1/Th2 differentiation. Int Immunopharmacol 2004;4(2):235–44.
[93] Dong TTX, Cui XM, Song ZH, Zhao KJ, Ji ZN, Lo CK, et al. Chemical assessment of roots of *Panax notoginseng* in China: regional and seasonal variations in its active constituents. J Agric Food Chem 2003;51:4617–23.
[94] Xu QF, Fang XL, Chen DF. Pharmacokinetics and bioavailability of ginsenoside Rb1 and Rg1 from *Panax notoginseng* in rats. J Ethnopharmacol 2003;84:187–92.
[95] Du QZ, Jerz G, Waibel R, Winterhalter P. Isolation of dammarane saponins from *Panax notoginseng* by high-speed counter-current chromatography. J Chromatogr A 2003;1008:173–80.
[96] Lau AJ, Woo SO, Koh HL. Analysis of saponins in raw and steamed *Panax notoginseng* using high-performance liquid chromatography with diode array detection. J Chromatogr A 2003;1011:77–87.
[97] Sun HX, Ye YP, Pan HJ, Pan YJ. Adjuvant effect of *Panax notoginseng* saponins on the immune responses to ovalbumin in mice. Vaccine 2004;22(29–30):3882–9.
[98] Sun HX, Ye YP, Pan YJ. Immunological adjuvant saponins from the roots of *Panax notoginseng*. Chem Biodivers 2005;2(4):510–5.
[99] Qin F, Ye YP, Sun HX. Haemolytic activities and adjuvant effect of notoginsenoside K from the roots of *Pananx notoginseng*. Chem Biodivers 2006;3(10):1144–52.
[100] Sun HX, Chen YH, Ye YP. Haemolytic activities and adjuvant effect of ginsenoside Re and notoginsenosides R_1 from the roots of *Panax notoginseng*. Chem Biodivers 2006;3(7):718–26.
[101] Yang ZG, Ye YP, Sun HX. Immunological adjuvant effect of ginsenoside Rh4 from the roots of *Pananx notoginseng* on specific antibody and cellular response to ovalbumin in mice. Chem Biodivers 2007;4(2):232–9.
[102] Yoshikawa M, Morikawa T, Yashiro K, Murakami T, Matsuda H. Bioactive saponins and glycosides. XIX. notoginseng (3): immunological adjuvant activity of notoginsenosides and related saponins: structures of notoginsenosides-L, -M, and -N from the roots of *Panax notoginseng* (Burk.) F.H. Chen. Chem Pharm Bull 2001;49:1452–6.
[103] Yang ZG, Chen AQ, Sun HX, Ye YP, Fang WH. Ginsenoside Rd elicits Th1 and Th2 immune responses to ovalbumin in mice. Vaccine 2007;25(1):161–9.
[104] Xie Y, Pan HJ, Sun HX, Li D. A promising balanced Th1 and Th2 directing immunological adjuvant, saponins from the root of *Platycodon grandiflorum*. Vaccine 2008;26(31):3937–45.
[105] Xie Y, Ye YP, Sun HX, Li D. Contribution of the glycidic moieties to the haemolytic and adjuvant activity of platycodigenin-type saponins from the root of *Platycodon grandiflorum*. Vaccine 2008;26(27–28):3452–60.
[106] Xie Y, Deng W, Sun HX, Li D. Platycodin D2 is a potential less hemolytic saponin adjuvant eliciting Th1 and Th2 immune responses. Int Immunopharmacol 2008;8(8):1143–50.
[107] Xie Y, Sun HX, Li D. Platycodin D is a potent adjuvant of specific cellular and humoral immune responses against recombinant hepatitis B antigen. Vaccine 2009;27(5):757–64.
[108] Xie Y, Sun HX, Li D. Platycodin D2 induces an improved and balanced Th1/Th2 immune response to HBsAg in mice. Int Immunopharmacol (INTIMP-D-08-00306), submitted for publication.
[109] Xie Y. Immunological adjuvant activity of saponins from *Platycodon grandiflorum*. Ph.D. thesis, Zhejiang University, Hangzhou, China; 2008.
[110] Mita A, Shida R, Kasai N, Shoji J. Enhancement and suppression in production of IgM-antibody in mice treated with purified saponins. Biomedicine 1979;31(8):223–7.
[111] Estrada A, Katselis GS, Laarveld B, Barl B. Isolation and evaluation of immunological adjuvant activities of saponins from *Polygala senega* L. Comp Immun Microbiol Infect Dis 2000;23(1):27–43.
[112] Katselis GS, Estrada A, Gorecki DK, Barl B. Adjuvant activities of saponins from the root of *Polygala senega* L. Can J Physiol Pharmacol 2007;85(11):1184–94.
[113] Nagai T, Suzuki Y, Kiyohara H, Susa E, Kato T, Nagamine T, et al. Onjisaponins, from the root of *Polygala tenuifolia* Willdenow, as effective adjuvants for nasal influenza and diphtheria–pertussis–tetanus vaccines. Vaccine 2001;19(32):4824–34.
[114] Sun HX. Isolation and evaluation of immunological adjuvant active saponins from *Polygala tenuifolium* Willd. Ph.D. thesis, Zhejiang University, Hangzhou, China; 2005.
[115] Yang ZZ. Detection of porcine circovirus type 2 infection and potentiation of saponins to DNA immunization. Ph.D. thesis, Zhejiang University, Hangzhou, China; 2008.
[116] Sun HX. Adjuvant effect of *Achyranthes bidentata* saponins on specific antibody and cellular response to ovalbumin in mice. Vaacine 2006;24(17):3432–9.
[117] Sun YX, Li MG, Liu JC. Haemolytic activities and adjuvant effect of *Anemone raddeana* saponins (ARS) on the immune responses to ovalbumin in mice. Int Immunopharmacol 2008;8(8):1095–102.
[118] Yang ZG, Sun HX, Fang WH. Haemolytic activities and adjuvant effect of *Astragalus membranaceus* saponins (AMS) on the immune responses to ovalbumin in mice. Vaccine 2005;23(44):5196–203.
[119] Kong X, Hu Y, Rui R, Wang D, Li X. Effects of Chinese herbal medicinal ingredients on peripheral lymphocyte proliferation and serum antibody titer after vaccination in chicken. Int Immunopharmacol 2004;4(7):975–82.
[120] Yesilada E, Bedir E, Calis I, Takaishi Y, Ohmoto Y. Effects of triterpene saponins from *Astragalus* species on in vitro cytokine release. J Ethnopharmacol 2005;96(1–2):71–7.
[121] Madl T, Sterk H, Mittelbach M, Rechberger GN. Tandem mass spectrometric analysis of a complex triterpene saponin mixture of *Chenopodium quinoa*. Am Soc Mass Spectrom 2006;17(6):795–806.
[122] Estrada A, Li B, Laarveld B. Adjuvant action of *Chenopodium quinoa* saponins on the induction of antibody responses to intragastric and intranasal administered antigens in mice. Comp Immunol Microbiol Infect Dis 1998;21:225–36.
[123] Dai JH, Iwatani Y, Ishida T, Terunuma H, Kasai H, Iwakula Y, et al. Glycyrrhizin enhances interleukin-12 production in peritoneal macrophages. Immunology 2001;103(2):235–43.
[124] Sun HX, Pan HJ. Immunological adjuvant effect of *Glycyrrhiza uralensis* saponins on the immune responses to ovalbumin in mice. Vaccine 2006;24(11):1914–20.
[125] Zhang C, Yang X, Xu L. Immunomodulatory action of the total saponin of *Gynostemma pentaphylla*. J Mod Dev Tradit Med 1990;10:69–70.
[126] Wang B, Ge ZD, Zhou AW, Chen MZ. Effects of gypenosides on immune function of rats in vitro. Tradit Chin Drug Res Clin Pharmacol 1999;10:36–7.
[127] Sun HX, Zheng QF. Haemolytic activities and adjuvant effect of *Gynostemma pentaphyllum* saponins on the immune responses to ovalbumin in mice. Phytother Res 2005;19(10):895–900.
[128] Matsuda H, Murakami T, Ikebata A, Yamahara J, Yoshikawa M. Bioactive saponins and glycosides. XIV. Structure elucidation and immunological adjuvant activity of novel protojujubogenin type triterpene bisdesmosides, protojujubosides A, B, and B1, from the seeds of *Zizyphus jujuba* var. *spinosa* (Zizyphi Spinosi Semen). Chem Pharm Bull 1999;47(12):1744–8.
[129] Tezuka Y, Honda K, Banskota AH, Thet MM, Kadota S. Kinmoonosides A–C, three new cytotoxic saponins from the fruits of *Acacia concinna*, a medicinal plant collected in Myanmar. J Nat Prod 2000;63(12):1658–64.
[130] Kukhetpitakwong R, Hahnvajanawong C, Homchampa P, Leelavatcharamas V, Satra J, Khunkitti W. Immunological adjuvant activities of saponin extracts from the pods of *Acacia concinna*. Int Immunopharmacol 2006;6:1729–35.
[131] Yoshikawa M, Murakami T, Komatsu H, Matsuda H. Medicinal foodstuffs. XII. Saponin constituents with adjuvant activity from hyacinth bean, the seeds of *Dolichos lablab* L. (1): Structures of lablabosides A, B, and C. Chem Pharm Bull 1998;46(5):812–6.
[132] Komatsu H, Murakami T, Matsuda H, Yoshikawa M. Medicinal foodstuffs. XIII. Saponin constituents with adjuvant activity from hyacinth bean, the seeds of *Dolichos lablab* L. (2): Structures of lablabosides D, E, and F. Heterocycles 1998;48:703–10.
[133] Tani C, Ogihara Y, Takeda T. Studies on the constituents of *Calliandra anomala* (Kunth) Macbr. IV. Structure analysis by HPLC retention time and FAB-MS spectrum. Chem Pharm Bull 1998;46(4):723–5.
[134] Nico D, Santos FN, Borja-Cabrera GP, Palatnik M, Palatnik de Sousa CB. Assessment of the monoterpene, glycidic and triterpene-moieties' contributions to the adjuvant function of the CP05 saponin of *Calliandra pulcherrima* Benth during vaccination against experimental visceral leishmaniasis. Vaccine 2007;25(4):649–58.
[135] Fleck JD, Kauffmann C, Spilki F, Lencina CL, Roehe PM, Gosmanna G. Adjuvant activity of *Quillaja brasiliensis* saponins on the immune responses to bovine herpesvirus type 1 in mice. Vaccine 2006;24:7129–34.

[136] Kumazawa Y, Takimoto H, Nishimura C, Kawakita T, Nomoto K. Activation of murine peritoneal macrophages by saikosaponin a, saikosaponin d and saikogenin d. Int J Immunopharm 1989;11:21–8.
[137] Ushio Y, Abe H. The effects of saikosaponin-d on yeast phagocytosis and degradation in peritoneal macrophages: related increase in Fc receptor expression and altered cytoplasmic organization. Jpn J Pharmacol 1991;56:167–75.
[138] Ushio Y, Abe H. The effects of saikosaponin on macrophage functions and lymphocyte proliferation. Planta Med 1991;57:511–4.
[139] Ushio Y, Oda Y, Abe H. Effect of saikosaponin on the immune responses in mice. Int J Immunopharm 1991;13:501–8.
[140] Kato M, Pu MY, Isobe KI, Iwamoto T, Nagase F, Lwin T, et al. Characterization of the immunoregulatory action of Saikosaponin-d. Cell Immunol 1994;159:15–25.
[141] Kato M, Pu MY, Isobe KI, Hattori T, Yanagita N, Nakashima I. Cell type-oriented differential modulatory actions of Saikosaponin-d on growth responses and DNA fragmentation of lymphocytes triggered by receptor-mediated and receptor-bypassed pathways. Immunopharmacology 1995;29:207–13.
[142] Sun HX. Haemolytic activities and adjuvant effect of *Bupleurum chinense* saponins on the immune responses to ovalbumin in mice. Vaccine 2006;24(9):1324–31.
[143] Finkelman FD, Holmes J, Katona IM, Urban Jr JF, Beckman MP, Park LS, et al. Lymphokine control of in vivo immunoglobulin isotype selection. Ann Rev Immunol 1990;8(1):303–33.
[144] Bomford R, Stapleton M, Winsor S, Beesley JE, Jessup EA, Price KR, et al. Adjuvanticity and ISCOM formation by structurally diverse saponins. Vaccine 1992;10(9):572–7.
[145] Oda K, Matsuda H, Murakami T, Katayama S, Ohgitani T, Yoshikawa M. Relationship between adjuvant activity and amphipathic structure of soyasaponins. Vaccine 2003;21(17–18):2145–51.
[146] Krivorutchenko YL, Andronovskaja IB, Hinkula J, Krivoshein YS, Ljungdahl-Stahle E, Pertel SS, et al. Study of the adjuvant activity of new MDP derivatives and purified saponins and their influence on HIV-1 replication in vitro. Vaccine 1997;15(12–13):1479–86.
[147] Oleszek WA. Chromatographic determination of plant saponins. J Chromatogr A 2002;967(1):147–62.
[148] Oleszek W, Bialy Z. Chromatographic determination of plant saponins—an update (2002–2005). J Chromatogr A 2006;1112(1–2):78–91.
[149] Abe I, Rohmer M, Prestwich GC. Enzymatic cyclization of squalene and oxidosqualene to sterols and triterpenes. Chem Rev 1993;93:2189–206.
[150] Haralampidis K, Trojanowska M, Osbourn AE. Biosynthesis of triterpenoid saponins in plants. Adv Biochem Eng Biotechnol 2002;75:31–49.
[151] Pillion DJ, Amsden JA, Kensil CR, Recchia J. Structure–function relationship among *Quillaja* saponins serving as excipients for nasal and ocular delivery of insulin. J Pharm Sci 1996;85(5):518–24.
[152] Rhodes J. Evidence for an intercellular covalent reaction essential in antigen-specific T cell activation. J Immunol 1989;143(5):1482–9.
[153] Palatnik de Sousa CB, Santos WR, Casas CP, Paraguai de Souza E, Tinoco LW, da Silva BP, et al. Protective vaccination against murine visceral leishmaniasis using aldehyde-containing *Quillaja saponaria* sapogenins. Vaccine 2004;22(19):2470–9.
[154] Pillion DJ, Recchia J, Wang P, Marciani DJ, Kensil CR. DS-1, a modified *Quillaja* saponin, enhances ocular and nasal absorption of insulin. J Pharm Sci 1995;84(11):1276–9.
[155] Liu G, Anderson C, Scaltreto H, Barbon J, Kensil CR. QS-21 structure/function studies: effect of acylation on adjuvant activity. Vaccine 2002;20(21–22):2808–15.
[156] Sun HX, Qin F, Ye YP. Relationship between haemolytic and adjuvant activity and structure of protopanaxadiol-type saponins from the roots of *Panax notoginseng*. Vaccine 2005;23(48–49):5533–42.
[157] Sun HX, Yang ZG, Ye YP. Structure and biological activity of protopanaxatriol-type saponins from the roots of *Panax notoginseng*. Int Immunopharmacol 2006;6(1):14–25.
[158] Glauert AM, Dingle JT, Lucy JA. Action of saponin on biological cell membranes. Nature 1962;196:952–5.
[159] Takechi M, Shimada S, Tanaka Y. Time course and inhibition of saponin-induced hemolysis. Planta Med 1992;58:128–30.
[160] Takechi M, Tanaka Y. Haemolytic time course differences between steroid and triterpenoid saponins. Planta Med 1995;61:76–7.
[161] Segal R, Milo-Goldzweig I, Schupper H, Zaitschek DV. Effect of ester groups on the haemolytic action of sapogenins—II. Esterification with bifunctional acids. Biochem Pharmacol 1970;19(8):2501–7.
[162] Segal R, Mansour M, Zaitschek DV. Effect of ester groups on the haemolytic action of some saponins and sapogenins. Biochem Pharmacol 1966;15(10):1411–6.
[163] Segal R, Shatkovsky P, Milo-Goldzweig I. On the mechanism of saponin hemolysis—I. Hydrolysis of the glycosidic bond. Biochem Pharm 1974;23:973–81.
[164] Matsuda H, Li Y, Murakami T, Ninomiya K, Yamahara J, Yoshikawa M. Effects of escins I a, I b, II a, and II b from horse chestnut, the seeds of *Aesculus hippocastanum* L., on acute inflammation in animals. Chem Pharm Bull 1997;20:1092–5.
[165] Abe H, Sakaguchi M, Konishi H, Tani T, Arichi S. The effects of saikosaponins on biological membranes. 1. The relationship between the structures of saikosaponins and haemolytic activity. Planta Med 1978;34:160–6.
[166] Ronnberg B, Fekadu M, Behboudi S, Kenne L, Morein B. Effects of carbohydrate modification of *Quillaja saponaria* QH-B fraction on adjuvant activity cholesterol-binding capacity and toxicity. Vaccine 1997;15(17/18):1820–6.
[167] Sanders MT, Brown LE, Deliyannis G, Pearse MJ. ISCOM-based vaccines: the second decade. Immunol Cell Biol 2005;83(2):119–28.
[168] Sjölander A, Drane D, Maraskovsky E, Scheerlinck JP, Suhrbier A, Tennent A, et al. Immune responses to ISCOM formulations in animal and primate models. Vaccine 2001;19(17–19):2661–5.
[169] Pearse MJ, Drane D. ISCOMATRIX® adjuvant for antigen delivery. Adv Drug Deliv Rev 2005;57(3):465–74.
[170] Drane D, Gittleson C, Boyle J, Maraskovsky E. ISCOMATRIX adjuvant for prophylactic and therapeutic vaccines. Expert Rev Vaccines 2007;6(5):761–72.