Adjuvant activities of saponins from traditional Chinese medicinal herbs

Xiaoming Song\(^a\), Songhua Hu\(^{a,b,*}\)

\(^a\) Department of Veterinary Medicine, College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang 310029, China
\(^b\) Key Laboratory of Animal Epidemic Etiology & Immunological Prevention of Ministry of Agriculture, Hangzhou, Zhejiang 310029, China

**Abstract**

New generation vaccines such as recombinant, antigen purified and DNA vaccines are poorly immunogenic due to the lack of an innate immune stimulus. Therefore, search of new adjuvants for these vaccines has become a topic of interesting. In new adjuvant development, saponins are outstanding candidates. Recently, increased attention has been received on plant-derived saponins in search of new adjuvant candidates from traditional Chinese medicinal herbs such as *Panax ginseng*, *Astragalus* species, *Panax notoginseng*, *Cochinchina momordica*, *Glycyrrhiza uralensis* and *Achyranthes bidentata*. Many of the saponins have been found to have adjuvant effects on purified protein antigens. The chemical structures of the saponins are related to their adjuvant activities, and influence the nature of the immune responses. Saponin adjuvants have been reported to stimulate secretion of a broad range of cytokines, suggesting that saponins may act by triggering innate immunity. As these plant-originated adjuvants may promote different branches of the immune system, they have the potential to be used in design of new vaccines so as to induce a desired immune response.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

The first generation of vaccines typically consists of live, but attenuated, or inactivated whole organisms, and has been very successful at inducing immune protection. The presence of pathogen-associated molecular patterns (PAMPs) provides these vaccines with built-in adjuvants (Rev. in [1]). Unfortunately, some problems remain, for instance: (1) inactivated vaccines have generally proven ineffective at inducing cell-mediated immunity (CMI), (2) some live attenuated vaccines cause disease in immunosuppressed individuals, (3) some pathogens are difficult or even impossible to grow in culture and (4) many traditional inactivated vaccines (e.g., *Bordetella pertussis*) also contain components that can cause undesirable effects. Therefore, it is needed to develop new generation vaccines, such as recombinant, antigen purified and DNA vaccines [2]. However, these vaccines are found to be poorly immunogenic due to the lack of an innate immune stimulus. Hence, the adjuvants for new generation vaccines should ensure that the vaccine resembles infection closely enough to initiate a potent immune response [1]. While adjuvants are potent immunostimulators, most of them cannot be used in the clinic because of unwanted side effects. Therefore, much research has been directed toward identification of the active components of the adjuvants and their subsequent modification to minimize side effects [3].

Saponins are a chemically heterogeneous group of steroid and triterpenoid glycosides present in a wide range of plant species
where they are distributed throughout the bark, leaves, stems, roots and even flowers [4,5]. A series of commercial veterinary vaccines as well as human vaccines have been formulated with saponins extracted from *Quillaja saponaria* (Quil A) and its reverse phase HPLC-released saponin Q5-21. Saponins are immunostimulatory adjuvants [1] composed by triterpene, steroidal or alkaloid nuclei associated to carbohydrate moieties. Saponins of the plant *Q. saponaria* Molina contain two carbohydrate chains respectively attached to the triterpene C3 and C28, besides the hydrophobic moiety which is acylated to a C28 sugar attached residue. In the case of Q5-21 saponin of *Q. saponaria*, the three sugar units linked to C3 are glucuronic acid, xylose and galactose while the four associated to C28 are fucose, rhamnose, xylose and apiose (Rev. in [6]) and the hydrophobic chain is composed by two normonoterpene acylated to the C28 attached fucose unit [6]. The QS-21 normonoterpene moiety is related to the induction of the CTL CD8+ protective lymphocyte response [7]. Another unique feature of the Q5-21 saponin is the presence of an aldehyde group in triterpene C4, which is involved with the direct T lymphocyte stimulation, mimetizing the B7-1 co-stimulatory molecule and inducing the Th1 protective response [7,8]. Although saponins are not actually pathogen-derived and therefore not a PAMP, saponins function mainly through the induction of cytokines and may prove to have interactions with pattern recognition receptor (PRR) [2]. The unique capacity of Quil A to stimulate both the Th1 immune response and the production of cytotoxic T lymphocyte (CTL) against exogenous antigens makes them ideal for use in subunit vaccines and vaccines directed against intracellular pathogens as well as for therapeutic cancer vaccines [9,10]. A number of clinical trials have been performed using Q5-21 as an adjuvant, initially for cancer vaccines (melanoma, breast and prostate), and subsequently for infectious diseases, including HIV-1, influenza, herpes, malaria and hepatitis B [2]. The hemolytic properties of the saponins in vitro were considered in the past as an undesirable effect. Although QS-21 of *Q. saponaria* and the CP05 saponin of *Calliandra pulcherrima* are both triterpenoidal, they are very haemolytic, and the possible involvement of the long carbohydrate chains or the hydrophobic moieties in hemolysis has been indicated [11–14]. However, using them as adjuvants no direct contact with red blood cells occur, therefore the main restriction now is pain [15] and/or astheny and anorexy [16,17], local swelling or loss of hair, after injection of the most used saponins of *Q. saponaria* and of *C. pulcherrima* and induction of a mixed Th1/Th2 response [14,18,19] with its typical cytokines (interferon (IFN)-γ and IL-4 or IL-10) and IgG1 and IgG2a in mice or IgG2 in dogs. As a relatively safe medicine, traditional Chinese medicinal herbs have been recorded for use in treatment of human and animal diseases in China for long history. Many of them have been reported to contain saponins with adjuvant or immunomodulatory properties (see Table 1) [20–45]. Recently, increased research has been carried out on plant-derived saponins in search of new adjuvant candidates from traditional Chinese medicinal herbs such as *Panax ginseng*, *Astragalus* species, *Panax notoginseng*, *Cochininchina momordica*, *Glycyrrhiza uralensis* and *Achyranthes bidentata*.

### 2. *Panax ginseng*

As a traditional medicine, ginseng has been used for at least 5000 years. Archaeological studies have found a pictographic ginseng carved on animal bones as early as 3500 years ago. The first description of its medical use can be traced back to a bamboo book “Shen Nong Ben Cao Jing” (Shen Nong’s Herbal), the earliest pharmacutical monograph in Chinese history, compiled by an anonymous

| Herbs Botanical source Saponins Adjuvant effects Reference |
|-----------------|-------------------------------------------------|-----------------------------------------------|--------|
| Ginseng Panax ginseng C.A. Mey. (Araliaceae) (root, stem and leaf) Crude saponins; Ginsenosides, e.g., Rg1, Rg2, Rg3, Rb1 and Re. • To enhance antibody responses to vaccination against PPV, *Erysipelothrix rh一次opathiae*, *Staphylococcus aureus*, NDV and FMDV; • To act synergistically with aluminum hydroxide and oil emulsion in promotion of immune response; • To promote production of IL-1, IL-2, IL-4, IL-10, IFN-γ and TNF-α; • To increase antibody responses to a model protein antigen OVA and NDV | [20–29] |
| Astragalus Astragalus membranaceus (Fisch.) Bge. var. mongholicus (Bge.) Hsiao or Astragalus membranaceus (Fisch.) Bge. (Leguminosae) (root) Crude saponins; Triterpene saponins, e.g., astragalosides I, II, IV, VII, brachyosides A–C and cyclocephalosides I, II. • To promote production of IL-1β, IL-2, IL-6 and TNF-α; • To activate NF-κB. |  |
| Notoginseng Panax notoginseng (Burk.) F.H. Chen (Araliaceae) (root) Crude saponins; Ginsenosides, e.g., Rh1, Rh4, Rg1, Re, Rb1 and Rd; Notoginsenosides, e.g., R1, R2, U, K and R4. • To promote antibody response to a model protein antigen OVA. | [34–39] |
| Momordica Momordica cochinchinensis (Lour.) Spreng. (Cucurbitaceae) (seed) Crude saponins; Momordica saponins I and II. • To promote the production of IL-2, IL-4, IL-5, IFN-γ and TNF-α; • To increase antibody responses to a model protein antigen OVA; • To enhance antibody responses to vaccination against FMDV and avian influenza virus; • To act synergistically with oil emulsion in promotion of immune response | [40–43] |
| Glycyrrhiza Glycyrrhiza uralensis Fisch., Glycyrrhiza inflata Batalz, or Glycyrrhiza glabra L. (Leguminosae) (root and rhizome) Crude saponins; Oleanane-type saponins, e.g., glycyrrhizin. • To enhance specific antibody and cellular responses against OVA in mice. | [44] |
| Achyrantes Achyrantes bidentata Bl. (Amaranthaceae) (root) Crude saponins • To enhance specific antibody and cellular responses against OVA in mice. | [45] |
| Platycodon Platycodon grandiflorum (Jacq.) A. DC. (Campanulaceae) (root) Crude saponins; Oleanane-type saponins, e.g., platycodin D and D3. • To enhance specific antibody and cellular responses against OVA in mice. | [46] |
30 ginsenosides have been identified in the active substances in total ginseng extracts. They are chemically triterpenoid glycosides of the dammarane series. More than 30 ginsenosides have been identified in *P. ginseng* [47]. Of these, protopanaxadiols and protopanaxatriols have attracted most attention. In the protopanaxadiols, the sugar moieties are attached to the ring of the triterpene dammarane at the three-position, (as in Rg3, Rd, Rc, Rb1 and Rb2), while in the protopanaxatriols, the sugar moieties are attached to the ring at the six-position (as in Rg1, Re and Rg2) (Fig. 1) [20]. Both types have a side-chain with or without sugar(s) attached at C20. The therapeutic effect of ginseng root (GS) may be related to its stimulation of the natural resistance against infections [48]. GS extracts consisting mainly of saponins have been found to possess various effects on the immune system, such as enhancement of lymphocyte proliferation, stimulation of macrophages in cytokine production, and improvement of phagocytic activity of macrophages and polymorphonuclear leukocytes [21,49–53]. Intraperitoneal administration of GS extracts in mice has been demonstrated to stimulate natural killer (NK) cells and cell-mediated immunity against Semliki Forest virus [22,54]. Ginsenoside Rg1 has been found to increase the lymphocyte proliferative responses to mitogenic stimulation and IL-1 and IL-2 production in macrophages in vitro [22].

Recent investigations have shown that GS saponins have adjuvant effects on the specific immune responses. Jie et al. [21] have reported that injection of sheep red blood cells in combination with oral administration of GS saponins has increased IgG and IgM responses in mice. Rivera et al. [23] have observed an increased antibody response to vaccination against porcine parvovirus (PPV) in guinea pigs when PPV antigen is mixed with a GS saponin. The enhanced antibody responses by GS favor the production of IgG2 isotype. Kong et al. [24] have found that GS saponin has promoted both humoral and cellular immune responses to Newcastle disease virus (ND) vaccine in chickens. Kenarova et al. [22] have reported an adjuvant effect of ginsenoside Rg1 on the immune response to sheep red blood cells in mice. GS saponin has been co-administered with other adjuvants to synergistically enhance the immune response. Rivera et al. [23,25] have reported an increased immune response of pigs to aluminum hydroxide (alum) adjuvanted porcine parvovirus (PPV) and *Erysipelothrix rhusiopathiae* vaccines by supplement of GS saponins. Sun et al. [26] have found that ginsenoside Rg1 acts synergistically with aluminum hydroxide in promotion of antibody responses as well as IL-5 and IFN-γ against OVA in mice. An enhanced immune response of dairy cattle to alum-adjuvanted *Staphylococcus aureus* vaccine by supplement of ginsenoside Rb1 was also observed [23,25,27]. Alum-adjuvanted vaccines induce higher IgG1 response than IgG2, while supplement of GS saponins mainly promotes production of IgG2 [23]. Song et al. [28] have recently reported that a GS saponin and oil emulsion act synergistically to promote the immune responses of mice to vaccination against foot-and-mouth disease virus (FMDV) serotype Asia 1.

Adjuvant effect of GS saponin may be related to its activation of innate immunity. It has been found that antibody response can be enhanced by injection of GS saponin at a separate site of the body from antigen [23], suggesting that GS might trigger the immune system in a non-specific manner. In another study for evaluation of ginsenoside Rb1 as an adjuvant in stimulation of the immune response to PPV vaccines in mice [29], production of cytokines including IFN-γ, IL-2, IL-4, IL-10 and tumor necrosis factor (TNF)-α has been effectively promoted, indicating that a balanced Th1 and Th2 response has been activated. While the study is rare on the relation between biological function and structures of saponins, adjuvant properties have been compared between different fractions of GS saponin. Sun et al. [20] have found that the adjuvant activity of ginsenosides Rg1, Re, Rg2, Rg3 and Rb1 is more potent than that of Rd, Rc and Rb2, and the former group of saponins may be responsible for the major constituents contributing to the adjuvant activities of total ginseng saponins. When ginsenosides are subjected to analysis by reverse phase HPLC, Rg2 is the chemical eluted first, followed by Rg1, Rg3, Re, Rd, Rc, Rb2 and Rb1 [55]. Interestingly, if the ginsenosides were divided into two groups based on the sequence eluted by HPLC: group I (Rg2, Rg1, Rg3 and Re) and group II (Rd, Rc, Rb2 and Rb1), those in group I are found stimulating much higher OVA-specific antibody responses than those in group II. Therefore, the adjuvant property of different GS fractions might be attributed to their different molecular conformation determined by the side sugar chains attaching to the dammarane skeleton, and the lack of sugars on the C20 or the presence of only one sugar attached probably characterizes a relative hydrophobicity of the saponin which then affects the adjuvant activity.

### 3. *Astragalus* species

So-called Huang Qi in the Chinese Pharmacopoeia [56] is the root of several *Astragalus* species such as *A. membranaceus* (Fisch.) Bge. and *A. membranaceus* Bge. var. mongholicus (Bge.) Hsiao. Polysaccharides and saponins are believed to be its pharmacologically active constituents. The root contains large amount of triterpene saponins such as astragalosides I-X, isoastragalosides I–IV and soyasaponin I [30] with immunomodulatory properties.

Previous studies were focused on aqueous extracts or polar fractions of the root which mainly contained polysaccharides, especially astragalans [57]. Recent investigations have shown that saponin part of the root also has immunomodulatory properties. For example, Yang et al. [31] have reported adjuvant effect of astragalus saponin (AS) on IgG1 and IgG2b response to OVA in mice. Kong et al. [24] have reported enhanced antibody response to ND vaccine in chickens by AS. Most research on AS found in English literatures was on its stimulatory effects on innate immunity. AS has been found to enhance phagocytosis by macrophages, T cell transformation and NK cell activity as reviewed by Rios and Waterman [57]. The enhanced cellular activities may be attributed to its activation of cytokine production. Yoshida et al. [32] have reported an increased production of IL-6 and TNF by peritoneal macrophages of C3H/HeN mice in vitro when cells are cultured with an aqueous extract of *A. membranaceus*. Sun et al. [58] have reported an increased free Ca²⁺ in ConA-activated T lymphocytes by AS. Verotta et al. [59] have isolated four cycloartane-type saponins from *A. peregrinus* and found that compound 2, (20R,24S)-epoxy-9β,19-cyclolanostan-3β,6α,16β,25-tetrol-3-O-α-L-rhamnopyranosyl-(1–4)-β-D-glucopyranoside and compound 3, (20R,24S)-epoxy-9β,19-cyclolanostan-3β,6α,16β,25-tetrol-3-O-α-L-rhamnopyranosyl-(1–2)-β-D-glucopyranoside, are able to stimulate the proliferation of mouse splenocytes either in presence or absence of concanavalin A stimulation. After investigation of 19 cycloartane-type triterpene glycosides for their effects on the human macrophage/monocyte using a transcription factor-based bioassay, Bedir et al. [33] have found only astragalose I can increase expression of nuclear factor kappa B-directed luciferase. The same compound also has enhanced mRNA expression of IL-1β and TNF-α. Yesilada et al. [30] have evaluated 13 cycloartenene- and 1 oleanan-type triterpene saponins isolated from Turkish species such as *A. brachyurus, A. cephalotes, A. microcephalus,* and *A. tropianus* for their in vitro effect on cytokine release. All the triterpene saponins have shown a high IL-2 inducing activity. IL-2 is a cytokine produced by activated T cells, and stimulates both the innate and acquired immune responses, including the release of secondary cytokines such as TNF, IL-1 and IL-6.
Fig. 1. Chemical structures of ginsenosides—Rg3 (Rg3), -Rd (Rd), -Rc (Rc), -Rb1 (Rb1), -Rb2 (Rb2), -Rg1 (Rg1), -Re (Re) and -Rg2 (Rg2) saponins from the roots of Panax ginseng.
4. *Panax notoginseng*

*P. notoginseng* (Burk.) F. H. Chen (Araliaceae) is a plant indigenous to the mountains of Yunnan and Guangxi provinces in China. The root has various pharmacological actions and is traditionally used for the treatment of cardiovascular diseases, inflammation, trauma, and haemorrhage. Extensive chemical studies on this drug have shown that dammarane-type saponins are the main bioactive principals [34,35]. The saponins contain protopanaxadiol and protopanaxatriol glucosides and chemically belong to notoginsenosides, accounting for 12% of the root [60–62]. Initially, a research group has reported that crude saponins extracted from the root of *P. notoginseng* (PNS) have significantly enhanced specific antibody and cellular responses against OVA in mice [36]. Subsequent purification of the extract by using ordinary and reversed-phase silica–gel, as well as Sephadex LH-20 chromatography has provided seven protopanaxatriol-type saponins (ginsenosides-Rh1, -Rh4, -Rg1, -Re, notoginsenosides-R1, -R2, -U) [37,38] and four protopanaxadiol-type saponins (ginsenosides-Rb1, -Rd, notoginsenosides-K, -R4) [37,39] (Fig. 2). Analysis of the relations between haemolysis, adjuvant activity and structures of these saponins has disclosed that the number, length and position of side sugar chains, and the type of sugar moiety might influence the adjuvant activity [38,39]. For example, glycosyl formation in C-6 of the glucopyranosyl group at the position C20 of aglycone with β-D-glucopyranosyl or β-D-xylopyranosyl (1→6)-β-D-glucopyranosyl group decreased the adjuvant activity of protopanaxadiol-type saponins, and the linkage of the side sugar chain at the position C3 could affect both lymphocyte proliferative response and antibody response (IgG1, IgG2a and IgG2b) induced by ovalbumin [39].

5. Other medicinal herbs

In addition to the herbs discussed above, many other traditional herbs containing saponins have demonstrated adjuvant properties, including *Semen Morindae*, *Radix Glycyrrhizae* and *Radix Aconitum Bidentatae*, etc. *S. Morinda* is the seed of *Momordica cochinchinensis* (Lour.) Spreng. mainly growing in Southeast Asian countries and southern China [63]. The seeds are traditionally used for the treatment of inflammatory swelling, scrofula, tinea, diarrhoea as well as supplicative skin infections such as sore, carbuncles, furuncles and boils in human beings and animals [64]. From the seeds, Iwamoto et al. [40] have isolated momordica saponins I and II. Chemical analysis has indicated that momordica saponin I is a triterpenoid saponin containing disaccharide chains, and momordica saponin II is structurally similar to quilliac acid. Recent investigations have shown that a crude saponin extracted from *Semen morinda* (ECMS) exerts an adjuvant effect on the immune response to OVA and FMV [41] in mice, and to influenza vaccination (H5N1) in chickens [42]. Saponins isolated from *Radix Glycyrrhizae* or *Radix Aconitum Bidentatae* platycodon grandiflorum have been reported to significantly enhanced OVA-specific immune responses in mice [43,44]. Oda et al. [65,66] have reported that soyasapogenins exhibits high adjuvant activity while the adjuvant activity of soyasapogenol is low.

6. Adjuvant properties and structures of saponins

Most reported saponins with adjuvant properties are pentacyclic triterpenoids such as QS-21, *Gypsophila* saponin and saponaside A. They usually have high hydrophilic–lipophilic balance (HLB) value. Results from analysis of the antibody responses and HLB of 8 purified soyasapogenins indicates that the adjuvant activity tends to increase with the length of the side sugar chain and the HLB value [65]. QS-21 as a representative of the saponins with strong adjuvant activity also has a high HLB value (HLB: 36.3). Other saponins with strong adjuvant activity as well as high HLB values include *Gypsophila* saponin (HLB: 39.4) [67], saponaside A (HLB: 20.3) [67], lablaboside F (HLB: 25.5) [68,69], and *Polygala* onjisaponins (HLB: 24.1) [70].

Studies on the relationship between adjuvant effects and chemical structures of protopanaxadiol and protopanaxatriol saponins from ginseng roots have revealed that molecular conformation determined by side sugar chains attaching to and the sites for the sugar(s) to attach in the dammarane skeleton may modify the adjuvant properties of ginsenosides [20]. Moreover, Sun et al. [38] have compared the structures and adjuvant activities of protopanaxatriol-type saponins (PTS) from the roots of *P. notoginseng* and found that the number, length and position of side sugar chains, and the type of glucosyl group in the structure of PTS not only affect their haemolytic and adjuvant activities, but also influence the nature of the immune responses.

Besides, other functional groups such as an aldehyde group are considered to play an important role in enhancing and modifying the immune response. Saponins with adjuvant properties extracted from *Q. saponaria* and *Gypsophila* spp. all have aldehydes in their triterpene frame. Closely related saponins with different carbohydrate moieties have similar activities when an aldehyde group is present [66,71]. The aldehydes have been shown to provide a co-stimulatory signal that leads to Th1 immunity [10,71], the acyl fatty acid moiety is responsible for CTL production and the toxicity of *Q.* saponins (with the exception of QS-7) [18]. Two mechanisms have been proposed for aldehyde-containing triterpene saponins and their derivatives to promote the adaptive immune response: (a) saponin adjuvant mediates the delivery of exogenous antigens directly into the APC’s cytosol for processing by the MHC class I pathway and presentation to TCR, resulting in T cell activation; (b) aldehyde-containing saponins react with amino groups on T cell surface receptor(s), forming an imine that provides T cell with a B7-CD28 independent co-stimulatory signal leading to T cell activation and Th1 immunity [7]. Formation of imine causes change in Na+ and K+ transport, together with signaling via the activated MAPK-ERK2 and increased Ca2+ mobilization, which favors higher IL-2 and IFN production [7,72]. Oliveira-Freitas et al. [73] have reported that normonopertene-deprived deacysapogenins extracted from *Quillaja saponaria* are non-toxic and less haemolytic but still capable of inducing antibody, DTH response and reduction of parasites in mice vaccinated against murine visceral leishmaniasis, supporting the relevance of the carbohydrate chains of QS-21 on immunomodulation. The other work by Nico et al. [13] have disclosed a major relevance of the carbohydrate moiety, mainly the C28 attached in most of the immunomodulatory properties of CP05 saponin and a minor contribution of the monoterpenic fraction. In addition, normotenopentenes in QS-21 and CP05 saponins have been shown to provide significant contributions to the adjuvant function and haemolytic activity [13].

The moieties attaching the nuclei may influence the immune response stimulated. Marciani et al. [74] obtained RP18-1 and RP18-2 from unfractionated GPI-0100 containing semi-synthetic derivatives of deacylated Quillaja saponins (DS saponins) by reverse phase low-pressure liquid chromatography (RP-LPLC). They found that the fraction RP18-1 contained DS saponin adducts of N-dicyclohexylurea, and stimulated Th2 immunity with production of IgG1, while the RP18-2 fraction contained the dodecylamide derivatives of DS saponins and stimulated Th1 immunity with production of IgG2a, IFN-γ, IL-2, and CTL.

7. Possible mode of action exerted by saponin adjuvants

As discussed above, many saponins from traditional Chinese medicinal herbs have the potential to promote both humoral and
cellular immune responses. Although the mechanisms have yet to be elucidated, a clearer understanding is emerging. Saponins have been found to enhance phagocytosis, promote IL-1 production by peritoneal macrophages, and stimulate secretion of cytokine such as IL-2, IL-4, IL-6, IL-10, IFN-γ and TNF-α [29,30,32,37,39,75]. Such broad range of cytokines is consistent with the mixed Th1/Th2 responses as observed in an evaluation of GS saponin in stimulation of the immune response to PPV vaccines in mice [29]. These findings suggest that saponins may exert their adjuvant activities by activating innate immunity.

Adjuvant activity may be initiated when saponin binds and activates the specific receptors on lymphocytes and antigen-presenting cells (APC). Most studies on the immunostimulatory activities of saponins have been carried out with preparations derived from Q. saponaria [5]. Soltysik et al. [10] have demonstrated that the adjuvancy of Quillaja saponins is dependent on a functional aldehyde-containing group present within the individual molecules and aliphatic side-chains. Quil A has been shown to intercalate into cell membranes through interaction with structurally similar cholesterol, forming ‘holes’ or pores [76]. It is currently unknown if the adjuvant effect of saponins is related to pore formation, which may allow antigen to gain access to the endogenous pathway of antigen presentation, promoting a CTL response [1,77]. Recent studies on other plant-originated saponins also have showed that the adjuvant properties are related to their chemical structures. These findings suggest that their adjuvant activities

Fig. 2. Chemical structures of ginsenosides-Rh1 (Rh1), -Rh4 (Rh4), notoginsenosides-R1 (R1), -R2 (R2), -U (U), -K (K) and -R4 (R4) saponins from the roots of Panax notoginseng with immunomodulatory effects.
may be mediated by the interaction of their moieties with the cell membrane, possibly via specific receptors, although this has not yet been confirmed. Production of TNF-α and IFN-γ is induced by a ginseng extract in spleen cells and peritoneal macrophages of C3H/HeJ mice but is impaired in C3H/HeJ mice carrying a defective toll-like receptor-4 (TLR-4) gene, suggesting that ginseng extract may contain an agent that enhances innate immunity through production of proinflammatory cytokines via TLR-4 [78]. However, little is known about the molecular mechanisms on the immunomodulatory properties of most herbal saponins. Meanwhile, the type of immune response that is generated after immunization of a saponin-adjuvanted vaccine depends not only on the adjuvant itself, but also on the factors like the antigen, administration route and immunization schedule.

Saponin molecules have three distinct areas with respect to polarity: hydrophilic–hydrophobic–hydrophilic [79]. The hydrophilic part is carbohydrates, and the hydrophobic part is sapogenin. It is thought that this three-block structure enables the formation of non-bilayer structures such as ISCOMs [79] and ginsenoside-based ISCOM-like nanoparticles (ginsomes; unpublished data) from saponins, cholesterol and phospholipid. As the particulate already has a built-in adjuvant, the ISCOM-like ginsome-based vaccines are able to induce both Th1 and Th2 responses, with production of a variety of Th1 and Th2 cytokines. This has been extensively reviewed elsewhere [80,81].

Concluding remarks

New generation vaccines such as recombinant, antigen purified vaccines have been used in the design of new vaccines so as to induce a desired immune response. As these plant-originated adjuvants may promote different branches of the immune system, they have the potential to be used in the design of new vaccines so as to induce a desired immune response.

Acknowledgement

Support from the Ministry of Science and Technology of China (No. 2008BADB4B06-2) and the National Natural Science Foundation of China (No. 30771592) are gratefully acknowledged.

References


Xu QF, Fang XL, Chen DF. Pharmacokinetics and bioavailability of ginsenoside Rb1 and Rg1 from Panax notoginseng in rats. J Ethnopharmacol 2003;84(February (2–3)):187–92.


Sun HK, 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(December (48–49)):5533–42.


Tsoi AY, Ng TB, Fong WP. Antioxidative effect of a chymotrypsin inhibitor from Glycyrrhiza uralensis and Glycyrrhiza uralensis root saponins on the immune responses to ovalbumin in mice. Vaccine 2006;24(March (11)):1914–20.


