Revista Brasileira de Farmacognosia (2020) 30:467–473
https://doi.org/10.1007/s43450-020-00080-y

REVIEW

Quillaic Acid–Containing Saponin-Based Immunoadjuvants Trigger Early Immune Responses

Idris Arslan [1]

Received: 9 May 2020 / Accepted: 8 July 2020 / Published online: 20 August 2020

Abstract
Immunoadjuvants have major effects on the immune responses in vaccine formulations. Many specific adjuvants have been studied, including mineral salts, emulsions, cytokines, non-ionic block copolymers, carbohydrate polymers, muramyl dipeptides, and saponin-based adjuvants. Here, we focus on quillaic acid–containing saponin-based adjuvants and their mechanisms of action. Interestingly, when analyzing the adjuvants capable of forming immune-stimulating complexes, one of the characteristics shared by the majority of them is the presence of quillaic acid as the aglycone. This suggests that quillaic acid might be a strategic molecule for immunoadjuvant activity and a powerful candidate in the development of novel adjuvants.

Keywords Quillaic acid · Immunoadjuvant · Saponin · ISCOM · QS-21

Introduction

Vaccination has been one of the most effective public health measures to reduce infectious diseases and associated mortality, particularly in children. A variety of vaccines generally require the addition of adjuvants to trigger sufficient immunological stimulation and ensure safety when facing challenges. Adjuvants have often been used in human vaccine formulations for nearly a century in an effort to increase immunogenicity of the vaccine, mainly by stimulating innate immunity, facilitating controlled inflammation, and improving adaptive immune responses. However, the major troubles for adjuvant use in human vaccines include limiting the residual toxicity and adverse side effects of induced inflammation (Petrovsky 2015), for which mitigation is generally prevented by a limited understanding of the mechanisms of action (den Brok et al. 2016; Marty-Roix et al. 2016). This is why so few adjuvants are approved for humans, but in clinical trials, many formulations are being evaluated (Oleszycka and Lavelle 2014; Leroux-Roels et al. 2016).

Adjuvants have major effects on immune responses and can tilt the immune system in favor of a Th1- or Th2-type response (Livingston et al. 1994). In the vaccine industry, it is highly desirable to have a flexible adjuvant that can induce the correct form of immune response to antigens to provide an optimal defense against each type of infection (McNeela and Mills 2001). Thus, one of the key challenges in adjuvant production is learning how to selectively induce the correct form of immune response to each type of infection. On the other hand, the correct adjuvant should have low toxicity and side effects to allow for use in human formulations (Aguilar and Rodríguez 2007). Although several hundred specific adjuvants have been studied for research or use in novel vaccine designs over the past few decades, including mineral salts, microorganism-derived adjuvants, emulsions, cytokines, non-ionic block copolymers, carbohydrate polymers, muramyl dipeptides, and nucleic acid-based adjuvants, the overwhelming majority have failed to obtain approval for human use, with limitations including lack of efficacy, undesired local, and/or systemic toxicity, difficulty of manufacture, poor stability, and prohibitive cost (McCluskie and Weeratna 2001; Aguilar and Rodríguez 2007; Apostólico et al. 2016).

✉ Idris Arslan
idris.arslan@beun.edu.tr

[1] Biomedical Engineering, Faculty of Engineering, Bülent Ecevit University, Incivez, 67100 Zonguldak, Turkey

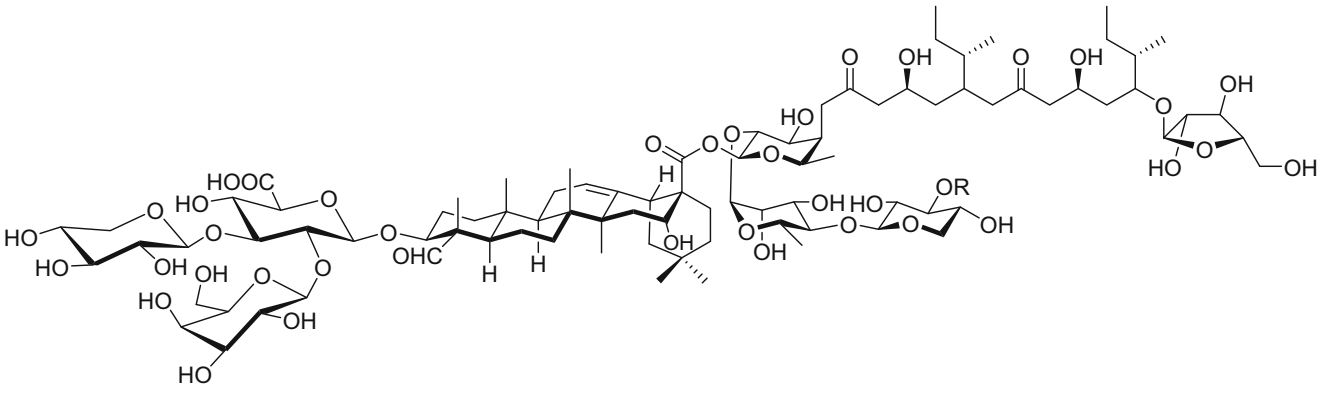
Saponins are a widespread class of natural compounds found in many plant species. They consist of a hydrophobic pentacyclic triterpene (C_{30}) or tetracyclic steroidal (C_{27}) backbone and one or two hydrophilic glycoside moieties attached it. Due to this structural composition, saponins are amphiphilic glycoconjugates that make soap-like foams in water that exhibit a number of different biological and pharmacological properties, such as ion channel inhibitory, opioid receptor modulatory, immunomodulatory, antitumor, antiinflammatory, antiviral, antifungal, hypoglycemic, genotoxicity, hypocholesterolemic, and cytotoxicity-enhancing properties. Saponins are commonly used in beverages, confectioneries, and cosmetic and pharmaceutical products (Sparg et al. 2004; Arslan et al. 2012, 2013; Arslan 2014; Arslan 2017; Arslan and Ili 2015; Nazir et al. 2015). Saponin not only has stimulating effects on specific immunity components but also results in certain non-specific immune reactions, such as inflammation (Haridas et al. 2001; de Oliveira et al. 2001) and proliferation of monocytes (Delmas et al. 2000; Yui et al. 2001).

Quillaic Acid as the Aglycone: *Quillaja* Sapogenin

Quillaic acid is a pentacyclic triterpenoid, and its basic structure includes olean-12-ene substituted by hydroxy groups at positions C-3 and C-16, an aldehyde group at position C-23, and a carboxy group at position C-28. Interestingly, the basic structures of all adjuvant saponins include a lipid-soluble quillaic acid as the aglycone and a water-soluble sugar moiety. Amphiphilic structure is a significant characteristic for stability and drug delivery, and many immunoadjuvants possess this structure (Oda et al. 2000). Thus, quillaic acid might be a strategic molecule for immunoadjuvant activity.

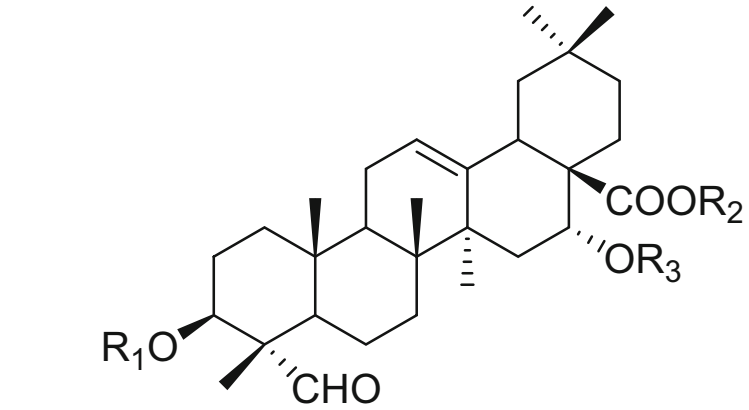
The adjuvant capacity of saponins was first described by Galea and Tzortzakis (1932). Quillaic acid–containing triterpene saponins were isolated from a water-extractable fraction from the South-American tree, *Quillaja saponaria* Molina, in the family Quillajaceae. One example is Quil A®, a highly purified concentrated mixture of approximately 25 different saponin molecules which have the same triterpene backbone. These saponins have been commonly used as adjuvants for many years in various veterinary uses (Dalsgaard 1974; Sun et al. 2009). However, their use in human vaccines has been restricted due to undesirable side effects, such as systemic toxicity, local reactions, and hemolytic activity (Sun et al. 2009; Sivakumar et al. 2011).

QS-21 (**1** and **2**) is another example of a quillaic acid–containing saponin-based adjuvant extracted from the bark of *Q. saponaria*. It is currently being evaluated in clinical trials due to its ability in tailoring Th1-biased immune responses, leading to protection against cancers and intracellular pathogens (Ragupathi et al. 2011; Buglione-Corbett et al. 2014; Didierlaurent et al. 2017, Bigaeva et al. 2016). However, the high toxicity and unintended hemolytic effects of Quil A® and QS-21 (**1**, **2**) have been highlighted as the key limitations to their use in human vaccination (Waite et al. 2001). QS-21 includes quillaic acid with branched trisaccharide and one unbranched tetrasaccharide attached, as well as a dimeric acyl group attached to the first sugar (fucopyranose) of a tetrasaccharide by an ester linkage.



1 R=Api (65%)
2 R=Xyl (35%)

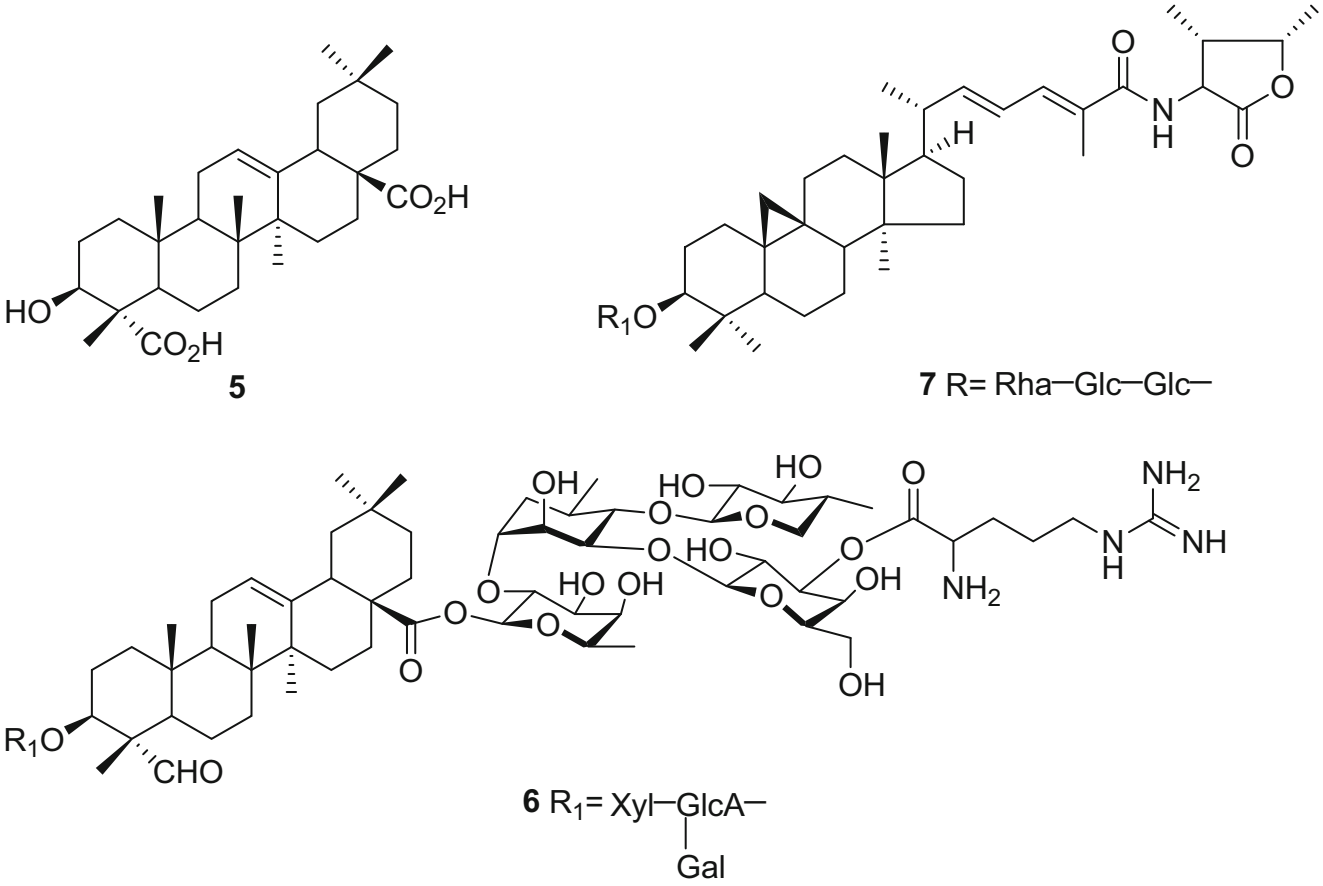
Some studies have found potential adjuvant saponins from the leaves of *Q. brasiliensis* (A.St.-Hil. & Tul.) Mart., an endemic tree species from Brazil, Uruguay, Argentina, and Paraguay. These natural products share structural and biological activities with saponins from *Q. saponaria* (Kauffmann et al. 2004) but induce lower levels of toxicity than Quil A® *in vivo* (Fleck et al. 2006). Two of the *Q. brasiliensis* saponin fractions, QB-90 (**3**) and QB-80 (**4**), were shown to increase secretion of Th1-associated cytokines ((IFN-γ and IL-2) and antigen-specific IFN-γ production by $CD4^+$ and $CD8^+$ T cells when used as vaccine adjuvants (Cibulski et al. 2017; Cibulski et al. 2016). Moreover, the adjuvant potential of *Q. brasiliensis* saponins has been confirmed in experimental vaccines against bovine herpes virus types 1 and 5 (BoHV), human poliovirus, bovine viral diarrhea virus (BVDV), and rabies in mice (Fleck et al. 2006; Silveira et al. 2011; de Costa et al. 2014; Yendo et al. 2016; Cibulski et al. 2017). Phytochemical studies of the *Q. brasiliensis* saponins led to the isolation of 27 bidesmosidic saponins bearing four types of triterpenic aglycones, which were identified by electrospray ionization ion trap–multiple stage mass spectrometry (ESI-IT-MS^n) with either quillaic acid, gypsogenin, phytolaccinic acid, or 23-*O*-acetyl-phytolaccinic acid as the aglycone (Wallace et al. 2017).



3 R_1= Xyl–GlcA– (Gal) ; R_2= Xyl–Ara–Rha– (Glc) ; R_3=OH

4 R_1= Xyl–GlcA– (Gal–Gal) ; R_2= Xyl–Ara–Rha– (Glc) ; R=H

Gypsophila is a genus of flowering plants in the carnation family, Caryophyllaceae, which is an especially rich source of triterpene saponins. The most common basic structures of aglycones isolated from the *Gypsophila* genus are mainly quillaic acid, as in nebuloside A (**3**) from Arrost's baby's breath (*G. arrostii* Guss), gypsogenin, as in nebuloside B (**4**), and gypsogenic acid, as in *G. trichotoma* (**5**) (Arslan et al. 2013; Krasteva et al. 2014; Arslan 2014). The main structural difference between quillaic acid and gypsogenin backbones is that quillaic acid has an additional hydroxyl group at the position C-16. The aglycones of *Gypsophila* saponins are similar to those of *Quillaja* in that they share an aldehyde group at position C-23, and amongst the variety of molecular species found in mixtures, there are also some with branched sugar chains at the same positions C-3 and C-28. Although *Gypsophila* saponins are similar to those of *Quillaja* and adjuvant-active (Bomford et al. 1992), there is no comprehensive research on specific immunomodulatory effects of fractions or pure compounds obtained from *Gypsophila* species.



Immune-Stimulating Complex

Immune-stimulating complexes (ISCOMs) are spherical cage-like particles (typically 40 nm in diameter) that are spontaneously formed when mixing cholesterol, phospholipids, and *Quillaja* saponins. They have been reported to have both humoral and cellular immune responses. Thus, they are mainly used as a vaccine adjuvant in order to induce a stronger immune response and longer protection. ISCOMs are capable of promoting long-lasting functional antibody responses as well as powerful T cell responses, including enhanced cytokine secretion and CTL activation (Sun et al. 2009).

With the presence of an instinctive adjuvant (Quil A), ISCOMs have the character of a particulate carrier system like virus particles due to the size and surface protein orientation. They are recognized as more immunogenic than other lipid-based systems, such as liposomes and micelles. In addition, the matrix component alone (ISCOMATRIX adjuvant; a saponin-containing molecule grid described without antigen) was also shown to be a potent adjuvant when physically mixed with free antigen.

A variety of antigens have been tested to improve the reliable delivery mechanism with ISCOM and ISCOMATRIX adjuvants. Some of them are currently undergoing clinical trials, such as HSV (Mastrolorenzo et al. 1995). Nanotechnology provides many vaccine-delivery mechanisms, such as VLPs, ISCOMs, liposomes, emulsions, polymeric micro/NPs, and self-assembling micelles. These mechanisms have emerged as leading candidates and may also be used as adjuvants. Quillaic acid–containing saponins such as Quil A, QS-21, *Gypsophila*, and *Saponaria* saponins are able to form ISCOM-type micellar nanometric structures, which are even more effective vaccine adjuvants (Bomford et al. 1992).

Saponin Protein Interactions and Mechanisms of Action

It is currently unknown whether the adjuvant effect of saponins is associated with pore formation, which may enable antigens to enter the endogenous antigen presentation pathway and promote a cytotoxic T lymphocyte response (Sjölander et al. 2001). There are many examples in the literature regarding the different saponin-based adjuvants of acylated or nonacylated, steroidal or monodesmosidic, bidesmosidic, and tridesmosidic triterpene-based saponins from natural sources. Recently, it was reported that *N*-

triterpene saponins are glycoconjugates that include a nitrogen atom in an aminoacyl moiety attached to a polycyclic triterpene core structure via peptide bonds, which are termed aglycones or sugar chains (Arslan and Cenzano 2020).

Phytochemical studies of *Gypsophila trichotoma* Wender led to the isolation of two new GOTCAB-based aminoacyl saponins, and both of them were arginine ester derivatives, *e.g.*, *Gypsophila trichotoma* aminoacyl triterpene saponin (**6**) (Gevrenova et al. 2006). In some cases, saponins may be acylated with organic acids or amino acid derivatives, such as 4-hydroxyisoleucine γ-lactone (**7**) (Zhao et al. 1996; Mohamed et al. 2015). This information suggests that both the polycyclic triterpene core structure and sugar chains of saponins can enzymatically and irreversibly bind to antigens via a peptide bond. The incorporation of saponins into cell or endosomal membranes might expose the incorporated antigen to cytosolic proteases.

Saponinum album from *G. paniculata* enhanced the cytotoxicity of a saporin-based chimeric toxin (up to 385,000-fold) and the toxicity of saporin (up to 100,000-fold) with *N*-glycosidase activity (Weng et al. 2008). Similarly, throughout the last decade, our group has been studying nebuloside A (**5**), a quillaic acid–containing saponin. Toxicity-enhancing effects on type-I RIPs (ribosome inactivating proteins) have been demonstrated without causing toxicity by itself at 15 μg/ml (Arslan et al. 2013). Recent discoveries have demonstrated that soapwort saponins trigger clathrin-mediated endocytosis of both type-I RIPs and type-I-based immunotoxins (Weng et al. 2008). Since saponins were classified as membrane active compounds, soapwort saponins were thought to function primarily by interaction of the aglycone with plasma membrane sterols (cholesterol) accompanied by aggregation of the carbohydrate moieties. This may induce the rearrangement of phospholipid bilayer and pore formation, possibly followed by translocation of antigens through these pores (Lacaille-Dubois and Wagner 2001).

Structure Activity Relationship

Due to the characteristic lability of esters, bidesmosidic and/or acylated saponins are readily converted into their monodesmosidic and/or deacylated forms by mild alkaline hydrolysis. The adjuvanticity of saponins depends on the sugar chain, acyl residue, presence/absence the epoxy-framework, and the aglycone backbone (Oda et al. 2000). It was also thought to be related to branched, unbranched sugar chains or aldehyde groups like quillaic acid (Bomford et al. 1992), or acyl residue-bearing aglycone (Kensil 1996). The aldehyde group of quillaic acid can easily react with amino groups of proteins to form a Schiff base.

Conclusion

Quillaic acid–containing saponins such as *Quillaja*, *Gypsophila*, and *Saponaria* saponins are able to form micellar nanometric structures ISCOM-type that are even more effective vaccine adjuvants (Bomford et al. 1992). The aglycones of *Gypsophila* saponins such as quillaic acid and gypsogenin are similar to those of *Quillaja* in that they share an aldehyde group at position C-23, and amongst the variety of molecular species found in these mixtures, some have branched sugar chains at the same positions C-3 and C-28 in *Gypsophila* saponins. Additionally, a group of saponins are triterpenoid carboxylic acids and also called GOTCAB (**G**lucuronide **O**leanane-type **T**riterpenoid **C**arboxylic **A**cid 3, 28-*O*-**B**idesmosides) saponins. Because they include a glucuronic acid unit at the first sugar attached to aglycone at the position of 3C, both *Quillaja* and *Gypsophila* saponins are GOTCAB-type saponin molecules. The immunoadjuvant effect of quillaic acid–containing *Gypsophila* saponins should be investigated due to their similarity to *Quillaja* saponins. Interestingly, when examining the adjuvant effect of saponins from *Quillaja brasiliensis*, *Q. saponaria* (Quil A, QS-21), *Gypsophila*, and *Saponaria*, all of them contain quillaic acid as the aglycone. This strongly suggests that quillaic acid might be a strategic molecule for immunoadjuvant activity and a powerful candidate in the development of novel adjuvants.

Funding Information This article was financially supported by Zonguldak Bulent Ecevit University, Scientific Research Unit (2019-3997-1044-01).

Compliance with Ethical Standards

Conflict of Interest The author declares that there is no conflict of interest.

References

Aguilar JC, Rodríguez EG (2007) Vaccine adjuvants revisited. Vaccine 25:3752–3762. https://doi.org/10.1016/j.vaccine.2007.01.111

Apostólico JS, Lunardelli VAS, Coirada FC, Boscardin SB, Rosa DS (2016) Adjuvants: classification, *modus operandi*, and licensing. J Immunol Res 2016:1–16. https://doi.org/10.1155/2016/1459394

Arslan I (2014) Simenoside A, a new triterpenoid saponin from *Gypsophila simonii* Hub.-Mor. Chem Biodivers 11:445–450. https://doi.org/10.1002/cbdv.201300118

Arslan I (2017) A new acylated and oleanane-type triterpenoid saponin from *Gypsophila arrostii* roots. Int J Food Prop 20:507–513. https://doi.org/10.1080/10942912.2016.1168437

Arslan I, Cenzano AM (2020) *N*-triterpene saponins in cancer therapy: a review of the mode of action. Rev Bras Farmacogn 30:1–6. https://doi.org/10.1007/s43450-020-00033-5

Arslan I, Ili P (2015) Genotoxicological assessment of nebuloside-A a triterpenoid saponin compound on whole blood DNA. Int J Food Prop 18:2374–2379. https://doi.org/10.1080/10942912.2014.971185

Arslan I, Celik A, Chol JH (2012) A cytotoxic triterpenoid saponin from under-ground parts of *Gypsophila pilulifera* Boiss. & Heldr. Fitoterapia 83:699–703. https://doi.org/10.1016/j.fitote.2012.02.005

Arslan I, Celik A, Melzig MF (2013) Nebulosides A-B, novel triterpene saponins from under-ground parts of *Gypsophila arrostii* Guss. var. *nebulosa*. Bioorgan Med Chem 21:1279–1283. https://doi.org/10.1016/j.bmc.2012.12.036

Bigaeva E, Doorn EV, Liu H, Hak E (2016) Meta-analysis on randomized controlled trials of vaccines with QS-21 or ISCOMATRIX adjuvant: safety and tolerability. PLoS One 11:e0154757. https://doi.org/10.1371/journal.pone.0154757

Bomford R, Stapleton M, Winsor S, Beesley JE, Jessup EA, Price KR, Fenwick GR (1992) Adjuvanticity and ISCOM formation by structurally diverse saponins. Vaccine 10:572–577. https://doi.org/10.1016/0264-410x(92)90435-m

Buglione-Corbett R, Pouliot K, Marty-Roix R, Li W, West K, Wang S, Morelli AB, Lien E, Lu S (2014) Reduced MyD88 dependency of ISCOMATRIXTM adjuvant in a DNA prime-protein boost HIV vaccine. Hum Vaccin Immunother 10:1078–1090. https://doi.org/10.4161/hv.27907

Cibulski SP, Siveira F, Mourglia-Ettlin G, Teixeira TF, Santos HF, Yendo AC, Costa F, Fett-Neto AG, Gosmann G, Roehe PM (2016) *Quillaja brasiliensis* saponins induce robust humoral and cellular responses in a bovine viral diarrhea virus vaccine in mice. Comp Immunol Microbiol Infect Dis 45:1–8. https://doi.org/10.1016/j.cimid.2016.01.004

Cibulski S, Rivera-Patron M, Suarez N, Pirez M, Rossi S, Yendo AC, Costa F, Gosmann G, Fett-Neto A, Roehe PM, Silveira F (2017) Leaf saponins of *Quillaja brasiliensis* enhance long-term specific immune responses and promote dose-sparing effect in BVDV experimental vaccines. Vaccine 36:55–65. https://doi.org/10.1016/j.vaccine.2017.11.030

Dalsgaard K (1974) Saponin adjuvants. 3. Isolation of a substance from *Quillaja saponaria* Molina with adjuvant activity in food-and-mouth disease vaccines. Arch Gesamte Virusforsh 18:349–360

de Costa F, Yendo ACA, Cibulski SP, Fleck JD, Roehe PM, Spilki FR, Gosmann G, Fett-Neto AG (2014) Alternative inactivated poliovirus vaccines adjuvanted with *Quillaja brasiliensis* or Quil-A saponins are equally effective in inducing specific immune responses. PLoS One 9:1–7. https://doi.org/10.1371/journal.pone.0105374

de Oliveira CAC, Perez AC, Merino G, Prieto JG, Alvarez AI (2001) Protective effects of *Panax ginseng* on muscle injury and inflammation after eccentric exercise. Comp Biochem Physiol C Toxicol Pharmacol 130:369–377. https://doi.org/10.1016/s1532-0456(01)00262-9

Delmas F, Di Giorgio C, Elias R, Gasquet M, Azas N, Mshvildadze V, Dekanosidze G, Kemertelidze E, Timon-David P (2000) Antileishmanial activity of three saponins isolated from ivy, alpha-hederin, beta-hederin and hederacolchiside A(1), as compared with their action on mammalian cells cultured *in vitro*. Planta Med 66:343–347. https://doi.org/10.1055/s-2000-8541

den Brok M, Büll C, Wassink M, Graaf AM, Wagenaars JA, Minderman M, Thakur M, Amigorena S, Rijke EO, Schrier CC, Adema GJ (2016) Saponin-based adjuvants induce cross-presentation in dendritic cells by intracellular lipid body formation. Nat Commun 7:13324. https://doi.org/10.1038/ncomms13324

Didierlaurent AM, Laupeze B, Pasquale AD, Hergli N, Collignon C, Garçon N (2017) Adjuvant system AS01: helping to overcome the challenges of modern vaccines. Expert Rev Vaccines 16:55–63. https://doi.org/10.1080/14760584.2016.1213632

Fleck JD, Kauffmann C, Spilki F, Lencina CL, Roehe PM, Gosmann G (2006) Adjuvant activity of *Quillaja brasiliensis* saponins on the immune responses to bovine herpesvirus type 1 in mice. Vaccine 24:7129–7134. https://doi.org/10.1016/j.vaccine.2006.06.059

Galea M, Tzortzakis H (1932) Essai d'immunisation antiaphteuse du cobaye à l'aide d'un virus aphteux saponiné. C R Soc Biol 109:21–23

Gevrenova R, Voutquenne-Nazabadioko L, Harakat D, Prost E, Henry M (2006) Complete ^{1}H and ^{13}C NMR assignments of saponins from roots of *Gypsophila trichotoma* Wend. Magn Res Chem 44:686–691. https://doi.org/10.1002/mrc.1827

Haridas V, Arntzen CJ, Gutterman JU (2001) Avicins, a family of triterpenoid saponins from *Acacia victoriae* (Bentham), inhibit activation of nuclear factor-kappa B by inhibiting both its nuclear localization and ability to bind DNA. Proc Natl Acad Sci U S A 98:11557–11562. https://doi.org/10.1073/pnas.191363498

Kauffmann C, Machado AM, Fleck JD, Provensi G, Pires VS, Guillaume D, Sonnet P, Reginatto FH, Schenkel P, Gosmann G (2004) Constituents from leaves of *Quillaja brasiliensis*. Nat Prod Res 18:153–157. https://doi.org/10.1080/14786410310001608055

Kensil CR (1996) Saponins as vaccine adjuvants. Crit Rev Ther Drug Carrier Syst 13:1–55

Krasteva I, Yotova M, Yosifov D, Benbassat N, Jenett-Siems K, Konstantinov S (2014) Cytotoxicity of gypsogenic acid isolated from *Gypsophila trichotoma*. Pharmacogn Mag 10:430–433. https://doi.org/10.4103/0973-1296.133299

Lacaille-Dubois MA, Wagner H (2001) Saponins, in: Atta-Ur-Rahman (Ed.), Studies in natural products chemistry series, vol. 21, Elsevier Science, pp. 633–687

Leroux-Roels G, Marchant A, Levy J, Van Damme P, Schwarz TF, Horsmans Y, Jilg W, Kremsner PG, Haelterman R, Clément F, Gabor JJ, Esen M, Hens A, Carletti I, Fissette L, Da Silva FT, Burny W, Janssens M, Moris P, Didierlaurent AM, Van Der Most R, Garçon N, Van Belle P, Van Mechelen M (2016) Impact of adjuvants on CD4+ T cell and B cell responses to a protein antigen vaccine: results from a phase II, randomized, multicenter trial. Clin Immunol 169:16–27. https://doi.org/10.1016/j.clim.2016.05.007

Livingston PO, Adluri S, Helling F, Yao TJ, Kensil CR, Newman MJ, Marciani D (1994) Phase I trial of immunological adjuvant QS-21 with a GM2 ganglioside-keyhole limpet haemocyanin conjugate vaccine in patients with malignant melanoma. Vaccine 14:1275–1280. https://doi.org/10.1016/s0264-410x(94)80052-2

Marty-Roix R, Vladimer GI, Pouliot K, Weng D, Buglione-Corbett R, West K, JD MM, Chee JD, Wang S, Lu S, Lien E (2016) Identification of QS-21 as an inflammasome-activating molecular component of saponin adjuvants. J Biol Chem 291:1123–1136. https://doi.org/10.1074/jbc.M115.683011

Mastrolorenzo A, Tiradritti L, Salimbeni L, Zuccati G (1995) Multicentre clinical trial with herpes simplex virus vaccine in recurrent herpes infection. Int J STD AIDS 6:431–435. https://doi.org/10.1177/095646249500600611

McCluskie MJ, Weeratna RD (2001) Novel adjuvant systems. Curr Drug Targets Infect Disord 1:263–271. https://doi.org/10.2174/1568005014605991

McNeela EA, Mills KHG (2001) Manipulating the immune system: humoral versus cell-mediated immunity. Adv Drug Deliv Rev 51:43–54. https://doi.org/10.1016/S0169-409X(01)00169-7

Mohamed SM, Backheet EY, Bayoumi SA, Jain S, Cutler SJ, Tekwani BL, Ross SA (2015) Potent antitrypanosomal triterpenoid saponins from *Mussaenda luteola*. Fitoterapia 107:114–121. https://doi.org/10.1016/j.fitote.2015.10.011

Nazir M, Harms H, Loef I, Kehraus S, El-Maddah F, Arslan I, Rempel V, Müller CE, König GM (2015) GPR18 inhibiting amauromine and the novel triterpene glycoside auxarthonoside from the sponge-derived fungus *Auxarthron reticulatum*. Planta Med 81:1141–1145. https://doi.org/10.1055/s-0035-1545979

Oda K, Matsuda H, Murakami T, Katayama S, Ohgitani T, Yoshikawa M (2000) Adjuvant and haemolytic activities of 47 saponins derived from medicinal and food plants. Biol Chem 381:67–74. https://doi.org/10.1515/BC.2000.009

Oleszycka E, Lavelle EC (2014) Immunomodulatory properties of the vaccine adjuvant alum. Curr Opin Immunol 28:1–5. https://doi.org/10.1016/j.coi.2013.12.007

Petrovsky N (2015) Comparative safety of vaccine adjuvants: a summary of current evidence and future needs. Drug Saf 38:1059–1074. https://doi.org/10.1007/s40264-015-0350-4

Ragupathi G, Gardner JR, Livingston PO, Gin DY (2011) Natural and synthetic saponin adjuvant QS-21 for vaccines against cancer. Expert Rev Vaccines 10:463–470. https://doi.org/10.1586/erv.11.18

Silveira F, Cibulski SP, Varela AP, Marqués JM, Chabalgoity A, de Costa F, Yendo ACA, Gosmann G, Roehe PM, Fernández C, Ferreira F (2011) *Quillaja brasiliensis* saponins are less toxic than Quil A and have similar properties when used as an adjuvant for a viral antigen preparation. Vaccine 29:9177–9182. https://doi.org/10.1016/j.vaccine.2011.09.137

Sivakumar SM, Safhi MM, Kannadasan M, Sukumaran N (2011) Vaccine adjuvants- current status and prospects on controlled release adjuvancity. Saudi Pharm J 19:197–206. https://doi.org/10.1016/j.jsps.2011.06.003

Sjölander A, Drane D, Maraskovsky E, Scheerlinck JP, Suhrbier A, Tennent J, Pearse M (2001) Immune responses to ISCOM formulations in animal and primate models. Vaccine 19:2661–2665. https://doi.org/10.1016/s0264-410x(00)00497-7

Sparg SG, Light ME, van Staden J (2004) Biological activities and distribution of plant saponins. J Ethnopharmacol 94:219–243. https://doi.org/10.1016/j.jep.2004.05.016

Sun HX, Xie Y, Ye YP (2009) Advances in saponin-based adjuvants. Vaccine 27:1787–1796. https://doi.org/10.1016/j.vaccine.2009.01.091

Waite DC, Jacobson EW, Ennis FA, Edelman R, White G, Kammer R, Anderson C, Kensil CR (2001) Three double blind, randomized trials evaluating the safety and tolerance of different formulations of the saponin adjuvant QS-21. Vaccine 19:3957–3967. https://doi.org/10.1016/s0264-410x(01)00142-6

Wallace F, Bennadji Z, Ferreira F, Olivaro C (2017) Analysis of an immunoadjuvant saponin fraction from *Quillaja brasiliensis* leaves by electrospray ionization ion trap multiple-stage mass spectrometry. Phytochem Lett 20:228–233. https://doi.org/10.1016/j.phytol.2017.04.020

Weng A, Bachran C, Fuchs H, Melzig MF (2008) Soapwort saponins trigger clathrin-mediated endocytosis of saporin, a type I ribosome-inactivating protein. Chem Biol Interact 176:204–211. https://doi.org/10.1016/j.cbi.2008.08.004

Yendo ACA, de Costa F, Cibulski SP, Teixeira TF, Colling LC, Mastrogiovanni M, Soulé S, Roehe PM, Gosmann G, Ferreira FA, Fett-Neto AG (2016) A rabies vaccine adjuvanted with saponins from leaves of the soap tree (*Quillaja brasiliensis*) induces specific immune responses and protects against lethal challenge. Vaccine 34:2305–2311. https://doi.org/10.1016/j.vaccine.2016.03.070

Yui S, Ubukata K, Hodono K, Kitahara M, Mimaki Y, Kuroda M, Sashida Y, Yamazaki M (2001) Macrophage-oriented cytotoxic activity of novel triterpene saponins extracted from roots of *Securidaca inappendiculata*. Int Immunopharmacol 1:1989–2000. https://doi.org/10.1016/s1567-5769(01)00126-6