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Review

Saponins as immunoadjuvant agent: A review

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Saponins are triterpene or steroid glycosides widely distributed in the plant and animal kingdom and include a large of number of biologically active coumpounds. Most of them have surface-active and cholesterol-binding properties. They have been shown to exhibit many biological and pharmacological activities as antiphlogistic, antiallergic, cytotoxic, antitumor and antitumor-promoting, antiviral, antihepatotoxic, molluscicidal, antibacterial, antiparasitic and antifungal activities, and especially immunoadjuvant activities. Adjuvants have been used to improve vaccine efficacy since the early 1929s. Nowadays many new vaccines are under development and there is a desired more effective adjuvants. Some triterpene saponins, especially those originating from *Quillaja saponaria*, are potent immunoadjuvat vaccines.This work will make an overview on the immunomodulatory properties of some adjuvants, especially the saponins, and will explain the main mechanisms of action by which they act.

Key words: Saponins, pharmacological activities, immunoadjuvant activity.

INTRODUCTION

Saponins are a group of natural products that are widely distributed in the plant kingdom and the animal kingdom (Milgate et al., 1995), and have as common ownership foaming when shaken with water (Basu et al., 1967). The formed foam is a result of the formation of a colloidal solution, being stable and lasting action of diluted mineral acids, differing from the common soaps. This property is more characteristic of this group of substances, which derives its name (from the Latin *sapone* = soap) (Simões et al., 2010). Another important property of this group of substances is related to the ability of precipitating cholesterol, due to its characteristic amphipathic molecule having a lipophilic and hydrophilic portion (Lacaille-Dubois and Wagner, 1996). This detergent action was recognized a hundred years ago, when extracts from plants such as *Saponaria officinalis* was used to make soap (Osbourn, 1996).

Saponins have attracted much attention in recent years because of their various biological properties, some of which are harmful and others beneficial to human health (Mahato et al., 1991). It has also been widely used by the pharmaceutical industry as a raw material for the synthesis of steroidal drugs such as contraceptives, plus numerous pharmacological properties, used in herbal medicine and cosmetics (Sparg et al., 2004).

Chemically, saponins are triterpenoid, steroidal or steroidal glycoalkaloids molecules that have one or more sugar chains in their structure. Hydrolysis yields units of sugars and sugar-free portion, the aglycone, also called sapogenin (Santos et al., 1997).

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The term adjuvant comes from the Latin word *adjuvare*, which means support and is defined as a substance which accelerate, prolong and enhance the quality of specific immune responses when incorporated into a vaccine formulation. The aluminum as aluminum phosphate and aluminum hydroxide is the only FDA-approved adjuvant immune; however, new and more effective adjuvants have been substituted, therefore increase the number of antibodies and cellular responses. Some of these new adjuvants demonstrated ability to increase the immunogenicity of vaccines against infectious diseases and cancer (Dzierzbicka et al., 2006; 2003; 2011).

Any material which increases or entailing immune response to an antigen is considered an adjuvant. Immunoadjuvants are substances, and can be natural or synthetic compounds. The use of adjuvants in vaccines is particularly important when the antigen has low immunogenicity. This applies to both subunit antigens consisting of peptides and recombinant peptides, the structure and conformation are less complex than viruses and bacteria inactivated intact. Adjuvant utility is dependent on its safety and ability to stimulate immunity for long periods (Resende et al., 2004).

The use of adjuvants began in 1916 when Moignic Pinoy and mineral oil used in vaccine *Salmonella typhimurium* are dead. In 1926, Glenny demonstrated the adjuvant activity of aluminum compounds. Ten years later, in around 1930, Freund developed water in oil emulsion containing killed *Mycobacteria*. In 1956, Johnson demonstrated the adjuvant activity lipopolysaccharides. Already in 1974, Lederer and colleagues identified muramyl dipeptide as the smallest component of mycobacterial adjuvant. More than 300 synthetic derivatives of MDP have been made in the search for molecules which have adjuvant activity (Vogel, 1995).

The adjuvant vaccine has several advantages because they increase the performance of vaccines, enhance the immunogenicity of weak immunogens through purification and recombinant antigens, reduce the amount of antigen or the frequency of immunizations required to promote adequate protection, increase the effectiveness of vaccines in infants, and immunocompromised adults, and promote T cell proliferation and cell-mediated immunity (Wardowska et al., 2006; 2009; Dzierzbicka et al., 2012; Samsel et al., 2014).

During the past eighty years, some adjuvant formulations have been developed, and few have been tested in clinical trials and most of them was never accepted for vaccination due to toxicity and adverse effects; however, currently, adjuvants have received great attention due to the generation of new vaccines developed (well purified and synthetic subunits), which are more secure, but less immunogenic and to their ability to selectively modulate the humoral and/or cellular immune response (Mota et al., 2006).

CLASSIFICATION AND MECHANISM OF ACTION OF ADJUVANTS

The existence of many molecules with adjuvant properties and heterogeneity of the biological effects of these substances hinder the classification and division into distinct groups that would make it easier to select the appropriate to be used according to the desired purpose adjuvant. Several proposals for classification have been suggested by different authors, but many have several disadvantages and one of the most common is the inclusion of a compound in more than one of the classification categories (Mota et al., 2006) (Table 1).

Adjuvants have diverse mechanisms of action and can be chosen depending on the antigen, based on the route of administration, and with respect to the desired immune response (Vogel, 1995) (Table 2).

The first mechanism action of adjuvant is associated with immunomodulation, which refers to the ability of many adjuvants to modify the system of cytokines. In general, only immunomodulatory adjuvants exert an adjuvant effect when in the presence of an immunogen. Immunomodulation may result in general in a direct regulation of the immune system, as well as cytokines. Therefore, there are two responses which can be observed (responses Th1 cell and Th2), antibody production. The Th1 response promotes the fixation of complement factors, related to hypersensitivity reactions and is associated with interferon gamma (IFN-γ) and interleukin (IL-2). Since Th-2 response results in high circulating and secretory antibody often, immunoglobin E (IgE), IL-4, IL-5, IL-6, and possibly IL-10. Th1 and Th2 responses are mutually inhibitory (Cox and Coulter, 1997).

The second mechanism of action of adjuvant is associated with increased antigen presentation time as nonionic block copolymers, which act as adhesive molecules, binding to the antigen via complement factors hydrophilic moieties. This presentation helps expose the epithets of immune cells. Particulate adjuvants as some liposomes and microspheres can protect the proteolytic destruction of the stomach, allowing the antigen to pass intact into the intestine and associate with the lymphoid system. This presentation mechanism also refers to interactions with antigen presenting cells and possibly also macrophages. The antigen enters the cell through a process of endocytosis or pinocytosis, and once inside the cell are processed into small peptides which complex with factor histocompatibility class II (MHC-II), which are grouped in the endoplasmic reticulum and processed in the Golgi complex. The resulting complex is then transported to the surface of the antigen presenting cell (APC) where the peptide is displayed molecule in association with MHC class II. APC will secrete IL-1, which will attract CD4 + T cells through their T cell receptor (TCR) peptide complementary to the complex of MHC class II (Vogel, 1995).

Table 1. Classification of adjuvants.

Adjuvant	Example
Alum gel	Aluminum hydroxide/Aluminum phosphate, calcium phosphate
Bacteria adjuvant	Monophosphoryl lipid A, muramyl peptides
Particular adjuvants	Complexes immunostimulatory (ISCOMS), liposomes, biodegradable microspheres
Adjuvant oil/water emulsion	Freund's incomplete adjuvant (FIA), saponins (QS-21)
Synthetic	Blocks nonionic copolymers, analogues of muramyl peptide, synthetic lipid A, synthetic polynucleotides

Table 2. Mechanism of action of adjuvants.

Action	Adjuvant type	Benefit
Immunomodulation	Generally small molecules or proteins which modify the cytokine system.	Regulation of immune response. Selection of Th1 or Th2.
Presentation	Generally amphipathic or complex molecules that interact with the immunogen in natural conformation.	Increase the antibody response with a higher response
Induction of CTL	Particles that bind to an immunogen; Emulsions of oil / water for peptide binding to MHC-I on the cell surface.	Process cytolytic proteins. Simple process if the peptide is known.
Direction	Particular adjuvants which bind immunogen. Adjuvants which saturate Kupffer cells. Adjuvants with carbohydrates that are targets of lecithin macrophage receptors.	Efficient use of adjuvant and immunogens can determine the type of response if the target is selective
Deposit	Emulsion water/oil, microspheres and nanospheres	Efficient, powerful for a single dose of vaccine

The third mechanism of action is associated with the induction of cytotoxic T lymphocyte responses (CTL). This induction of CTL responses requires that the antigen be processed in the cytosol of the cell, which in-corporates the peptides with the major histocompatibility complex class I (MHC-I) and are thus expressed on the surface (Cox and Coulter, 1997).

The fourth mechanism is related to the ability of an adjuvant to immunogen to deliver the effector cells of the immune system, typically via antigen presenting cell. Alternatively, delivery to macrophages may be increased when the adjuvant has sugar groups (eg saponins), or other cell surface receptor molecules that recognize, or the immunogen can be linked to a polymer of mannose or other carbohydrates (Cox and Coulter, 1997).

The fifth and last mechanism of adjuvant action is identified as "depot effect", since adjuvants in gel form, such as aluminum hydroxide or a basic emulsion associated antigen effectively increases the biological and immunobiological half-life (Vogel, 1995-).

CHARACTERISTICS OF AN IDEAL ADJUVANT

Some characteristics for the selection of an adjuvant are antigen, the animal species to be vaccinated, the route of administration and side effects. Ideally, the adjuvants are stable with time of use, biodegradable, presenting a cheap production, immunologically inert, promoting a cellular and persistent humoral immune response with a small amount of antigens, effective in children and low toxicity (Aguilar et al., 2007).

The benefits of incorporating adjuvants in vaccine

formulations to enhance immunogenicity must overcome the risks that these agents may cause adverse reactions. Local adverse reactions include injection site inflammation or, rarely, induction of granulomas. Systemic reactions observed in laboratory animals include pain, fever, arthritis and others. The reactions can be caused by the interaction with the antigen or adjuvant may be due to the type of response or even the type of cytokine. Thus an extensive study of the toxicity of these adjuvants is to be performed, as well as the formulation of the vaccine, to ensure safety of the candidate vaccine for testing in Phase 1 clinical trials to be conducted (Vogel, 1995).

Obtaining new adjuvants

The studies leading to new adjuvants are critical, since they play a crucial role in determining the magnitude and direction of the immune response by mechanisms including increased antigen presentation, capture, distribution and selectivity of the target. Moreover, obtaining new adjuvants is stimulated by a number of factors, including the poor immunogenicity of pure antigens and DNA vaccines, low immune response in certain age groups, such as the elderly with poor response to antigens from *Haemophilus influenzae* and a better understanding of the mechanisms of immune response and release of new routes that have been explored, such as intradermal, and intranasal mucosa (Mota et al., 2006).

According to the Committee for Proprietary Medicinal Products (CPMP), the adjuvants present in vaccines must demonstrate compatibility with the antigens, stable and efficient adsorption (in the case of adsorbed antigens) and acceptable toxicity. In these aspects, it is necessary a comparative study with appropriate animal model in the presence and absence of adjuvant, evaluating the safety profile of the antigen-adjuvant combination and the route of administration chosen. For the study of new adjuvants, a model that uses a standard antigen, such as ovalbumin or haemagglutinin of *H. influenzae* has been suggested. As for studies to evaluate the toxicity and safety of adjuvants, some pharmacological and toxicological parameters are cited: observation of reactions at the injection site, fever or other systemic effects, including those mediated by the immune system (hypersensitivity response), teratogenicity and genotoxicity; adjuvant effects on the immune response; study of hypersensitivity reactions to repeated dose toxicity; study of the distribution; autoimmune diseases; carcinogenesis; biodegradation and immunogenicity (Gupta and Siber, 1995).

A major problem of obtaining adjuvants is due to limited suitable animal models and problems with experimental tests immunogenicity. The limited adjuvanticity occurs because some adjuvants are effective for specific antigens and ineffective for others. There are many animal models for diseases for which some vaccines are being developed, in addition, different species respond differently to the various adjuvants and even within the same animal species, different strains respond differently. A major obstacle to the development of new adjuvants is the toxicity that has restricted the release and use of new adjuvants. The balance between safety and adverse effects is assessed differently for a therapeutic vaccine. In the first case, only adjuvants that induce minimal adverse effects are accepted, as for therapeutic use, higher levels of adverse effects are accepted (Mota et al., 2006).

Several formulations have been tested and have a strong adjuvant potential, such as emulsions, liposomes, microspheres, saponins, immunostimulatory complexes (ISCOMs), among others. One of the problems found in vaccines with oil adjuvants is that frequent use may result in undesirable side effects such as granulomas and cysts, which are attributed to several factors including oil and impurities in the case of complete and incomplete Freund's adjuvant, many side effects are credited to mineral oil, because it is not biodegradable, forming ulcerative lesions at the injection site. Due to these effects, a long variety of oils have been tested, such as squalene, squalane and vegetable oils (Mota et al., 2006).

The liposomes are vesicles and single membrane bilayer composed of cholesterol and phospholipids, which have a good ability to release and target antigen presentation cells to stimulate CTL and are safe, however, they have difficulty in manufacture and incorporation of immunogen, in addition to requiring immunomodulators to be effective in many situations (Cox and Coulter, 1997). Since the derivatives of aluminum adjuvants are relatively weak, they are unable to stimulate cell-mediated immunity and exhibit adverse effects correlated with increased IgE production and neurotoxicity (Aguilar et al., 2007).

Saponins with immunoadjuvant activity

Triterpenoid saponins from *Quillaja saponaria*, have been extensively studied and shown to be a strong adjuvant potential which appears to be associated with their ability to induce cytokine production. Quil A saponin purified adjuvant showed high capacity and has been used in veterinary vaccines, but due to the hemolytic activity and local reactions, it use in humans has not been authorized. QS-21 (Figure 1) of purified saponin Quil A adjuvant is a powerful Th1 response through the stimulation of cytokines (IL-2 and INF-γ) production of IgG2a and IgG2b antibody response inducing an antigen through the stimulation of specific CTL via MHC-I and above all, shows no hemolytic activity (Mota et al., 2006). The unique ability of Quil A saponin and its purified QS-21 to stimulate immune responses both with consequent production of CTLs against exogenous antigens makes them ideal for use in vaccines against intracellular pathogens,

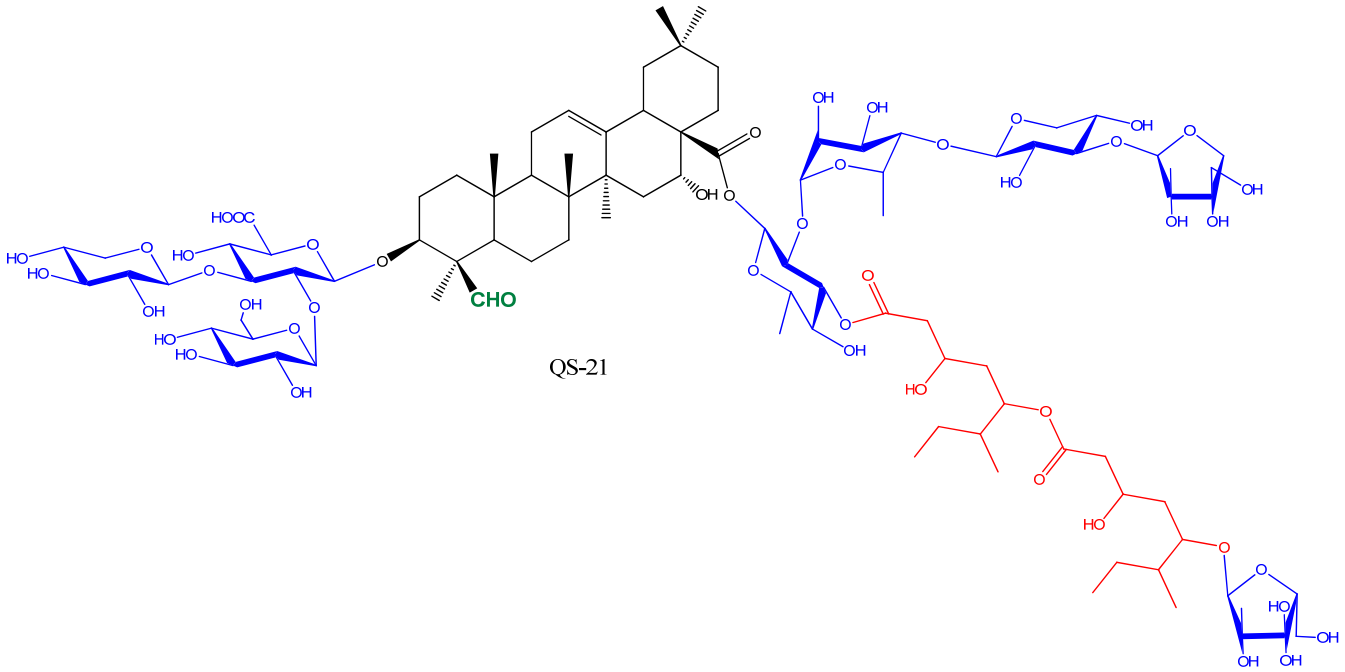

Figure 1. Structure of QS-21 from *Quillaja saponaria* Molina. Source: Da Silva et al. (2005).

as well as cancer vaccines; however, *Quillaja* saponins exhibit high toxicity, hemolytic effects and instability in aqueous phase (Sun et al., 2009).

Purification and structure-activity relationships of saponins with adjuvant activity has been speculated in recent years. Purification performed with reversed-phase HPLC fractions produced ten fractions. The major peak fractions were represented by QS-7, QS-17, QS-18 and QS-21. All saponin adjuvant activity showed, however, the toxicity is discrepant between them. QS-18 was lethal in mice at doses less than 25 mcg, while the QS-21 showed only lethality above 500 µg (Kensil et al., 1991).

The high toxicity and undesirable hemolytic effects of Quil A and QS-21 has been the main point of the restriction of its use in human vaccination. The haemolytic activity of Quil A and QS-21 is correlated with the sugar side chains attached to the aglycone, as well as the acyl residue of monoterpenes (Sun et al., 2009).

QS-21 consists of a core triterpene (quillaic acid), with an aldehyde and two sugar chains, one of which is acylated by means of ester bond (normally monoterpenes) lipophilic aliphatic acids bound fucose (Marciani, 2003). The presence of fatty acids favors the interactions between the saponin and membrane cholesterol promoting hemolysis. However a lipophilic side chain acylated showed a significant stimulation of CTL against exogenous proteins and instability under physiological conditions. Deacylation of the lipophilic side chain of QS-21 favored the reduction of toxicity being able to promote a Th2 response, however, not able to promote a Th1 response (Sun et al., 2009).

Quil A has been used as a new design for therapeutic cancer vaccines. The Leishmune vaccine formulated with adjuvants from Quil A, decreased the amount of clinical cases, but increased the amount of lymphocytes specific for Leishmania treated dogs, indicating their therapeutic potential against visceral leishmaniasis. Induction of immune response against cancer antigens is generally difficult because these antigens are autoantigens.

The structure of QS-21 allows us to easily understand its mechanism of action since it enhances both humoral immunity and the cell. The stimulation of the Th2 response is related to balance the hydrophobic and hydrophilic, respectively represented by the core triterpene and the sugar chains, while stimulation of Th1 response is related to the presence of structures acylated in this case, acylated normal monoterpenes, for carrying out the deacylation, if it is found that there is loss of stimulation of Th1; Th2 response is however maintained. A major distinguishing feature of this saponin is a costimulatory signal Th1 response through the aldehyde grouping present in the aglycone, thus facilitating the production of CTL against exogenous antigens (Marciani, 2003).

The saponin isolated from the leaves of *Calliandra pulcherrima*, pulcherrima saponin (CP-05) (Figure 2) showed immunoadjuvant activity in models of immunization of visceral leishmaniasis. This activity is related to the structural similarity with QS-21. Both feature a triterpenoid nucleus, two chains of sugars distributed in two distinct points of the aglycone and acylated structures. However, QS-21 provides a costimulatory signal

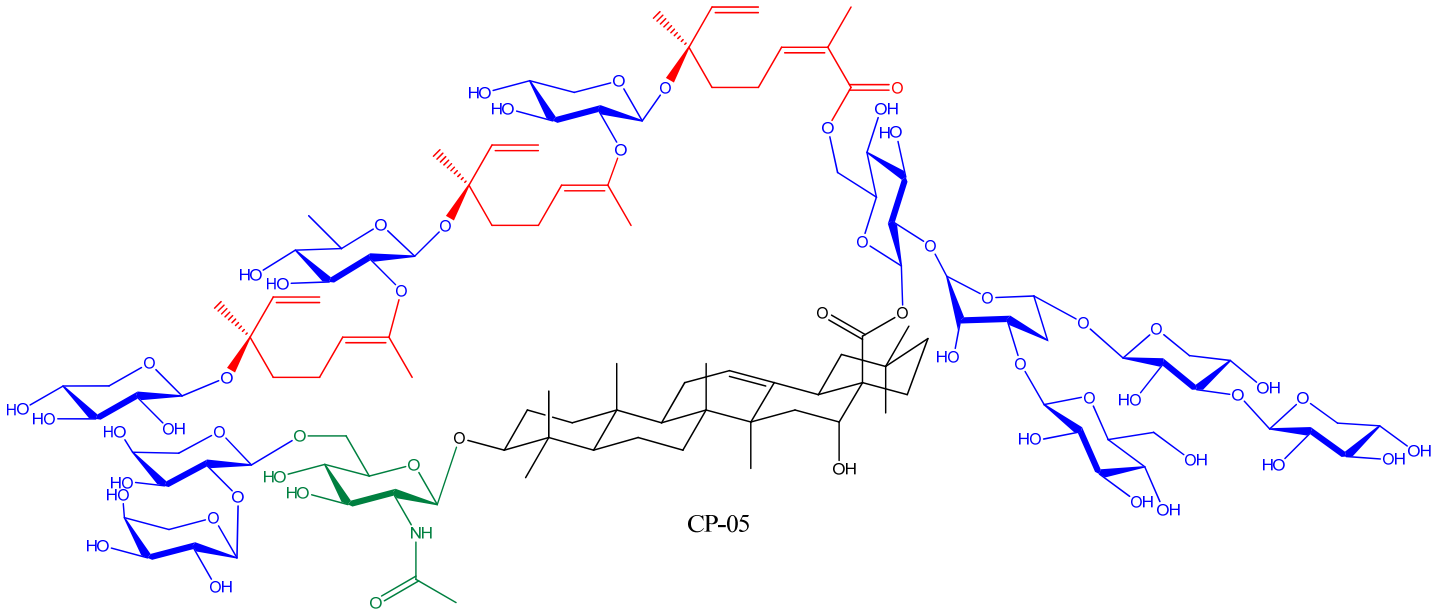

Figure 2. Structure of CP-05, Pulcherrimasaponin, from *Calliandra pulcherrima*.
Source: Da Silva et al. (2005)

Th1 response, the aldehyde grouping, since the CP-05 has N-acetylglucosamine (GlcNAc), which is recognized by the mannose receptor on the surface of the APC membrane, thereby contributing to the stimulation of the Th1 response. Another difference between these two saponins is related to the type of acylated structure, QS-21 nor-acylated two monoterpenes, while the CP-05 acylated monoterpenes and there is a larger number, acylated three monoterpenes (da Silva et al., 2005).

STRUCTURE-ACTIVITY RELATIONSHIPS OF SAPONINS IMMUNOADJUVANTS

Effect of functional groups on the adjuvant activity of saponins

The adjuvanticity of saponins depends on the hydrophobic-hydrophilic balance between its aglycone and their sugar chains. The adjuvant activity of saponins was correlated, at first, with the aldehyde groups present in the aglycone. QS-21 derivatives modified at the carboxyl group of glucuronic acid remained adjuvant effect by stimulating the production of antibodies, while derived from the modified QS-21 carbonyl of the aldehyde present in the aglycone showed no adjuvant activity in cellular and humoral response. These results demonstrate the importance of the aldehyde grouping in immunoadjuvant activity of QS-21. One possible mechanism is through formation of a Schiff base between the aldehyde and the free amino groups on the surface of target cells (Sun et al., 2009). The proportion of conformational isomers of the triterpene aldehyde QS-21E is crucial to the integrity of the Th1 response. Axial aldehyde is more important for the humoral immune response, since the equatorial aldehyde for the protective cellular immune response (Nico et al., 2007).

The lipophilic acyl residues (normally monoterpenes) around the aglycone were responsible for the induction of CTL against exogenous proteins. In contrast with most of the saponins of the genus *Quillaja* (QS-17, QS-18 and QS-21) are acylated at the 4-position of fucose hydroxyl. QS-18 and QS-21 induced deacylated lower total IgG response to bovine serum albumin in comparison with the acylated forms deacylated and QS-21 failed to stimulate the production of CTLs anticopros or ovalbumin (OVA). This suggests that the acylation is highly critical for Th1-type responses (CTL and IgG2a), however, less critical to Th2 type responses (Sun et al., 2009).

Although the aldehydes, carboxylic acyl residues and some structure in the molecule groups are essential role of the adjuvant properties of *Quillaja* saponins, many free aldehyde groups, and acyl residues, such as soy saponins are capable of promoting a strong adjuvant activity. Furthermore, most feature escin acyl residues do not exhibit adjuvant activity. Thus, the aldehyde group and the acyl residues of saponin molecule are not considered essential for adjuvant activity of general formula (Sun et al., 2009).

Similar potency of humoral responses induced by formulations of QS-21 and CP-05 with FML antigens are related to their structural similarity and its chemical composition. The presence of the aldehyde grouping QS-21 and CP-05 in the absence may explain the induction of strong Th1 response through the production of IgG2a subtype antibody (Sun et al., 2009). In contrast, CP-05

has a N-acetylglucosamine (GlcNAc) which when recognized by the mannose receptor on the surface of the APC membrane promote the stimulation of a Th1 response (Da Silva et al., 2005).

Nico et al. (2006) conducted a comparative study of the immune response of Balb/c FML antigen of *L. donovani* between the intact saponin *Calliandra pulcherrima* (CP-05) and their derivatives in mice. The mice were immunized with the FML antigen of *L. donovani* along the CP-05 and each compound derived from it. The derivative of CP-05 devoid of lipophilic acyl residues (monoterpenes) (BS), the derivative of CP-05 lacking the sugar chain attached to C-28 (HS) and sapogenin (Figure 3) were tested. The results showed that the acylated monoterpenes surrounding the aglycone are not required for the induction of Th1 global protection, because the BS derivative was able to present a potent protective effect on antibody production and secretion of IFN-γ. The sugar attached to C-3 and C-28 of the aglycone is responsible for induction of humoral response against the antigen. Sugar attached to C-28 is important for the induction of IgG2a, for animals treated with HS showed dimuição derivative or loss of antibody titers, however, this same derivative was able to increase the total IgM and IgG1 response, and contribute partially to answers of IgG and IgG2b. Sapogenin of CP-05 did not show adjuvant activity, which confirms the necessity of a hydrophobic and hydrophilic balance of molecules saponins.

Influence of a sugar chain in the adjuvant activity of saponins

Oda et al. (2003) concluded that adjuvant activity tends to increase the length of the sugar chains and their ability surfactant through observations of the immune response of saponins derived from soybeans. The importance of glycemic chains was also observed in the saponins *Calliandra pulcherrima*, CP-05, where the HS saponin, had lower response to vaccine antigens FML, indicating that the integrity of the sugar linked to the C-28 chain is essential for these functions.

A study of the relationship between the hemolytic activity and adjuvant activity of saponins from *Panax notoginseng* showed that the number, length, position of the sugar chains and links and the type of carbohydrate residues in the structure of saponins does not affect the potential adjuvant especially its effects are significant in the nature of immune responses. In contrast, it was found that the type saponins platicodigenin increasing the sugar residues attached to the C-3 of the aglycone chain decrease the immunoadjuvant action.

Most adjuvants has an amphipathic structure, especially the saponins which have a hydrophobic region represented by the sapogenin and other hydrophilic represented by sugar chains attached to the aglycone. This hydrophobic-hydrophilic balance is essential for adjuvanticity of these substances, more than any present in the aglycone functional group (Sun et al., 2009).

Structure-activity relationships of saponins with hemolytic activity

In the past it was stated that saponins could not be used as adjuvants due to their hemolytic properties. However, there are some saponins that have low or no hemolytic action. The hemolytic activity is influenced by the affinity of the aglycone with cholesterol membrane. The degree of activity or affinity depends on the type of algicona, the presence of sugar chains attached to the aglycone and the presence of monoterpenes (Santos et al., 1997). Takechi and Tanaka (1992), who observed saponins with steroidal nucleus are more hemolytic than triterpenoidais saponins and steroidal saponins within the monodesmosidic saponins are more hemolytic than bidesmosidic ones.

QS-21 has a very high hemolytic activity, despite its aglycone by triterpenoidal. Fatty acids linked to QS-21 are responsible for their toxicity and its hemolytic action. Deacylated saponins QS-21 and CP-05 showed low hemolysis and zero toxicity. Thus, the presence of hydrophobic monoterpenes could favor the interaction between saponin and cholesterol membrane, and promotes hemolysis. Reduction of toxicity of *Quillaja* saponins may be achieved by the degradation of carbohydrate treated with sodium periodate. Therefore, one can not evaluate the hemolytic activity of saponins across functional groups and chains of sugars, but through the global conformation of the molecule (Sun et al., 2009).

Immunostimulating complexes - ISCOM™ and ISCOMATRIX™

The cage structure ISCOMATRIX™ adjuvant consists of a molecule of saponin, cholesterol and phospholipids, which are held together by hydrophobic forces. The saponin used in producing the immunostimulating complex is called ISCOPREP™ which is purified by *Quilaja saponaria*. The saponin was prepared by ISCOPREP™ reproducible processes. The complexing saponin with cholesterol and phospholipid to form the ISCOMATRIX™ adjuvant, which is beneficial for endless reasons, among the main ones including: reduction of hemolytic activity of saponins, increased stability and increased ability to associate with a large number of antigens. The adjuvant ISCOMATRIX™ has been extensively characterized and its raw materials used are well defined, making a suitable technology for the development of vaccines in humans. The manufacturing process is simple, optimized and well-spaced and controlled. It is extremely stable holding both physical and biological characteristics for many years when stored

at 2 to 8°C (Pearse and Drane, 2004).

The original ISCOM™ was produced by incorporating antigens during complex formation. This limits the use of technology in areas such as hydrophobic membrane proteins, besides the manufacturing process is difficult to control. Thus, ISCOMATRIX™ has been immuno-stimulatory complex of choice for the development of vaccines in humans. The immunostimulating complexes and ISCOM™ ISCOMATRIX™ present in vaccines has shown a potent induction of antibody response in large numbers of studies using a considerable amount of antigens of many animal species, including humans and non-human primates. The ISCOMATRIX™ induced a rapid antibody response that the clinical point of view, is a great advantage in epidemic situations when a rapid response is needed, particularly in unprotected individuals, to decrease and prevention of morbidity. The adjuvant induces ISCOMATRIX™, the balance of the Th1/Th2 response and is a potent inducer of antibody production, as well as responses of CD4+ and CD8+ cells. This broad immune response is dependent on the induction of multiple immune mediators of innate and adaptive response, cellular processes and relationship between these elements. ISCOMATRIX™ stands out for cytokine induction, which is a major component of the immune response. The production of IL-1, a pro-inflammatory mediator, was the first cytokine response in vaccines observed ISCOM™. Subsequently, other cytokines were observed using the responses from both complexes, such as IL-2, IL-4, IL-5, IL-6, IL-10, IL-12 and INF-γ (Pearse and Drane, 2004).

The production of inflammatory mediators by immune effector cells is recognized as a critical role in the induction of primary immune response. IL-12 is responsible for the recruitment and cell activation, which makes it essential for adjuvanticity of these complexes. The ISCOM™ is a potent activator of the innate immune response, because they stimulate the production of IL-12, MHC-II and CD86 molecules expressed on antigen-presenting cells. However, ISCOM™ have some limitations for liquid formulations relied on, since the production of vaccines with the ability to be freeze dried are advantageous due to increased stability and half-life, as they are more easily stored without the application cooling (Skene and Sutton, 2006).

The saponin component of adjuvants and ISCOM™ and ISCOMATRIX™ is indispensable due to its immuno-modulatory properties. Saponins alone have potential adjuvants; however, CTL induction is achieved when the antigen and adjuvant are associated. The production of CTLs in ISCOMATRIX™ adjuvants vary and apparently depend on antigen. The mechanism by which CTL induction occurs is still unclear, but it is known to relate with the delivery of antigen. ISCOM™ occurs with a better delivery of the antigen by presenting molecules MHC-I. Therefore, ISCOM™ induces a CTL response that best fit ISCOMATRIX™ (Pearse and Drane, 2004).

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