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Updated insights into the mechanism of action and clinical profile of the immunoadjuvant QS-21: A review

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ABSTRACT

Background: Vaccine adjuvants are compounds that significantly enhance/prolong the immune response to a co-administered antigen. The limitations of the use of aluminium salts that are unable to elicit cell responses against intracellular pathogens such as those causing malaria, tuberculosis, or AIDS, have driven the development of new alternative adjuvants such as QS-21, a triterpene saponin purified from Quillaja saponaria.

Purpose: The aim of this review is to attempt to clarify the mechanism of action of QS-21 through either receptors or signaling pathways in vitro and in vivo with special emphasis on the co-administration with other immunostimulants in new adjuvant formulations, called adjuvant systems (AS). Furthermore, the most relevant clinical applications will be presented.

Methods: A literature search covering the period 2014–2018 was performed using electronic databases from Sci finder, Science direct, Medline/Pubmed, Scopus, Google scholar.

Results: Insights into the mechanism of action of QS-21 can be summarized as follows: 1) in vivo stimulation of Th2 humoral and Th1 cell-mediated immune responses through action on antigen presenting cells (APCs) and T cells, leading to release of Th1 cytokines participating in the elimination of intracellular pathogens. 2) activation of the NLRP3 inflammasome in mouse APCs with subsequent release of caspase-1 dependent cytokines, IL-1β and IL-18, important for Th1 responses. 3) synthesis of nearly 50 QS-21 analogs, allowing structure/activity relationships and mechanistic studies. 4) unique synergy mechanism between monophosphoryl lipid A (MPL A) and QS-21, formulated in a liposome (AS01) in the early IFN-γ response, promoting vaccine immunogenicity. The second part of the review is related to phase I-III clinical trials of QS-21, mostly formulated in ASs, to evaluate efficacy, immunogenicity and safety of adjuvanted prophylactic vaccines against infectious diseases, e.g. malaria, herpes zoster, tuberculosis, AIDS and therapeutic vaccines against cancer and Alzheimer’s disease.

Conclusion: The most advanced phase III clinical applications led to the development of two vaccines containing QS-21 as part of the AS, the Herpes Zoster vaccine (HZ/su) (Shingrix™) which received a license in 2017 from the FDA and a marketing authorization in the EU in 2018 and the RTS,S/AS01 vaccine (Mosquirix™) against malaria, which was approved by the EMA in 2015 for further implementation in Sub-Saharan countries for routine use.

Introduction

Vaccines are the most effective and least expensive methodology for preventing diseases caused by infectious pathogens. Recently emerging or re-emerging diseases such as the severe acute respiratory syndrome (SARS) in 2003, H1N1 influenza pandemic in 2009 or Ebola virus in 2014 have made the research on vaccines very attractive in the last decades (Lee and Nguyen, 2015). Modern subunit vaccines comprising homogeneous molecular antigens have been developed to prevent and treat many human diseases, but they are weakly immunogenic and must be administered with an adjuvant to elicit a potent immune response. Adjuvants whose name originates from the Latin word adjuvare...
Adjuvants (meaning to help) are substances used in combination with a specific antigen (Ag) to produce a more potent and persistent immune response against a specific disease than the Ag alone. The aim is to confer long-term protection, and with the additional benefit that less antigen and fewer injections are needed (Lee and Nguyen, 2015). Adjuvants are used in many vaccines, but their mechanisms of action have not been fully elucidated. They include diverse classes of compounds such as mineral salts, microbial products, emulsions, saponins, cytokines, polymers, microparticles and liposomes (Awate et al., 2013).

Up to now, few adjuvants have been approved for use in humans (Del Giudice et al., 2018). Aluminium salts, which have been extensively used as adjuvants in vaccines for more than 80 years, induced robust antibody responses but weak Th1 cell type responses, which are instrumental for protection against many pathogens. The new generation of vaccines using purer components for safer vaccines (e.g., subunits vaccines with highly purified recombinant antigens) are less immunogenic in contrast to live attenuated or inactivated whole-cell vaccines. Therefore, they required the induction of strong cellular responses including CD4+ T-helper cells (Th) and CD8+ cytotoxic T lymphocytes (CTL) in addition to antibody (Ab) responses. Thus, the development of new adjuvants is necessary for vaccines that require a cell-mediated immune response (CMI). Among them, the triterpene glycosides represent an interesting class of clinically relevant adjuvants, particularly those of Quillaja saponaria (QS). These amphiphatic plant glycosides possess a variety of pharmacological activities including immunoadjuvant, antitumor, anti-inflammatory, and antimicrobial properties which have been extensively studied in many in vitro and in vivo bioassays (Lacaille-Dubois and Wagner, 1996, 2000, 2017; Lacaille-Dubois, 1999, 2005). Despite the apparent success of QS-21 as vaccine-adjuvant in the last years, there is an urgent need for a deeper understanding of its mode of action which could accelerate the development of new vaccine strategies. This requires an interdisciplinary approach between chemists, biochemists, molecular biologists and immunologists. The following review will summarize updated insights into the modes of action, the structure/activity relationships allowing detailed mechanistic studies, and the clinical status of the vaccine adjuvant QS-21.

**General mechanism of action of adjuvants**

With a growing understanding of the innate immune system (response first mediated by antigen presenting cells (APCs)) and its role in activation and modulation of adaptive immune responses (Ag-specific B and T cell responses) (Didierlaurent et al., 2017; Di Pasquale et al., 2015), the mechanisms of action of adjuvants are being elucidated. They can act on one or more of the following targets to increase response to Ags: (1) sustaining release at the injection site (depot effect), (2) transient secretion of cytokines and chemokines, (3) recruitment of various immune cells (neutrophils, monocytes, eosinophils, macrophages and Dendritic Cells (DCs) at the injection site leading to a local immune-competent environment, (4) expression by the recruited APCs of various Pathogen Recognition Receptors (PRRs) both on their surface (Toll-like receptors, TLRs, C-type lectin receptors, CLRs), and intracellularly (Nucleotide Oligomerization Domain (NOD)-like receptors (NLRs) and Retinoic Inducible Gene-1 (RIG)-like receptors (RLRs)), which are recognized and/or activated by adjuvants, (5) maturation and activation of recruited APCs which up-regulate the expression of Major Histocompatibility Complex (MHC)-I and/or MHC-II and activation of co-stimulatory signals CD40, CD80/86, (6) increased capacity of APCs for Ag processing and presentation by MHC, (7) migration of the mature APCs to the draining lymph nodes (dLNs) to interact with Ag-specific B or T lymphocytes (through receptor-ligand interactions, MHC-T cell receptor (MHC-TCR), CD40-CD40L, CD80/86-CD28) which are activated to produce potent Ab-secreting B cells and/or effector CD8+ T cell responses (Awate et al., 2013).

Researchers continue to characterize the adaptive immune response and to clarify the respective roles of vaccine-induced immune effectors (Fig. 1). They include Abs produced by B lymphocytes that bind specifically to a toxin or a pathogen, memory B cells produced only when B cells received T cell help having the ability to respond rapidly on re-exposure of the same antigen, cytokote CD8+ T lymphocytes that limit the spread of infectious agents by killing infected cells or secreting specific cytokines, and CD4+ T helper (Th) lymphocytes. Various Th subsets have been defined through their cytokine profiles and ability to induce B cell and CD8+ T cell responses (Reed et al., 2013; Siegrist 2013). The Th1-type CD4+T cells essentially release the...
proinflammatory cytokines interleukin-2 (IL-2), interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), which participate in the elimination of intracellular pathogens both directly (cytokine response) or indirectly via CD8 T cell activation and differentiation in CTLs (cellular immune response) and the generation in mice of the complement-fixing Abs IgG2a and IgG3. The Th2-type CD4 T cell response is characterized by secretion of IL-4, IL-5 and IL-6 providing B cell help and the preferential induction of IgG1, IgE, and IgA in mice, and IgE and IgG4 in humans (humoral response) (Guy, 2007).

Structure of QS-21 and proposed immunoadjuvant mechanism of action

The saponin QS-21 has been isolated from Quillaja saponaria tree bark (Kensil et al., 1991) and is one of the most potent adjuvants known which is currently used in exploratory, and a few licensed, vaccines. QS-21 is a mixture of two isomeric molecules, QS-21 Apiose (QS-21 Api), and QS-21 Xylose (QS-21-Xyl) (2:1), each having four domains: the triterpene quillaic acid, a branched trisaccharide linked at C-3 through an O-heterosidic bound, a linear tetrasaccharide linked at C-28 through an ester bond, and a glycosylated pseudodimeric acyl chain attached through a labile ester linkage at C-4 of the fucose unit of the tetrasaccharide (Fig. 2). This compound showed a potent adjuvant activity against a wide variety of Ags with low toxicity in preclinical studies in mice, guinea pigs, monkeys and baboons. It was shown to stimulate Th1 and Th2 immune responses, when added to vaccine (Kensil, 1996). This adjuvant is a candidate for many clinical trials of vaccines directed against infectious diseases, cancer, and other. However, the exact role of QS-21, either through activation of receptors or signaling pathways, is poorly characterized. Some hypotheses advanced that QS-21 may facilitate Ag uptake into APCs by binding to cell surface lectins through its carbohydrate domains, leading to specific cytokine profiles that enhance the T and/or B cells’ response (Marciani, 2003). An effective mechanism of action in which QS-21 apparently acts on both DCs and T cells was proposed (Marciani, 2018) (Fig. 3). Exogenous Ags and QS-21 enter DCs by cholesterol-dependent endocytosis. The high affinity of QS-21 for endosomal membrane cholesterol resulted in the destabilization of the membrane (Lorent et al., 2014) leading to pore formation, thus facilitating the escape of the antigen to the cytosol for further processing inside the cell into peptides. They are then loaded to MHC-I and presented on the DC surface to naïve CD8 T cells to yield CTLs. The role of aldehyde in QS-21 was highlighted in the T cell by forming an imine with the amino group of the T cell surface receptor such as CD2, providing a costimulatory signal to the T cell. This signal together with the mitogen-activated protein kinase (MAPK) ERK2 resulting from the activation of the TCR-MHC interaction, and the changes of the ionic balance Na+/K+, stimulates T cell activation resulting in the secretion of Th1 cytokines (Marciani, 2018) (Fig. 3).

Recently, studies in mouse APCs (DCs and macrophages) identified QS-21 as an activator of the NLRP3 inflammasome. QS-21 in combination with the TLR4-agonist 3-deacyl-4′-monophosphoryl lipid A (MPLA), was shown to activate the ACS-NLRP3 inflammasome, a multi-protein complex and cause subsequent release of caspase-1 dependent proinflammatory cytokines Il-1β/Il-18 that can promote Th 17 cell maturation or drive INF-γ-mediated Th1 responses, respectively (Marty-Roix et al., 2016). Thus, inflammasome signaling has the potential to direct the development of T helper subsets. However, the role of this signaling pathway in vivo remained to be clarified.

Structure activity relationships and semi-synthetic analogs

Although QS-21 has been used in many clinical investigations of vaccines, several drawbacks inherent to the natural product, such as its chemical instability, scarcity, heterogeneity, dose-limiting toxicity and poorly understood mechanism of action have limited its widespread clinical advancement, except for the recent malaria (Mosquirix™) and shingles (Shingrix™) vaccines developed by GlaxoSmithKline (GSK). To overcome these limitations, structural modifications of the natural product through chemical synthesis have been undertaken during the last decade, leading to the preparation of nearly 50 saponin analogs, which have provided key insights into structure/activity relationships (SAR) which are summarized in Fig. 4 (Fernández-Tejada et al., 2016). These achievements allowed the identification of new novel saponins probes with potent adjuvant activities, increased stability, and decreased toxicity, and the investigation into their mechanisms of action (Fernández-Tajeda et al., 2016; Fernández-Tejada, 2017). Firstly, the total synthesis of each isomeric QS-21 produced this potent adjuvant in high purity and homogeneous composition. Then, some QS-21 variants (SQS-101, SQS-102, SQS-103) were chemically synthesized with more stable amide linkages in the acyl chain instead of the unstable native esters (Fig. 5). Their immunological evaluation in mice demonstrated adjuvant activities comparable to QS-21, as assessed by measuring Ab responses by ELISA one week after booster injection (day 72) and significantly lower toxicity than the natural product, except for SQS-102. Further modifications in the acyl chain with a dodecanedioic acid and truncation of the tetrasaccharide led to a trisaccharide variant with potent adjuvant activity and whose toxicity issue is not completely resolved (Fernández-Tejada et al., 2016). This simplification led to further structural variations at the linker (ester linkage at C-28) between the triterpene and the linear oligosaccharide in order to
modulate the distance and orientation of these two domains. The variants exhibit striking differences in adjuvant activity and toxicity in mouse vaccination models, yielding new insights into the structural requirements for adjuvant activity. Molecular dynamics simulations of these compounds and QS-21 Api have revealed distinctive conformational features correlating with adjuvant activity, which may help in the design of new analogs (Walkowicz et al., 2016). The modifications of the tetrasaccharide into a trisaccharide variant, and the acyl chain into a terminal amine variant enables the synthesis of novel acyl chain variants bearing fluorescent and iodinated substituents for subsequent imaging and in vivo biodistribution studies (Fernández-Tejada et al., 2014). They were adjuvant-active in several mouse-vaccination models and less toxic than QS-21. Early studies of biodistribution and fluorescence imaging allowed investigations into the enigmatic mechanism of action of these saponins. Preliminary results in vaccinated mice suggested the potential role of these fluorescent probes in Ag ovalbumin (OVA) trafficking by APCs from the site of injection to the LNs for its presentation to the immune system and yielded some mechanistic understanding of the potentiation of the immune response (Fernández-Tejada et al., 2014). Extensive structure/function studies have shown that aryl iodide derivatives with a free C-3 OH display a good efficacy/toxicity profile. It was concluded that the trisaccharide at C-3 was not required for adjuvant activity. Modifications at C-4 aldehyde and C-16 hydroxyl have shown that echinocystic acid aryl iodide derivatives lacking the C-4 aldehyde but retaining the C-16 alcoholic function (Fig. 6) exhibited potent adjuvant activity and no toxicity in a preclinical vaccination model using a four-component vaccine (MUC1-KLH, OVA, GD3-KLH) when tested at doses of 20 and 50 μg. These data indicated that the C-4 aldehyde previously suggested to participate in Schiff's base formation with a presumed T cell surface receptor target (Marciani, 2003), is not required for adjuvant activity and revealed the previously unknown role of the C-16 alcohol in enhancing activity. This opens the door to semi-synthesis from easily available alternative precursors and allowed detailed mechanistic studies (Fernández-Tejada et al., 2014).

QS-21 and new adjuvant formulations

Adjuvant systems

One of the promising approaches to improving efficiency of newly developed vaccines is given by the combination of several adjuvants...
into single formulations, such as the adjuvant systems AS01, AS02, AS015 (Garçon et al., 2011). AS01, developed more than 20 years ago, is a liposome-based adjuvant comprising two immunostimulants, MPL and QS-21. Liposomes are synthetic nanospheres consisting of phospholipid bilayers that can encapsulate antigens and act as antigen delivery vehicles (Garçon et al., 2011). MPL is a detoxified synthetic derivative of the lipopolysaccharide (LPS) from the bacteria Salmonella minnesota and induces activation of innate immunity via TLR-4, stimulating NF-kB transcriptional activity. It stimulates the induction of Ag-specific T cells producing interferon-γ (IFN-γ) and IgG2A antibodies. QS-21 from Q. Saponaria (licensed by GSK from Antigenics Inc., a wholly owned subsidiary of Agenus Inc., Lexington, MA, USA) in the early evaluations as an adjuvant was suggested to promote high Ag-specific Ab responses and CD8+ T cell responses in mice and high Ag-specific Ab responses in humans (Didierlaurent et al., 2014). QS-21, as an amphiphilic compound is known for its hemolytic properties, which can be abrogated through formulation in liposomes in the presence of cholesterol such as in AS01 (Beck et al., 2015; Garçon and Di Pasquale, 2017). This resulted in an acceptable tolerability profile for its use in human vaccines. AS02 contains MPL/QS21 in an oil-in-water emulsion. AS015, combining QS-21, MPL, and CpG 7909 (an oligodeoxynucleotide and TLR-9 agonist), in a liposome is the most complex combination of adjuvants which has been used for applications in cancer immunotherapy.

**Mechanisms of action of QS-21 when formulated in liposomes**

The mechanism of action of QS-21 in a liposome is poorly understood despite the clinical efficacy of AS01. Therefore, some studies were carried out in order to tentatively elucidate the adjuvant effect of QS-21 in such a formulation. It was observed after an intramuscular (im) immunization in mice against two model antigens, Hepatitis B surface antigen (HBsAg) and OVA that QS-21, rapidly accumulated in CD169⁺ resident macrophages of the dLN, where it induces caspase-1 activation. These early events then organized the recruitment of innate immune cells and activation of DCs and subsequent T cell priming. Further analysis showed that the adjuvant effect of QS-21 depended on the integration of caspase-1 and MyD88 pathways at least in part through local release of HMGB1 (High-Mobility-Group protein B1) (Detienne et al., 2016).

A recent study reported that QS-21 directly activated human monocyte-derived dendritic cells (mo-DCs) and promoted a pro-inflammatory transcriptional program (Welsby et al., 2017). In this model, QS-21 was endocytosed via a cholesterol-dependent mechanism and accumulated in lysosomes where it induces their destabilization through pore formation and potential release of their contents. This resulted in inflammasome activation, Syk- (a tyrosine kinase) and cathepsin-B (a cysteine protease) dependent cell activation and cytokine production in mo-DCs. Lysosomal disruption could also affect Ag processing and translocation to the cytosol for presentation on MHCs. Finally it was shown that cathepsin B contributes to the adjuvant properties of QS-21 on both CD4⁺ and CD8⁺ Ag-specific T-cell responses in vivo.

**Mechanism of action of AS01: synergistic effect of QS-21 and MPL**

The Adjuvant Systems containing MPL/QS21 in combination with HBsAg were shown to induce very strong and persistent humoral and cellular immune responses in healthy adults, AS01B inducing the strongest and most durable specific CMI after two doses, without serious adverse events. The CD4⁺ response was characterized in vitro by high lymphoproliferation, high IFN-γ and moderate Il-5 production. This response was confirmed ex vivo by detection of IL-2 and IFN-γ producing CD4⁺ T cells and in vivo by measuring increased levels of IFN-γ in the serum (Vandepapelière et al., 2008). Therefore AS01B was selected as lead AS for the development of vaccines. Experiments were conducted in mouse models with a recombinant varicella-zoster virus (VZV) glycoprotein E (gE) and the fluorescently labeled QS-21 incorporated in AS01 (HZ (shingle) vaccine formulation). AS01 induces a rapid and transient activation of the innate immune system, both at
the injection site and in the dLN, leading to the generation of high number of efficient Ag-loaded DCs in the dLN to promote Ag-specific adaptive immune responses (Didierlaurent et al., 2014). A comparative study of the immunogenicity of different formulations containing the subunit gE was carried out in a VZV-primed mouse model (Dendouga et al., 2012). This model was chosen in order to mimic the clinical setting, in which the large majority of persons at elevated risk for HZ are VZV sero-positive. An HZ candidate vaccine comprising gE (5 μg) and AS01B (5 μg each of MPL and QS-21) was shown to be highly immunogenic in mice, inducing higher responses of CD4⁺ T cells expressing IL-2 and/or INF-γ and higher humoral responses compared to other gE/AS01 formulations with different Ag doses (0.5, 2.5, 10 μg) or with lower doses of adjuvant (2.5 and 1.25 μg each of MPL and QS-21, corresponding to AS01A and AS01B, respectively) (Dendouga et al., 2012). Furthermore, the contribution of each component of AS01A to the induction of cellular and humoral response was assessed after immunization of mice with gE either unadjuvanted, or adjuvanted with QS-21 (5 μg), MPL (5 μg) (both in liposomes), and AS01. The results showed that the gE-specific cytokine (IFN-γ, IL-2) producing CD4⁺ T cell responses induced by gE/liposome + QS21 and gE/liposome + MPL were of similar and low magnitude, whereas the response induced by gE/AS01B was significantly higher than gE/liposome + QS21 (8.7 fold difference) and gE/liposome + MPL (7.5 fold difference). This clearly showed that QS-21 and MPL acted synergistically to induce a high frequency of gE-specific CD4⁺ T cells. For the anti-gE antibody responses, an additive effect, rather than a synergistic effect was observed (Dendouga et al., 2012; Didierlaurent et al., 2017). These results suggested that the gE/AS01B vaccine candidate was appropriate for further clinical assessment.

An investigation of the molecular and cellular mechanism of AS01 adjuvanted vaccines was undertaken in order to clarify how immunostimulators function in combination. (Coccia et al., 2017). Results from in vivo and clinical studies showed that the combination of MPL/QS-21 in AS01 resulted in the stimulation of de novo pathways that were not triggered by the individual components. Mice were immunized twice, 2 weeks apart, with the RTS,S antigen of the malaria vaccine, formulated either without adjuvant, with MPL, with QS-21 (both included in liposomes) or with AS01. RTS,S is a recombinant protein consisting of repeated sequences of the Plasmodium falciparum circumsporozoite protein (CSP-repeat region, (R)) and T-cell epitope domain (T) linked to the hepatitis B surface antigen (HBsAg) (S), and HBsAg alone (S) (Moris et al., 2018). The CSP-specific immune response was measured after the second dose. The results clearly showed that MPL and QS-21 act synergistically to induce strong antibody and Ag-specific CD4⁺ T cell responses. In contrast, very low levels of CD8⁺ T cell responses were generated against CSP. Further investigations highlighted the key role of the early release of IFN-γ by innate resident cells (NK and CD8⁺ T cells) in the LN, draining the injection site, which is essential for the development of the CD4⁺ T cell response (Th1 immunity). This was confirmed by measuring the levels of the cytokine after immunization of mice with BHs adjuvanted with AS01 or with its components. AS01 induced the early production of IFN-γ with a peak in concentration at 6 h post immunization (300 pg/ml), whereas MPL or QS-21 alone failed to significantly induce IFN-γ. This effect results from the MPL/QS-21 synergy and is controlled by macrophages, IL-11 and IL-18 (Coccia et al., 2017).

Clinical applications

QS-21 containing adjuvants have been assessed in more than 100 human clinical trials. AS01 is a key component of the recently developed malaria and HZ vaccines and other candidate vaccines under development against infectious diseases, and cancer (Coccia et al., 2017; Didierlaurent et al., 2017; Garçon and di Pasquale, 2017). Recent clinical studies of prophylactic vaccines against malaria, HZ, HIV, tuberculosis from an efficacy, immunogenicity and safety points of view (Table 1) and of therapeutic vaccines against melanoma, NSCLC and Alzheimer’s disease, will be reported below.

Prophylactic vaccines

Malaria

Despite effective medicines, insecticide-treated nets and indoor insecticide spraying, malaria killed 445,000 people in 2016, particularly sub-Saharan African children (World Malaria Day, 2018). The Ag of the candidate malaria vaccine RTS,S targets the CSP which is expressed on the plasmodium sporozoite during the pre-erythrocytic stage of its life-cycle, the stage between mosquito bite and liver infection. RTS,S/AS02, was the first malaria vaccine candidate, in which an adjuvant system was used (Garçon et al., 2007). However, RTS,S/AS01 was shown in a phase II trial to be well tolerated, to induce strong humoral and cellular immune response and to improve protection against Plasmodium falciparum challenge in comparison to RTS,S/AS02 (Kester et al., 2009). Namely, it induced high levels of anti-CSP IgG titers and CSP-specific polyfunctional CD4⁺ T cells expressing IL-2, IFN-γ, TNFα or CD40L which have been associated with protection in the experimental malaria challenge model in adults. Other clinical trials in children and adults confirmed these observations and no serious adverse events were reported (Agnandji et al., 2010; Olotu et al., 2011; Ansong et al., 2011; White et al., 2013; Levast et al., 2014; Leroux-Roels et al., 2014a). Therefore the development of RTS,S/AS02 was stopped and RTS,S/S/AS01 was consequently selected for phase III development (Garçon and Di-Pasquale, 2017). Analyses of data in term of efficacy, immunogenicity and safety of Phase I-III trials including adults, infants, and children from sub-Saharan countries were reviewed (Agnandji et al., 2015). Clinical trials in children with newly adjuvanted vaccines led to the conclusion of clinical safety except for some unexplained cases of meningitis in one phase III AS01 adjuvanted malaria vaccine trial, which warrant further safety evaluations (Staats et al., 2016). Important insights into the molecular mechanism of protective immunity against malaria were achieved involving additional immunogenicity analyses into the potential contributive roles of CSP-specific Abs and CSP-specific CMI to protection (Moris et al., 2018; Kazmin et al., 2017) leading to the identification of the NF-kB and IFN-γ pathways (van den Berg et al., 2017).

The first clinical large-scale Phase III trial (The RTS,S clinical trials partnership, 2014) evaluating a malaria vaccine involving 15,460 infants and young children was completed in December 2013 at 11 sites from 7 African countries (Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, and Tanzania). Safety and efficacy studies of the RTS,S/AS01 vaccine (Mosquirix™) showed that it prevented many cases of malaria over the 18 months after dose 3. Vaccine efficacy was higher in children (5–17 months) than in infants (6–12 weeks), but even at modest levels (45.7%). The final results in 2015 (The RTS,S clinical trials partnership, 2015) from the same trial, showed the enhanced efficacy by administration of a booster dose in both age categories, 18 months after the first three injections. With regards to risks, the safety of Mosquirix is similar to that of other vaccines, the most common side effects being fever, pain and swelling at the injection sites. Thus, the vaccine has the potential to make a substantial contribution to malaria control when used in combination with other effective control measures, especially in areas of high transmission. The ratio benefit/risk of Mosquirix was found to be acceptable by the European Medicines Agency’s committee for Medicinal Products for Human use (EMA/CHMP) which delivered a positive scientific opinion in 2015 for use outside the European Union (EU) (EMA Press release, 2015). Furthermore, the World Health Organization recommended a large-scale pilot implementation of the vaccine in young children with a four-dose schedule in African regions of moderate to high parasite transmission (WHO, 2016). Three countries, Ghana, Kenya, and Malawi were selected by the WHO in 2017 for this large-scale pilot program which started in 2018. It will be the first malaria
| Table 1 | Clinical trials involving newly adjuvanted prophylactic vaccines. |
|--------|---------------------------------------------------------------|
|        | Phase | Year          | Country       | Study populations | Nr of subjects | Summary |
| Malaria |       |               |               |                  |                | Reference |
|        | IIA   | 2003–06       | USA           | Healthy volunteers | 102            | RTS/S/AS01 (50% efficacy) well tolerated, safe, induced stronger humoral and cellular immune response, and improved protection over RTS/S/AS02. |
|        | II    | 2007          | Ghent, Belgium| Healthy naïve adults | 36             | Stronger immune response of RTS/S/AS01 as compared to RTS/S/AS02, acceptable safety profile. |
|        | II    | 2006–08       | Ghana         | 5–17 months old children | 540           | RTS/S/AS01 induced higher CD4+ T cells responses as compared to RTS/S/AS02, provided sustained protection from clinical malaria for at least 15 months. |
|        | II    | 2006–08       | Kenya, Korogocho, Tanzania | 15–60 months old children | 894         | RTS/S/AS01: higher immune response with CD8+ T cell and humoral immune responses. |
|        | IIB   | 2007–08       | Kenya, Korogocho, Tanzania | 15–60 months old children | 894         | RTS/S/AS01 E provides sustained protection from clinical malaria for at least 15 months. The frequency of RTS/S-induced CSP-specific T-effector memory cell responses were associated with protection. |
|        | II    | 2007–09       | Ghana, Gabon, Tanzania | 6–10 weeks infants | 511         | RTS/S/AS01 E integrated in the EPI programme of immunization showed a favorable safety and immunogenicity profile. |
|        | III   | 2009–14       | Multicentric (11 sites in Africa) | 6 weeks to 17 month infants | 15,460    | RTS/S/AS01: 3 doses at month 0, 1, 2. 18 months following vaccination: substantial contribution to malaria control. |
| Herpes Zoster | I/II | 2004–05       | Ghent, Belgium | Adults 18–30 years | 20           | GbS-A143 highly immunogenic with CD4+ T cell and humoral immune responses. Clinically acceptable safety profile. |
|        | I/II  | 2007–08       | Czech Republic, Germany, Sweden, Netherlands | Adults >60 years | 135         | GbS-A143, the formulation containing 25 μg, 50 μg, or 100 μg of the adjuvanted vaccine was immunogenic and well tolerated in older adults. This vaccine induced robust CD4+ T cell and humoral immune responses that persisted up to 3 years after vaccination. |
|        | II    | 2007–10       | Czech Republic, Germany, Sweden, Netherlands | Adults >60 years | 715         | GbS-A143, the formulation containing 25 μg, 50 μg, or 100 μg of the adjuvanted vaccine was immunogenic and well tolerated in older adults. This vaccine induced robust CD4+ T cell and humoral immune responses that persisted up to 3 years after vaccination. |
|        | II    | 2011–13       | Czech Republic, Germany, Sweden, Netherlands | Adults >60 years | 129         | GbS-A143, the formulation containing 25 μg, 50 μg, or 100 μg of the adjuvanted vaccine was immunogenic and well tolerated in older adults. This vaccine induced robust CD4+ T cell and humoral immune responses that persisted up to 3 years after vaccination. |
|        | III   | 2010–15       | Germany, Sweden, Czech Republic | Adults >60 years | 15,411     | GbS-A143, the formulation containing 25 μg, 50 μg, or 100 μg of the adjuvanted vaccine was immunogenic and well tolerated in older adults. This vaccine induced robust CD4+ T cell and humoral immune responses that persisted up to 3 years after vaccination. |
|        | II    | 2016–18       | Germany, Sweden, Czech Republic | Adults >60 years | 13,900     | GbS-A143, the formulation containing 25 μg, 50 μg, or 100 μg of the adjuvanted vaccine was immunogenic and well tolerated in older adults. This vaccine induced robust CD4+ T cell and humoral immune responses that persisted up to 3 years after vaccination. |
|        | II    | 2016–18       | Germany, Sweden, Czech Republic | Adults >60 years | 70         | GbS-A143, the formulation containing 25 μg, 50 μg, or 100 μg of the adjuvanted vaccine was immunogenic and well tolerated in older adults. This vaccine induced robust CD4+ T cell and humoral immune responses that persisted up to 3 years after vaccination. |
| Tuberculosis | I/I  | 2006–07       | Ghent, Belgium | Healthy, HIV-negative, adolescents | 110        | M72/AS01 and M7/AS02: TB vaccine formulations clinically well tolerated and induced high magnitude of polyfunctional MF-72-specific CD4+ T cell, humoral and cellular immune responses, which were similar to those observed after 6 months post-initial vaccination, with which were similar to those observed after 6 months post-initial vaccination. |
|        | II    | 2010–12       | Germany, Sweden, Czech Republic | Adults >60 years | 300         | M72/AS01: 3 doses at month 0, 1, 2. 18 months following vaccination: substantial contribution to tuberculosis control. |
|        | II    | 2009–10       | TB endemic region | Adults >60 years | 60         | M72/AS01: clinically acceptable safety and immunogenicity profile. Robust T cell (CD4+ and CD8+ T cell responses) and Ab responses. Good candidate for advancement into efficacy trials. |
First introduced in 1921, the Bacillus Calmette-Guérin (BCG) vaccine is the only currently licensed vaccine available to protect against tuberculosis (TB), a disease caused by the bacteria *Mycobacterium tuberculosis* (*M. tb*). It protects children from meningeal and severe forms disseminated of TB and death, but offers limited protection against pulmonary TB in adolescents and adults, which is the form of the disease responsible for the vast majority of transmission and TB-related morbidity and mortality. Therefore, there is an urgent need for a new and improved vaccine against TB for controlling this disease that continues to pose a global health threat with 1.7 million deaths in 2016.
M. tuberculosismunogenic (Van Der Meeren et al., 2018). The M72/AS01 candidate vaccine contains the recombinant fusion protein derived from the two immunogenic M. tuberculosis Ags (Mtb32A and Mtb39A) in AS01. A first phase I/II randomized, controlled clinical study with M72/AS01 was carried out in Belgium in healthy adults volunteers. The results showed that the vaccine was clinically well tolerated and induced high and persistent (up to three years) specific Th1 CD4+ T-cell (co-expressing CD40L, IL-2, TNF-α and IFN-γ) and Ab responses, supporting its further clinical evaluation in TB-endemic settings (Leroux-Roels et al., 2013). Similar conclusions were drawn in a phase II controlled trial conducted on healthy, HIV uninfected adolescents living in an area with high TB burden in South Africa (Penn-Nicholson et al., 2015). In a phase II study M72/AS01 given to infants after or concomitantly with expanded-Programme-on-Immunization (EPI) vaccines had an acceptable safety profile. These results suggested no interference of immunogenicity profiles occurred following co-administration of M72/AS01 and EPI vaccines. Two M72/AS01 doses elicited higher immune responses than one dose (Ikodo et al., 2014). In a large phase Ib trial conducted on 3600 HIV-uninfected adults latently infected with Mtb in three African countries, M72/AS01E was shown to provide 54% protection for Mtb-infected adults against active pulmonary TB, without evident safety concerns (van der Meeren et al., 2018). These results suggest further evaluations of M72/AS01 as a possible vaccination strategy against TB.

**HIV-infections**

There were approximately 36.9 million people living with HIV at the end of 2017 with 1.8 million people becoming newly infected in 2017 (HIV/AIDS, Key facts, 19 July 2018). While treatments are available to treat or to prevent HIV infections, there is no vaccine currently licensed. Despite ongoing prevention efforts, there is an urgent need for a safe and effective prophylactic vaccine. Prevention of infection through induction of neutralizing Abs, induction of strong cellular immune responses in order to delay progression and reduce the transmission rate in high risk population, are the major aims for an HIV vaccine. Although clinical attempts were disappointing, they contributed valuable insights into immune protective immunity.

A randomized double-blind study conducted on healthy HIV-seronegative adults showed that a vaccine formulation containing gp 120, Nef and Tat Ags and AS01E induced strong Ab response and CD4+ T cell responses characterized by high lymphoproliferative capacity and IL-2 production, that were maintained up to 18 months after the last vaccine dose. A CD8+ T cell response was not observed (Leroux-Roels et al., 2010). This study conducted with different adjuvants underlined the superiority of responses with AS01E in comparison with other adjuvants, confirming previous findings. Another candidate HIV-1 vaccine consisting of a recombinant fusion protein (F4) encoding four HIV-1 clade B Ags (p17, p24, reverse transcriptase (RT) and Nef) adjuvanted with AS01 was studied in a phase I/II trials with healthy HIV-seronegative volunteers (Van Braeckel et al., 2011). This vaccine was shown to be immunogenic with an acceptable safety profile. After two doses of 10 μg, strong polyclonal (CD40L, IL-2, TNF-α, IFN-γ phenotype) and persistent CD4+ T cell responses were induced, suggesting that this vaccine merits further evaluation both in a prophylactic setting and as a potentially disease-modifying therapeutic vaccine in HIV-infected subjects. A phase I clinical study in such subjects has been initiated (Harrer et al., 2014). A phase II study was conducted with the F4/AS01E candidate vaccine on healthy adults in order to evaluate the effect of chloroquine on the specific CD8+ T cell responses and the safety profile of a booster dose (Leroux-Roels et al., 2014b). The results showed that a booster dose, administered alone or two days after chloroquine induced a strong Ab immune response and robust F4-specific CD4+ T cell response, but no significant CD8+ T cell response. This suggest that two primary doses of the F4/AS01E vaccine induce long-lasting memory B cell and CD4+ T cell responses in healthy adults, confirming previous findings of the phase I/II study (Leroux-Roels et al., 2014b). This vaccine was shown to be immunogenic with a clinically acceptable safety profile, but does not show significant viral efficacy in antiretroviral therapy-naive HIV-1-infected adults, results confirmed in a long term study (Dinges et al., 2016; Harrer et al., 2018).

**Therapeutic vaccines**

**Cancer**

The use of immunotherapeutic vaccines in cancer patients is aimed at inducing immune responses against existing tumors rather than protecting healthy individuals. They have been used in phase II and III trials against melanoma and lung cancer after surgical resection, and the disappointing results of the most recent studies will be shortly presented here. Enhanced production of ganglioside GM2 is observed in different human tumor types including melanoma. Anti-GM2 vaccines such as GM2 conjugated to a carrier keyhole limpet hemocyanin (KLH) and administered with QS-21 induced high IgM and IgG Ab responses but failed to show a beneficial effect in melanoma patients as it was shown in a phase III clinical trial in term of relapse free survival, distant metastasis-free survival and overall survival (Eggermont et al., 2013). A five-years survival occurs for less than 50% of the patients with completely resected non-small-cell-lung cancer (NSCLC). Therapeutic cancer vaccination has the potential for eliminating remaining cancer cells after complete resection, thereby decreasing relapse rates and improving survival (Vansteenkiste et al., 2016). Ag-specific immunotherapies enhance T-cell responses against specifically expressed tumor Ags. In this context, the use of MAGE-A3 (Melanoma AntiGen family A, 3) as Ag, which is expressed in a wide array of malignancies can be an application in cancer immunotherapy (Esfandiar and Ghafouri-Fard, 2015). The MAGE-A3/AS015 immunotherapeutic vaccine was assessed for clinical efficacy in an international, multicenter phase III study in surgically resected NSCLCs. This study confirmed the acceptable clinical safety profile of the vaccine, but did not improve overall survival. Therefore, its further development for use in NSCLCs has been stopped (Vansteenkiste et al., 2016). Similar conclusions were drawn from a phase III trial of a MAGE-A3/AS015 vaccine in patients with resected, MAGE-A3-positive, stage III melanoma, which led to the arrest of the clinical development of this vaccine for use in melanoma (Dreno et al., 2018).

**Alzheimer’s disease**

The pathogenesis of Alzheimer disease (AD) is associated with the accumulation of amyloid β (Aβ) and/or hyperphosphorylated tau proteins in the brain. Active immunotherapy of AD is the most extensively studied approach, in order to evaluate its ability to elicit anti-Aβ antibodies, to induce Aβ clearance, and reduce Aβ accumulation in the brain. Vaccination with a full length Aβ peptide (Aβ1−40) successfully elicited anti-Aβ-Aβ in human subjects with AD, but adverse effects such as meningoencephalitis were observed, attributed to T cell activation (Winblad et al., 2014). To avoid this safety issue, a N-terminal (Aβ1−28) peptide conjugated to a carrier protein called vanutide cridificar (ACC-001) was produced. In preclinical studies, it was shown to generate N-terminal Aβ Abs in adult nonhuman primates without inducing Aβ-directed T cell response, supporting further clinical studies. Several phase II trials in patients with mild to moderate AD were conducted in USA, Europe, and Japan by using 3 doses of ACC-001 (3, 10 and 30 μg) with and without the adjuvant QS-21 (50 μg) or placebo to investigate dose-range, safety, immunogenicity and long term treatment (Arai et al., 2015; Pasquier et al., 2016). ACC-001 + QS-21 elicited high, sustained anti-Aβ IgG Ab titers compared with ACC-001 alone, highlighting the role of QS-21 in this effect. No difference was observed among the three doses tested. Furthermore, ACC-001 at all dose levels with or without QS-21 was generally safe and well tolerated, but exploratory cognitive evaluations did not show differences between treatment groups and placebo. Similar safety, tolerability and anti-Aβ- IgG immunogenicity profiles were observed in long term phase II extensions studies, suggesting that side effects do not limit in principle anti-amyloid active
immunotherapy (Hüll et al., 2017). However, larger trials of adequate duration, with optimized dosing may be needed to attest efficacy of anti-Aβ vaccine therapy for AD.

Conclusion

This overview provides a summary of recent reports on the mechanism of action of QS-21, a promising triterpene glycoside vaccine adjuvant isolated from Quillaja Saponaria and the most relevant clinical applications. This compound showed a potent adjuvant activity stimulating humoral and cell-mediated immunity against a wide variety of Ags and presented advantages over aluminium salts by inducing a Th1-type immune response, necessary for controlling most intracellular pathogens. It is unlikely that a simple mechanism of action could explain its effects as an adjuvant.

This contribution highlights:

1) the role of QS-21 acting on both APCs and T cells. The interaction between the aldehyde function and T-cell receptors such as CD2, delivering co-stimulatory signals together with the presentation of the Ag peptides by APCs to the T-cell resulted in the activation of T lymphocytes and secretions of Th1 cytokines, an important immune effector mechanism for the elimination of infected cells.

2) a novel mechanism of action based on the activation of caspase 1-dependent NLRP3 inflammasome in mouse APCs, leading to the secretion of proinflammatory cytokine IL-1β/IL-18, which are important for Th1 responses.

3) the synthesis of nearly 50 structural analogs of QS-21 and detailed SAR studies leading to more easily available, and stable molecules with an improved activity/toxicity profile, among them a novel aryl iodinated simplified echinodermic derivative showing a potent adjuvant activity and considerably attenuated toxicity.

4) the signaling pathways of QS-21 when formulated in a liposome, such as the rapid accumulation in dLN resident CD169+ macrophages, where it induces caspase-1 activation and HMGB1 release. These events orchestrate the recruitment of innate immune cells and activation of DCs leading to T cell priming. A direct activation of DCs was also reported, resulting in release of cathepsin B which contribute to specific T cell responses in vivo.

5) the development of Adjuvant Systems (AS) including a combination of immunostimulants in a formulation, such as AS01 (QS-21/MPL in a liposome), in which they were shown to act synergistically in enhancing CMI responses.

6) the high number of clinical applications in terms of immunogenicity, efficacy, safety of QS-21 alone or in adjuvant systems in many prophylactic vaccines against complex pathogens, e.g. malaria, herpes zoster, tuberculosis, HIV, and therapeutic vaccines, e.g. cancer, AD.

7) the licensing by the US FDA in 2017 of a vaccine against shingles called HZ/su (Shingrix™) containing QsA/AS01 and its commercialization in the EU since March 2018.

8) the first malaria vaccine (Mosquirix™) containing RTS,S/AS01 receiving a regulatory approval by the EMA in 2015 for use outside of Europe and being currently under exploration in sub-Saharan regions for its use in routine.

9) the disappointing phase III clinical results in term of disease-free survival of patient with immunotherapeutic vaccines adjuvanted with QS-21/AS015 against NSCLC and melanoma despite promising results of phase II trials.

10) the encouraging results of a phase II trial confirming that the long term treatment with the immunotherapeutic vaccine vanutide cridificar (ACC-001) adjuvanted with QS-21 against Alzheimer’s disease was well tolerated with an acceptable safety profile.

Perspectives

The development of adjuvants in vaccines represents an area in constant evolution with substantial advances over the past two decades. QS-21 in ASs is a key component of multiple vaccines targeting infections and endemic diseases in developing countries. Recent research has led to the commercialization of Shingrix™ and the approval of Mosquirix™, two vaccines against HZ in adults and malaria in children, respectively, which contain QS-21 as a part of their formulation. To ensure a continuous supply of this exciting molecule, the researchers aimed in the near future to find an alternative innovative process of producing this compound on a large scale based on plant cell-culture. This might overcome some limitations of the current traditional techniques of extraction with tedious purification processes and chemical synthesis involving many complex reactions. However, the synthetic technology initially used for the first synthesis of QS-21, was subsequently applied to preparing novel structural analogs with potent activity and low toxicity, leading to SAR studies and a better understanding of the mode of action. Further work in this area could facilitate novel saponin design that will lead to the discovery of new adjuvants and improved adjuvant-antigen combinations for future vaccines, particularly against infectious tropical diseases such as Dengue, Leishmaniasis, and Chagas diseases, which continue to cause significant morbidity and mortality in developing countries.

Conflict of interest

There are no conflicts of interest associated with this publication.

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