

Vaccine adjuvants: role and mechanisms of action in vaccine immunogenicity

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Inactivated vaccines require adjuvants to stimulate an immune response. The choice of adjuvant or immune enhancer determines whether the immune response is effective, ineffective or damaging. Accordingly, there is a need for new adjuvants that stimulate the appropriate immunity, for example, T cell immunity for intracellular pathogens and cancer vaccines. In several adjuvants, the identification of chemical groups that interact with specific cell toll-like receptors (innate immunity) or receptors for co-stimulatory ligands (adaptive immunity), has enabled the establishment of structure–function relationships that are useful in the design of new adjuvants. Because of the crucial immunomodulating role of adjuvants, sub-unit vaccine development will remain dependent on new adjuvants.

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▼ Vaccines can be classified as either ‘live’ or ‘inactivated’. Live vaccines stimulate immunity via a transient infection caused by a replicating live organism, for example, the smallpox vaccine pioneered by Jenner in 1796 [1]. Although cost-effective and easy to produce, they carry the intrinsic risks associated with live pathogens. Inactivated vaccines that include killed organisms and isolated non-replicating sub-cellular components stimulate a lower level and shorter duration of immunity than that elicited by live vaccines. In 1916, Le Moignie and Pinoy [2] reported that mineral oil emulsions increased the immune response against an antigen. Alum, the only adjuvant approved for human use in America, was introduced by Glenny *et al.* in 1926 to enhance toxoid preparation activity [2]. Between 1924 and 1926, Ramon discovered that local inflammation caused by bacterial components correlated with an antibody titer increase and coined the term ‘adjuvant’ to describe compounds that enhance the antibody response against an antigen [2]. Immunological adjuvants can be defined as compounds that bias

the immune system toward Th1 or Th2 immunity and significantly enhance the immune response against an antigen. In inactivated vaccines, the adjuvant component often makes the difference between an effective and ineffective vaccine, regardless of the antigens used. The adjuvants used in human vaccines must fulfill stringent requirements (Box 1), but these are rarely met and, thus, the availability of suitable adjuvants is limited. However, advances in various scientific disciplines are now enabling a better understanding of the mechanisms of ‘adjuvanticity’, that will translate into the design of safer and more effective immune enhancers or potentiators. Cytokines and dendritic cells (DCs) are frequently considered as adjuvants, however, as they are an intrinsic part of the immune response [3], their immunostimulatory role will not be discussed here.

Although vaccines have been commonly used to stimulate protective immunity against infectious agents, there is a trend to apply information derived from genomics to develop therapeutic vaccines for various diseases, such as herpes, tuberculosis, multiple sclerosis and cancer [4]. These new products, produced by genetic engineering, would contain antigens that lack the contaminants that were responsible for both their immunogenicity and side effects in previous vaccines. New vaccines will require adjuvants to elicit, as well as enhance, the appropriate immune response, therefore, the development of adjuvants is pivotal to the development of vaccines.

Adjuvant classification

Adjuvants are classified according to their chemical nature, origin or physical chemical properties [2,5], yet, related compounds frequently

have divergent immunomodulating properties, for example, various saponins differ in their capacity to stimulate Th1 or Th2 immunity [6]. Thus, it would be advisable to first classify adjuvants according to their capacity to stimulate Th1 or Th2 immunity (associated with T cell and humoral immunity, respectively) and then sub-classify them according to their chemical structures. This system would help to establish useful structure–function relationships that would enable the identification of the pharmacophores that are responsible for immune modulation. Alternatively, adjuvants can be classified according to their capacity to stimulate either innate or adaptive immunity; this is demonstrated by significant differences in their cellular receptors and mechanisms of action (Box 2).

Mechanisms of immune stimulation

Adjuvants exert their effects by mechanisms that might act in a concerted manner. Structural characterization of several adjuvants and the identification of cellular receptors that are associated with their activities, such as toll-like receptors (TLRs) [7,8] and co-stimulatory ligand receptors [9–10], are eliciting a better understanding of their mechanisms of action at the molecular level. One mechanism is the depot formation that is observed with alum, emulsion-based, and insoluble adjuvants (known as particulate adjuvants) [5,11], where antigens and adjuvants are sequestered at the injection site and are released over time to stimulate antigen-presenting cells (APCs), such as macrophages and, particularly, DCs – ‘professional APCs’ [12]. Adjuvants also promote an increase in the number of antigen-specific lymphocytes in lymph nodes draining the injection site [13]. Targeting is the mechanism by which an adjuvant–antigen complex is delivered to APCs for processing [5,14]; particulate adjuvants bind to antigens, forming aggregates that are engulfed by APCs via endocytosis to form endosomes. More-effective targeting is achieved by using adjuvants with residues that are recognized by receptors on APCs. For instance, the mannose receptor that belongs to the endocytic-Pattern Recognition Receptors (PRRs) [15,16], binds compounds containing mannose (e.g. mannans), *N*-acetylglucosamine or fucose residues (e.g. some saponins) and sulfated oligosaccharides. Binding of these adjuvant–antigen complexes to PRRs initiates efficient receptor-mediated endocytosis and antigen processing [16].

Innate immunity processing

In vertebrates, antigen processing takes place via either innate or adaptive immunity [17]. In innate immunity, highly conserved pathogen-specific antigens that are absent from host T cells and are known as Pathogen-Associated Molecular Patterns (PAMPs), for example, lipopolysaccharide

Box 1. Required properties of an adjuvant

- Is non-toxic or has a negligible toxicity at the dose range for effective adjuvanticity
- Stimulates a strong humoral and/or T cell immune response
- Provides good immunological memory or long-term immunity
- Does not induce autoimmunity
- Is non-mutagenic, carcinogenic or teratogenic
- Is non-pyrogenic
- Is stable under broad ranges of storage time, temperature, and pH

(LPS), and unmethylated CpG-DNA are bound by germline-encoded PRRs. These PRRs can be: (i) secreted molecules found in blood and lymph that are associated with complement and opsonization, (ii) surface receptors on phagocytic cells that are associated with endocytosis, or (iii) TLRs, a family of receptors found on macrophages, DCs and epithelial cells that have been conserved from insects to mammals [7,8]. TLRs are transmembrane proteins with an extracellular domain containing leucine-rich repeats (LRRs) that recognize conserved motifs on pathogens, and a cytoplasmic domain similar to the corresponding domain of the interleukin-1 (IL-1) receptor [7,8,18]. Like the IL-1 receptor, TLRs induce signaling pathways, leading to activation of the nuclear factor- κ B (NF- κ B) in APCs, which results in the expression of various cytokine genes, production of co-stimulatory ligands B7-1 (CD80) and B7-2 (CD86), and activation of adaptive immunity [17,18] (Figure 1). It is the initial response of the innate immune system, stimulated

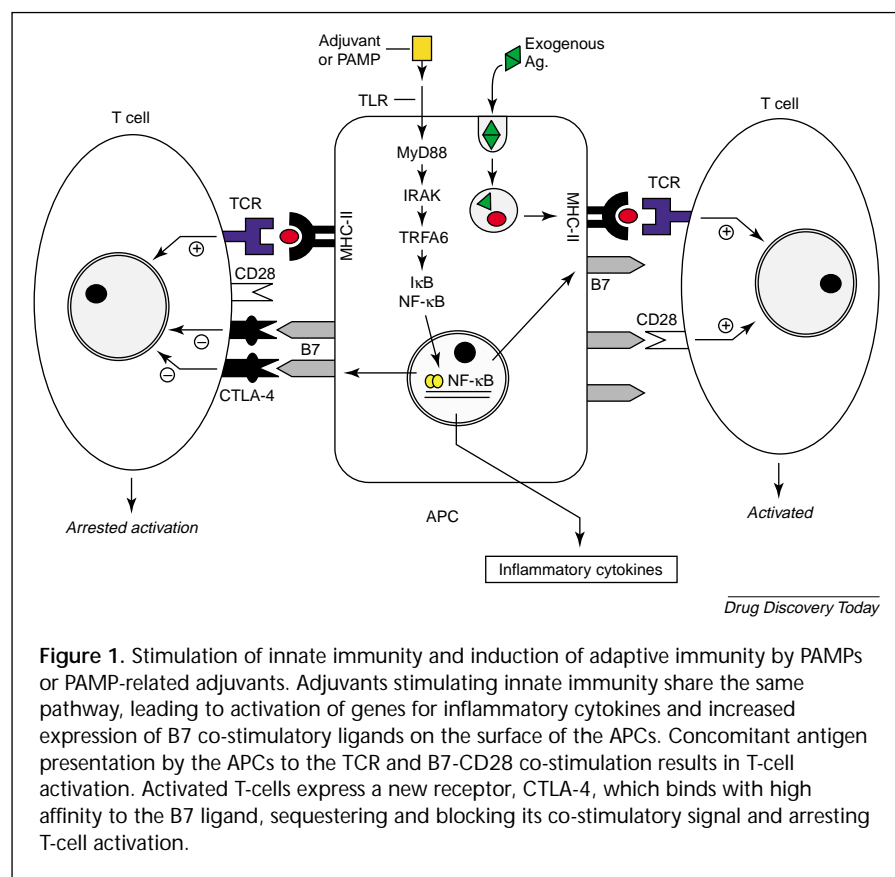
Box 2. Innate and acquired immunity adjuvants

Innate immunity adjuvants

- Microbial products (PAMPs) (e.g. CpG-DNA, LPS)
- Bind to broadly specific TLRs and PRRs on APCs
- Activate Nuclear Factor- κ B to:
 - Produce inflammatory cytokines
 - Increase B7 ligands production
- Adaptive immunity is activated

Adaptive immunity adjuvants

- Natural and synthetic products, such as cytokines, quillaja saponins, tucareol
- Bind to highly specific receptors on T cells
- Activate T cell by:
 - Co-stimulatory signals
 - Induction of cytokine-regulated genes



and CD8 receptors on the APC, respectively. Exogenous antigens (e.g. bacteria) are taken up by APCs via endocytosis and degraded in the lysosomes into peptides of 13–18 amino acids. These peptides, bound to MHC class II molecules that are embedded in the lysosome membrane to form MHC-II–peptide complexes, are delivered by exocytosis to the cell membrane for presentation to CD4⁺ T cells (Figure 1). Certain adjuvants, such as amphipathic non-ionic block copolymers, bind to exogenous antigens, preserving their tridimensional conformation (required for the production of neutralizing antibodies) during their internalization by APCs [5,20]. Depending on their size and the balance of hydrophilic (polyoxyethylene) to lipophilic (polyoxypropylene) chains, block polymers can deliver exogenous antigens to the class I (cytoplasm) or class II (phagosomes) pathways for processing [20]; they are effective antigen delivery systems that can be used with other adjuvants.

by PAMPs, that triggers and controls the adaptive immune system response. Most PAMPs have low-affinity for their TLRs and, thus, unidentified co-receptors might be necessary for specific recognition of the ligand [8]. The binding of PAMPs, such as LPS or bacterial CpG-DNA, initiating the activation of adaptive immunity, is indicative of their adjuvant effect acting via specific TLRs. Indeed, several natural or synthetic adjuvants, including monophosphoryl lipid A and lipoarabinomannan, that are bacterial cell-wall components, or the yeast polysaccharide glucan, are structurally closely related to different PAMPs [7,19].

Adaptive immunity processing

Processing and presentation of protein antigens by APCs to T or B cells is dependent on whether the antigen is intracellular (endogenous) or extracellular (exogenous) [3]. Endogenous antigens (e.g. viral antigens produced or delivered into the cell cytosol) are degraded into 8–13 amino acid peptides that are transported into the endoplasmic reticulum (ER) and loaded onto the Major-Histocompatibility-Complex (MHC) class I, to form MHC-I–peptide complexes. These complexes are exported via the Golgi apparatus to the APC surface for presentation and binding to CD8⁺ T cells, containing both T cell receptors (TCRs) and CD8 molecules that recognize both MHC-I–peptide complexes

T cell activation

CD4⁺ and CD8⁺ T cells recognize antigens that are present on APCs as complexes with MHC class II or I, respectively. The process mediated by the TCR leads to T cell activation and the production of effector cells that are capable of secreting cytokines [3]. Two signals are required for T cell activation: one is derived from the interaction of the TCR with the antigen–MHC complex, the other is a co-stimulatory signal delivered by the B7-1 or B7-2 ligand, present on the APCs, to the CD28 receptor on T cells. Several studies indicate that B7-1 signals preferentially promote development of Th1 cells and B7-2 signals, Th2 cells (reviewed in [9]). Following activation, T cells express a new surface antigen, CTLA-4, that binds tightly to B7 ligands, arresting T cell activation [9] (Figure 1). Failure to provide a CD28-based co-stimulatory signal leads to T cell anergy (unreactivity), which is also known as ‘immune tolerance’, or apoptosis [9]. This co-stimulatory signal provides an avenue for immune stimulation by adjuvants that can substitute for the B7-1 or B7-2 ligands [21]. Activated T cells produce two major types of effector cells: helper (Th) and cytotoxic (Tc) cells, derived from CD4 and CD8 cells, respectively. CD4 cells interact with APCs carrying MHC-II–antigen complexes to yield Th1 or Th2 cells. This selection appears to depend

on the origin of the activated DC that interacts with the CD4 cell [12]. Th1 cells produce pro-inflammatory cytokines, such as interferon gamma (IFN- γ), IL-2, and tumor necrosis factor β (TNF- β), and stimulates production of cytotoxic T lymphocytes (CTL). Th2 cells produce IL-4 and IL-10 cytokines, that favor antibody production and class-switching, and also inhibit Th cells from entering the Th1 path (reviewed in [22]).

Presentation by APCs of MHC-I-antigen complexes to CD8+ T cells results in activation of CD8 cells to produce CTLs; following recognition of MHC-I-antigen complexes, CTLs bind to target T cells and insert perforins into their cell membrane, delivering granzymes into the cell cytoplasm and initiating a process leading to cell apoptosis. An alternative CTL-mediated apoptotic process involves activation of Fas (CD95), a membrane protein belonging to the TNF receptor-like family that is present on the target T cell, by the ligand FasL, present on CTLs. Once Fas is activated, it triggers a cascade of events leading to caspase-mediated cell death. (For a review of pro-apoptotic mechanisms of CTL see [23]).

Innate immunity, TLR and PRR binding adjuvants

Binding of adjuvant PAMPs by TLRs stimulates innate immunity, which, in turn, activates adaptive immunity. Although TLRs activate an almost identical intracellular signaling pathway (TLR \rightarrow My88 \rightarrow IRAK \rightarrow TRAF6 \rightarrow I- κ B-kinase \rightarrow NF- κ B) [24] (Figure 1), different PAMPs stimulate different immune responses [25]. It could be the case that recognition of PAMPs requires an adaptor protein(s) [8], or that there is cooperation between different TLRs [26], or a combination of both of these mechanisms. In the following sections, some selected non-proteinic adjuvants of clinical value and their pharmacophores responsible for stimulating adaptive immunity are reviewed.

LPS and derivatives

LPS or endotoxin, a Gram-negative bacteria membrane component, has a hydrophilic polysaccharide and a lipophilic phospholipid (lipid A). The latter stimulates the excessive production of pro-inflammatory cytokines, leading to septic shock [27]. Studies using transfected cells and mouse mutants have shown that LPS-induced cell activation

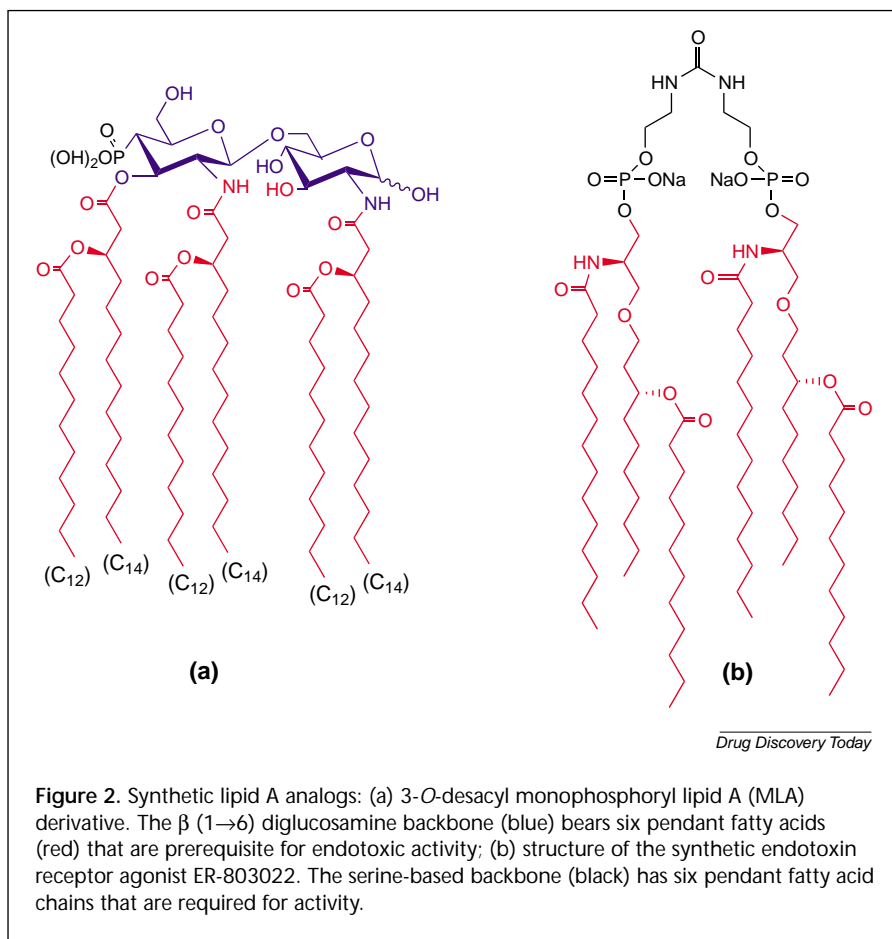


Figure 2. Synthetic lipid A analogs: (a) 3-O-desacyl monophosphoryl lipid A (MLA) derivative. The $\beta(1\rightarrow6)$ diglucosamine backbone (blue) bears six pendant fatty acids (red) that are prerequisite for endotoxic activity; (b) structure of the synthetic endotoxin receptor agonist ER-803022. The serine-based backbone (black) has six pendant fatty acid chains that are required for activity.

requires a tripartite receptor complex [28]. Initially, either free LPS or a complex of LPS-LPS-binding protein (LBP) bind to CD14, a protein that is either cell membrane-bound or is found in soluble form in the blood, triggering an association with TLR [29]. A third protein, MD-2, that binds tightly to TLR4, is also required for the signaling that leads to LPS-stimulated cell activation [28,29]. LPS can also be recognized, although less efficiently, by TLR2, which binds a variety of bacterial products [18]. Removal of a phosphate residue yields monophosphoryl lipid A (MPL), which retains adjuvanticity at a lower toxicity [2,30]. LPS receptor agonists or lipid A mimetics with TLR4-dependent immunostimulatory action have been synthesized [31], and based on work with these synthetic MPLs, it has been proposed that a hexaacylated $\beta(1\rightarrow6)$ diglucosamine having three 3-n-alkanoyloxytetradecanoyl residues or six fatty acid groups is required for adjuvanticity (Figure 2a) [31]. Later reports have demonstrated that the simplest lipid A pharmacophore consists of six lipid chains, sterically and structurally similar to those of lipid A, linked to a phosphate-containing acyclic backbone (Figure 2b) [32]. Using cells transfected with TLR4 and/or CD14, these non-saccharide phospholipids have been shown to share both

LPS receptors and presumably also MD-2 [32,33]. Although lipid A and its derivatives enhance CTL production, they do not appear to mediate CTL production against exogenous antigens, except when used with liposomes as a delivery system [34]. Lipid A mimetics stimulate Th1 immunity with no toxicity observed at low doses. Mucosal immunity is also stimulated but, apparently, only against highly immunogenic antigens [32]. With these properties, lipid A mimetics could be useful adjuvants

β -Glucans

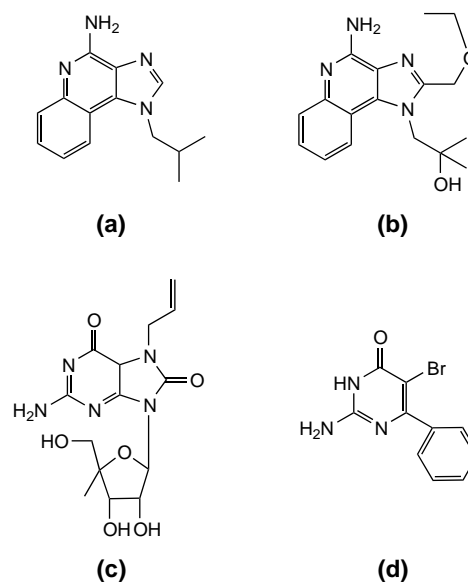
These fungal polysaccharides stimulate innate immunity via TLRs and other PRRs [35]. β -glucans are $\beta(1\rightarrow3)$ D-glucose polymers with or without $\beta(1\rightarrow6)$ linked $\beta(1\rightarrow3)$ glucan side-chains forming single- or triple-helix conformers [36]. β -glucans' immunomodulating properties are dependent on size, branching and conformation, with single helix conformers being the more effective ones [35]. High molecular weight (MW) β -glucans (>500 kDa) cross-link membrane complement receptor type three (CR3) from neutrophils and monocytes, triggering degranulation and cytokine release in the absence of target T cells. Low MW β -glucans bind to CR3, priming macrophages, neutrophils and NK cells to exert cytotoxic activity on iC3b-opsonized target T cells or pathogens [35]. Recent findings strongly suggest that linear β -glucans can activate macrophages via an unidentified receptor(s) possessing a toll/IL-1 receptor domain, like a TLR, to produce tumor necrosis factor (TNF)- α . A dominant-negative mutant of MyD88 [36] and a mutated NF- κ B binding site in the TNF- α gene promoter have shown that NF- κ B activation is crucial for TNF- α production.

Bacterial CpG-DNA

Bacterial DNA has unmethylated CpG motifs (uncommon in mammalian DNA), which are recognized by TLR9 with the concomitant activation of innate immunity and induction of cytokines that promote Th1 immunity [8]. In opposition to most TLRs, TLR9 seems to be intracellular [37], which would explain the lack of immunostimulation by immobilized CpG oligodeoxynucleotides (ODNs) [38]. The strong activation of cells transfected with human or mouse TLR9 by CpG-ODNs optimized for human or mouse signal initiation, indicates that CpG immunomodulating activity is species-specific [39]. Owing to dissimilarities between the amino acid sequences of the methyl-CpG-binding domain (MBD1) of TLR9 from various species, TLR9 affinity for CpG-ODNs depends on specific nucleotide flanking sequences that are optimal for each species [40]. Nuclease-resistant CpG-ODNs have been developed by replacing the phosphodiester with a phosphorothioate (PTO) backbone

and, based on the immune stimulatory properties hundreds of CpG-ODNs, they have been grouped into two distinct sets: 'D' and 'K' [41]. Type 'D' stimulates IFN- α and IFN- γ production (by plasmacytoid DCs and NK cells, respectively), poorly stimulates B cells and has a chimeric backbone, in which the central CpG core is a phosphodiester flanked by self-complementary PTO-polyguanosine (polydG) sequences. Type 'K' stimulates B cell proliferation and IL-6 production by monocytes, and can have a CpG motif containing a thymidine 5' and a TpT or TpA 3' [41] or can be a PTO-CpG-ODN.

However, a third class of PTO-CpG-DNA that stimulates both B cells and plasmacytoid DCs has recently been described [42]. The fundamental characteristics of this class include one or two TCGs at, or close to, the 5' end of the ODN and a region of at least ten bases, containing at least two additional CGs [42]. The PTO backbone, not the CpG-motif, apparently causes transient lymphadenopathy, therefore, polydG sequences that enhance cellular uptake and immunoreactivity of CpG-ODNs have been used at the 3' terminus [43]. According to the authors, polydG-CpG-ODNs do not induce lymphadenopathy or prolonged local cytokine release, limiting potential side effects [43]. The



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Figure 3. Structures of TLR7 ligands: (a) imiquimod and (b) resiquimod are imidazoquinoline agonists that vaguely resemble adenosine analogs but they lack an additional nitrogen present in purines and contain a third ring, part of the polycyclic imidazoquinoline; (c) loxoribine or 7-allyl-8-oxoguanosine is a guanine nucleoside analog; (d) bromopiridine or 2-amino-5-bromo-6-phenyl-4(3H)-pyrimidinone has a structure reminiscent of pyrimidines.

stimulation by CpG-ODNs of type 1 cytokines by APCs (type 1 cytokines are inducers of Th1 cell differentiation, and upregulation of molecules involved in antigen presentation and co-stimulation), enhances Th1 immunity with CTL production against soluble antigens [44]. Maximal antigen-specific Th1 immunity occurs when the CpG-ODN and antigen are delivered to the same APC as antigen-covalently linked conjugates [45]. Apparently, antigen-CpG-ODN-antigen conjugates are taken up by immature DCs via an enhanced DNA receptor-mediated endocytosis. Following cellular internalization, the CpG-DNA is recognized by TLR9 in late endosomes, where the adaptor molecule MyD88 is recruited, providing the signal for DC activation, which is required for CTL production [46]. Studies using TLR9-deficient mice have shown that TLR9 is not required for antigen cross-presentation but is essential for cross-priming or activation of naive CD8 T cells [46]. Considering the multiple immunological effects and safety of CpG-ODNs, they appear to have promising preventive and therapeutic applications in infectious diseases, cancer and allergic conditions [47].

Imidazoquinolines and other small synthetic compounds

A new group of immune response modifiers are the synthetic low-MW imidazoquinolines – imiquimod and resiquimod [48] – and other compounds, such as certain guanine nucleoside analogs (Figure 3) [49]. These molecules exert their stimulatory effects via TLR7, inducing cytokine production, and up-regulating co-stimulatory molecules and MHC I/II in DCs [49]. Resiquimod is also recognized by TLR8. Apparently, activation of immune cells via TLR7 requires endosomal maturation, while TLR8 activation does not [49]. Imidazoquinolines stimulate in DCs the production of IL-12 and high levels of IFN- α , cytokines with potent anti-viral and anti-tumor activities, as well as other pro-inflammatory Th1 cytokines [50]. It has been shown, using MyD88- and TLR7-deficient mice, that these synthetic compounds activate immune cells via a TLR7-MyD88-

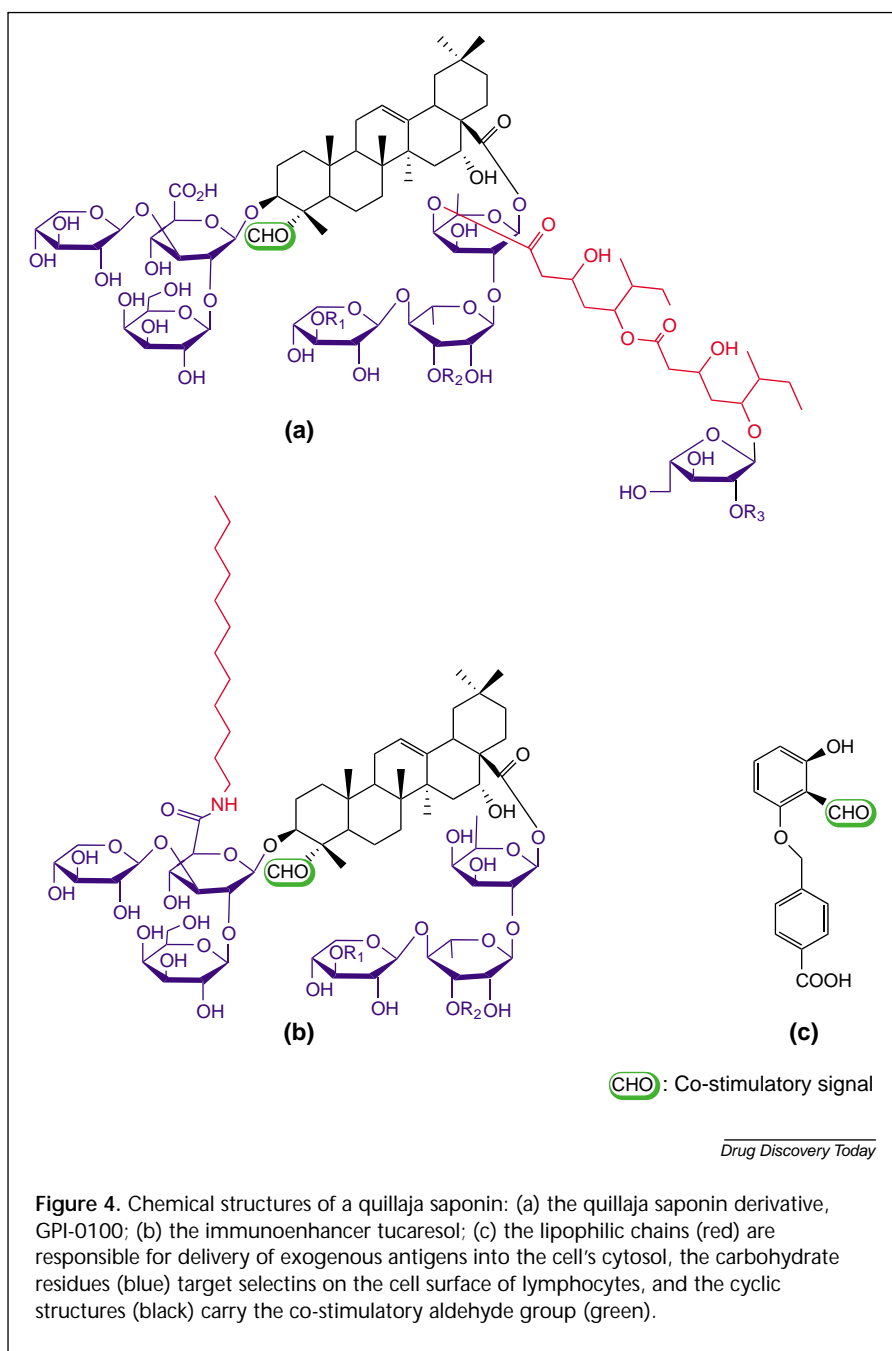


Figure 4. Chemical structures of a quillaja saponin: (a) the quillaja saponin derivative, GPI-100; (b) the immunoenhancer tucaresol; (c) the lipophilic chains (red) are responsible for delivery of exogenous antigens into the cell's cytosol, the carbohydrate residues (blue) target selectins on the cell surface of lymphocytes, and the cyclic structures (black) carry the co-stimulatory aldehyde group (green).

dependent signaling pathway [51]. Resiquimod is also capable of activating B cells in a manner similar to the signal provided by CD40 ligation, inducing the production of TNF- α and IL-6, both of which contribute to stimulate an antibody response [52]. Imiquimod is being used as a topical treatment for skin lesions caused by the papilloma virus infection (50).

Adaptive immunity adjuvants

Adaptive immunity adjuvants are largely endogenous cytokines (Box 2), plus the acylated quillaja saponins and their

semi-synthetic derivatives (GPI-0100) [6], and low-m.w. imine- or Schiff base-forming molecules [53]. Although the last three adjuvants stimulate Th1 immunity, only quillaja saponins (Figure 4a) and GPI-0100 (Figure 4b) facilitate CTL production against exogenous antigens [54,55]. Quillaja saponins have a triterpene nucleus with an aldehyde and two oligosaccharide chains, one of which is acylated by two coupled lipophilic aliphatic acids linked by an ester bond to a fucose (Figure 4a) [6,54]. Several aldehydes (e.g. tucaresol; Figure 4c) form imines with ϵ -amino groups of certain T cell surface receptor(s), providing the co-stimulation needed (besides TCR signaling) for T cell activation independent of B7-1 ligands [53,56]. Imine formation results in changes in Na^+ and K^+ transport that, together with TCR signaling via the mitogen activated protein kinase (MAPK) ERK2 and increased Ca^{2+} mobilization, favors higher IL-2 and IFN- γ production, and higher Th1 immunity [57]. Although quillaja saponins require aldehydes to stimulate Th1 immunity

[54], their deacylated saponins do not stimulate Th1 immunity or CTL production, but rather, Th2 immunity [58]. The lipophilic moiety appears to enhance aldehyde availability to T cells, and enables delivery of exogenous antigens to APCs for processing by the endogenous pathway, thus, yielding Th1 immunity with CTL production. In saponins, the antigen processing and presentation that are crucial for CTL production appear to require only macrophages, not CD4 $^+$ helper T cells [59]; whereas, deacylated saponin-antigen complexes will bind to APC lectins and be taken up by endocytosis, to yield Th2-associated antibodies. Although quillaja saponins are effective adjuvants, they have two drawbacks: toxicity and instability, this instability resulting in a change in the type of immunity stimulated by the vaccines [58].

By contrast, the new semi-synthetic saponin analogs (GPI-0100), carrying a lipophilic chain (dodecylamide) bound to the glucuronic acid residue (Figure 4b), are stable, safe and stimulate Th1 immunity with CTL production [55,60]. The presence or absence of the lipophilic chain is decisive as to which immunity is elicited; Th1 or Th2, respectively. Intranasal immunization with GPI-0100 vaccines stimulates mucosal and systemic immunity [61],

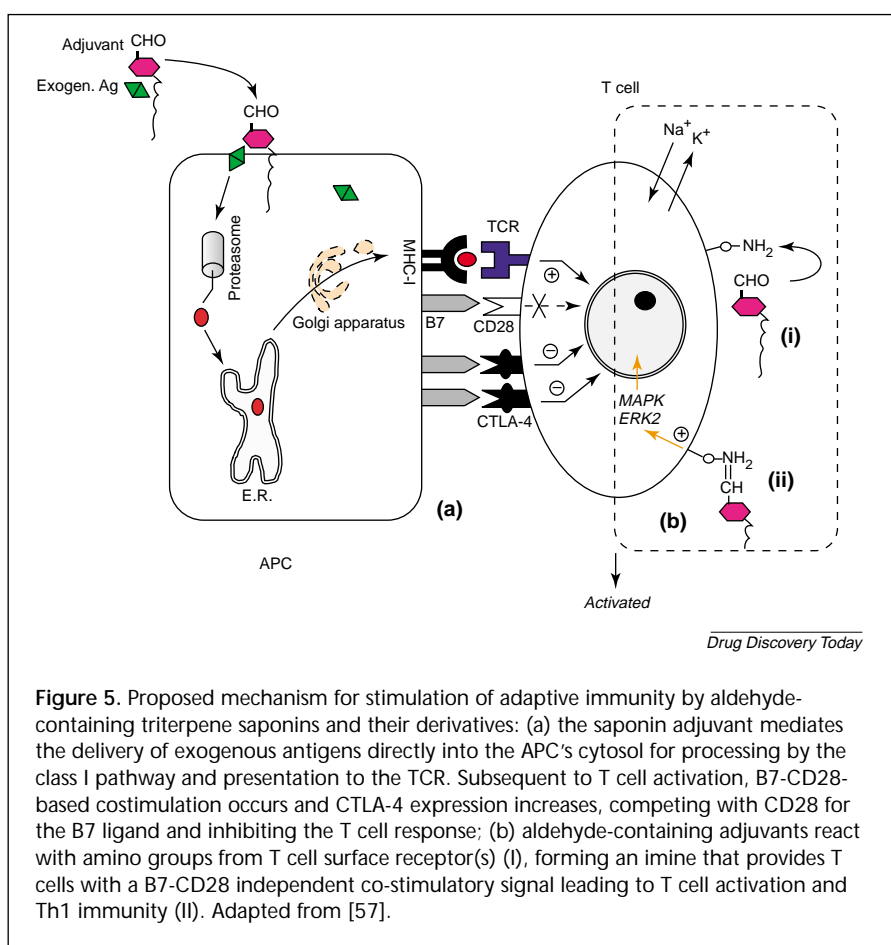
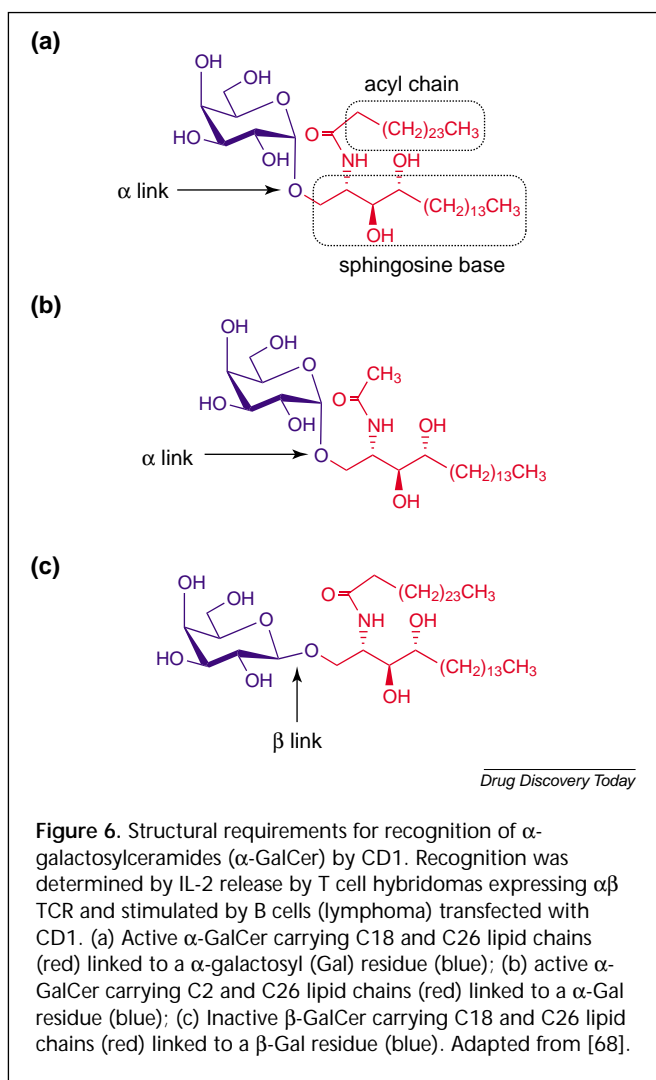


Figure 5. Proposed mechanism for stimulation of adaptive immunity by aldehyde-containing triterpene saponins and their derivatives: (a) the saponin adjuvant mediates the delivery of exogenous antigens directly into the APC's cytosol for processing by the class I pathway and presentation to the TCR. Subsequent to T cell activation, B7-CD28-based costimulation occurs and CTLA-4 expression increases, competing with CD28 for the B7 ligand and inhibiting the T cell response; (b) aldehyde-containing adjuvants react with amino groups from T cell surface receptor(s) (I), forming an imine that provides T cells with a B7-CD28 independent co-stimulatory signal leading to T cell activation and Th1 immunity (II). Adapted from [57].

effective against pathogens entering via mucosal membranes. GPI-0100 acts on both APCs and T cells, enabling antigen delivery to APCs for endogenous pathway processing, and providing a B7-1-nondependent co-stimulation to T cells that is free from CTLA-4 blocking (Figure 5). In confirmation of this property, an anti-CTLA-4 monoclonal antibody does not modify the GPI-0100-stimulated immune response [62], thus, it would be feasible to use GPI-0100 to overcome the downregulation of Th1 immunity by CTLA-4 [63,64] during active immunotherapy.

α -galactosylceramide

The glycolipid α -galactosylceramide (α CG) and its analogs are a unique class of immunoenhancers (Figure 6) that, when presented by the MHC class I-like molecule CD1d, are recognized by the $\alpha\beta$ TCRs of natural killer T cells (NKT cells), which are activated by the production of IL-4 and IFN- γ [65]. Studies using soluble forms of CD1d and $\alpha\beta$ TCR have shown that glycolipids bind to CD1d with low affinity and specificity, via hydrophobic interactions to form a complex [66,67]. This CD1d-glycolipid complex then binds to the $\alpha\beta$ TCR with high affinity, using rigid interacting surfaces apparently involving the glycolipid's sugar



moiety and invariant V α 14 chain of the TCR [66,67]. In effect, different lipids with one or two chains bind to $\alpha\beta$ TCR, however, the α -anomeric conformation of the sugar moiety is essential for binding (Figure 6) [68]. After initial activation, the NKT cells become polarized for the production of IL-4 to promote differentiation of naive antigen-specific CD4⁺T cells into Th2 cells, and to direct the adaptive immunity toward Th2 immunity [65]. Thus, α -galactosylceramides can be useful in the stimulation of Th2 immunity against extracellular pathogens, or in achieving protection from inflammatory diseases that are characterized by a pathogenic Th1 immunity [69].

The future of adjuvants in vaccine development

Despite the libraries of recombinant antigens available for use as vaccines, the only human viral recombinant sub-unit vaccine is hepatitis B. This lag in vaccine development reflects the use of adjuvants that elicit only Th2 immunity, such as alum, and a poor understanding of the adjuvant's

crucial role in immunomodulation. However, discoveries of innate and adaptive immunity receptors and their mechanisms of action, and access to structurally well-defined compounds, enable the rational design of novel Th1 or Th2 immunity adjuvants [6,47,69]. Compounds that stimulate immune responses characterized by production of Th1-cytokines, antibodies mediating ADCC, and CTLs against soluble antigens, will be important in the development of new preventive and/or therapeutic sub-unit vaccines against infectious diseases and cancer [70,71]. The effects of different adjuvants or modified adjuvants interacting with specific TLRs or co-stimulatory receptors can be either cumulative or synergistic, therefore, adjuvant combinations and/or modifications could yield improved immunity with the induction of specific responses, for example, CTL against virally infected cells [72].

As opposed to live vaccines, where the immune response depends on an individual's immunocompetence, new sub-unit vaccine adjuvants would be able to modulate the immune system to stimulate a safe and effective response under even immunodeficient conditions related to aging, certain congenital defects, or undergoing immune suppressive therapy. For these populations, which are extremely susceptible to infections and not eligible for immunization with live vaccines, the new adjuvants would provide safe and effective sub-unit vaccines. These new adjuvants or immune enhancers could be also used as therapeutic agents to switch adaptive immunity, for example, CpG and α -galactosylceramide, to Th1 and Th2, respectively. Co-stimulatory adjuvants, such as GPI-0100, could bypass blocking of Th1 immunity by either expression of CTLA-4 receptors or down-regulation of B7-1 ligand during cancer immunotherapy, while maintaining effective immunity. Availability of new adjuvants that are capable of stimulating both mucosal and systemic immunities would facilitate the development of sub-unit vaccines against pathogens that infect via the mucosal membranes from the urogenital, respiratory and digestive tracts. Adjuvants would also be useful in DNA vaccines, where stimulation of immunity is subjected to the same limitations that are described for natural infections and down-regulated by CTLA-4 [73]. Finally, the availability of new adjuvants would enable the large body of information derived from genomics to be effectively exploited in the development of novel vaccines.

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