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Pattern Recognition Receptors Based Immune Adjuvants: Their Role and Importance in Vaccine Design

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1. Introduction

Infectious, cancer and allergic diseases have always been scourge for humans and. disease prevention through immunization has been the most cost-effective health-care intervention available. Immunization has been a great public health success story. As immunization helps to inhibit the spread of disease, many people can be protected from illness and death. It has been proved beyond doubt that with the exception of pure drinking water, no other human endeavor rivals immunization in combating infectious diseases. Millions of lives have been saved, with considerably reduced mortality rates, millions have the chance of a longer healthier life. The purpose of prophylactic vaccination is to generate a strong immune response providing long term protection against infection. Vaccines have been described as weapons of mass protection as they mainly capitalize on the immune system's ability to respond rapidly to pathogens and eliminate them. The considerable success achieved in the eradication of smallpox and the reduction of polio, measles, pertussis, tetanus and meningitis, were among the most notable achievements of the 20th century (Wack and Rappuoli 2005). Unfortunately, for today's societal dreadful diseases which are major causes of morbidity and mortality, there are no effective vaccines. Some of the existing vaccines do not induce complete protection and therefore, the development of effective vaccines towards these diseases is needed. In this chapter, an attempt has been made to explain the role of pattern recognition receptors (PRR)based immune adjuvants for the development of safe and effective vaccines. We have also discussed the recent advances in the therapeutic and prophylactic application of PRR agonist and antagonists for the treatment of infectious diseases and cancer,. This topic was extensively studied in last one decade and thousands of high quality publication and high quality reviews are reported in the literature.

2. Need for immune adjuvants

Traditional vaccines mainly consisted of live attenuated pathogens, whole inactivated organisms, or inactivated bacterial toxins. Many traditional vaccines based on pathogen whole cells often contain components that can cause toxicity related side effects. As a result

of these safety limitations of conventional vaccines, several new approaches to vaccine development have emerged that may have significant advantages over more traditional approaches. These approaches include 1) recombinant protein subunits 2) synthetic peptides and 3) protein polysaccharide conjugates. By contrast, these vaccines although offering considerable advantages over traditional vaccines in terms of safety and cost of production, in most cases they have limited immunogenicity and therefore are used less as prophylactic or therapeutic vaccines on their own. These pitfalls have intensified the search of external agents that can synergistically boost the immune response of otherwise weakly immunogenic subunit vaccines. Such molecularly defined immune boosters, popularly known as *adjuvants*, ideally should constitute a non-immunogenic entity, however, able to stimulate humoral and cellular immunity in presence of a vaccine antigen and most importantly, being non-toxic, suitable for animals and human use.

3. Immune system: Innate and adaptive

The immune system in higher animals can be broadly classified into the innate and the adaptive immune systems (Janeway 2001; Janeway and Medzhitov 2002). The innate immune system was long thought to be a non-specific inflammatory response generated during exposure to foreign antigen. However, studies conducted in recent years indicate the innate immune response is able to discriminate between pathogen classes and direct innate and adaptive immune responses toward elimination of the invading pathogen (Akira, Uematsu et al. 2006; Hoebe, Jiang et al. 2006; Sansonetti 2006). The discovery of pathogen-associated molecular patterns (PAMPs) and pattern recognition receptors (PRRs) and the role they play in elimination of pathogen and activity as adjuvant has renewed interest in the importance of the innate immune system (Hopkins and Sriskandan 2005). Improved understanding of innate immunity in recent years, has led to the identification of immune pathways and adjuvant formulations more suitable for clinical advancement. The 2011 Nobel Prize for Medicine was awarded to three scientists who have done more than anyone to lay bare the two-tier structure of the immune system. One area of particular interest is the discovery of agonists that target the PRRs. Adaptive immune responses are essential for the control of pathogens that escape elimination by the innate immune response (Schwartz 2000). Because of its role in immune memory, the adaptive immune system's contributions to pathogen elimination and vaccine development have been widely studied. Adaptive immunity mediates a delayed, specific response to foreign antigen while innate immunity is not antigen specific and develops immediately following exposure to immune stimuli i.e., pathogens.

4. Pattern recognition receptors

The Pattern Recognition Receptors (PRRs) of the innate immune system serve an essential role in recognition of pathogen and directing the course as well as type of innate immune response generated following exposure to foreign antigen. PRRs are differentially expressed on a wide variety of immune cells (Iwasaki and Medzhitov 2004). Engagement of PRRs invokes the cascades of intracellular signaling events that further induce many processes such as activation, maturation and migration of other immune cells and the secretion of cytokines and chemokines (Hoebe, Janssen et al. 2004; Medzhitov 2007; Kumar, Kawai et al.

2009; Blasius and Beutler 2010; Kawai and Akira 2010; Takeuchi and Akira 2010). This creates an inflammatory environment in tandem, that leads to the establishment of the adaptive immune response (Iwasaki and Medzhitov 2004). PRRs consist of non-phagocytic receptors such as **toll-like receptors (TLRs)** and **nucleotide-binding oligomerization domain (NOD) proteins** and receptors that induce phagocytosis such as **scavenger receptors, mannose receptors** and **β -glucan receptors**. In last decade, several natural, natural derived (PAMPs) and synthetic ligands of PRR belonging to the diverse structural class have been identified (Kumar, Kawai et al. 2011) and reported in the literature possessing potential immunomodulatory properties. In spite of thousand of molecules identified as potential PRR agonist properties, a hand full number of these are now in clinical or late preclinical stages of development as immune adjuvant for vaccines (Kanzler, Barrat et al. 2007; Makkouk and Abdelnoor 2009; Mbow, Gregorio et al. 2010; Basith, Manavalan et al. 2011). Various vaccine R&Ds and research group around the world are currently exploring the use of natural ligands or synthetic ligands as well-defined PRRs as adjuvants, either alone or with as formulations with other ingredients for various subunit vaccines being developed against cancers, infectious and allergic diseases. Furthermore, TLR antagonists derived from the modifications of natural ligands also appear quite promising for a number of inflammatory and autoimmune diseases.

4.1 Toll like receptors

Vaccine adjuvants are perhaps the most extensively explored applications for TLR agonists. In last decade, efforts an increasing focus has been to use natural ligands or synthetic agonists for well-defined TLRs as adjuvants, either alone or with various formulations. A number of these are now in clinical or late preclinical stages of development for multiple applications and have been the subject of research to clarify the basis of their adjuvant activity. TLR are type I membrane glycoproteins, characterized by a cytoplasmic Toll/interleukin-1 receptor homology (TIR) signaling domain and an external antigen recognition domain comprising 19–25 tandem leucine-rich repeat (LRR) motifs (Rock, Hardiman et al. 1998). TLRs were initially discovered in fruitfly, *Drosophila melanogaster*, and have been defined as factors involved in the embryonic development (Lemaitre, Nicolas et al. 1996; Hoffmann 2003) and resistance of the fly *Drosophila* to bacterial and fungal infection. Being a major component in innate immunity TLRs are known to play a significant role in innate-adaptive cross talks (Pandey and Agrawal 2006; Rezaei 2006; Kanzler, Barrat et al. 2007; Romagne 2007). First human TLR was discovered in 1997 by Medzhitov *et al.* and after that research in this field has exploded so rapidly that all TLRs have been cloned and many of their ligands (PAMPs) and associated signaling pathways have been identified. TLRs recognize broad classes of PAMPs and are emerging as a central player in initiating and directing immune responses to pathogens. Till date, ten TLR (TLR1–10) are reported in humans and subdivided according to their localization in cell compartments. TLR1, 2, 4, 5, 6 and 10 are expressed on the cell surface and recognize PAMPs derived from bacteria, fungi and protozoa. TLR3, 7, 8 and 9 are expressed in intracellular compartments with the ligand-binding domains sampling the lumen of the vesicle and recognize nucleic acid PAMPs derived from various viruses and bacteria (Janeway and Medzhitov 2002; Akira and Hemmi 2003; Akira, Uematsu et al. 2006). Generally, natural ligands of TLR fall into three broad categories: **lipids and lipopeptides**

(TLR2/TLR1; TLR2/TLR6; TLR4), **proteins** (TLR5) and **nucleic acids** (TLR3, 7, 8, 9). TLRs forms both homo- and heterodimers to enable functioning and downstream signaling activation resulting in ligand recognition (PAMPS) of diverse structure from various sources. TLR2 preferentially forms heterodimers with either TLR1 or TLR6, whereas the other TLRs appear to associate as homodimers. Various natural (microbial) and synthetic ligands of functional TLRs as well as cellular localization of TLRs are discussed in Table 1.

PRR (Cellular localization)	Microbial Ligands	Synthetic Ligands
TLR1/TLR2 (Cell surface)	Triacyl lipopeptide (Pam3CSK4)	Pam2CSK/Pam3CSK4 analogues
TLR2/TLR6 (Cell surface)	Diacyl lipopeptides (Pam2CSK4), Lipoteichoic acid, Zymosan, porins, MALP2, Bacterial peptidoglycan, Lipoarabinomannan	Pam2CSK/Pam3CSK4 analogues
TLR3 (Endosome)	ssRNS and dsRNA virus, Respiratory syncytical virus, Mmurine cytomegalovirus	Poly I:C; poly A:U
TLR4 (Cell surface)	LPS; Mannan; Phospholipids; Envelope proteins (MMTV, RSV)	Monophosphoryl lipid A and its analogues
TLR5 (Cell surface)	Flagellin	---
TLR7 (Endolysosome)	ssRNA (viral), RNA from bacteria from group B streptococcus	GU-rich oligoribonucleotides; Loxoribin; Imiquimod; Resiquimod; Adenosine and Guanosine derivative
TLR8 (Endolysosome)	ssRNA (viral)	GU-Rich oligoribonucleotides; Adenosine and Guanosine derivative; Resiquimod
TLR9 (Endolysosome)	DNA (bacterial/viral)	Deoxynucleotides with unmethylated CpG motifs
TLR10 (Cell surface)	Unknown	----

Table 1. Pattern Recognition Receptors and their ligands

Ligand binding to TLR appears to result in conformational changes and possibly dimerization, leading to recruitment of crucial adaptor proteins. These Toll/interleukin-1

receptor homology (TIR) domain-containing molecules include myeloid differentiation primary-response protein 88 (MyD88), used by nearly all TLR, TIR domain-containing adaptor protein (TIRAP), TIR domain-containing adaptor protein inducing interferon (IFN)- β (TRIF), and TRIF-related adaptor molecule (TRAM). Engagement of these adaptors activates a series of signal transduction molecules including interleukin (IL)-1R-associated kinases (IRAKs), tumor necrosis factor receptor (TNFR)-associated factor 6 (TRAF6), transforming growth factor (TGF)- β -activated kinase (TAK1), and the inhibitor of nuclear factor- κ B (I κ B)-kinase complex. These events lead ultimately to activation of mitogen-activated protein (MAP) kinases and nuclear translocation of the transcription factor NF- κ B, key regulators of many inflammatory response pathways. A second discrete pathway, used by intracellular TLR, leads to activation of IFN regulatory factors (IRF), particularly IRF-7, leading to high levels of type I IFN production. Differential adaptor use by different TLR and cell type-specific signaling pathways leads to many variations on this theme. Thus, the response to TLR signaling can include cell differentiation, proliferation or apoptosis, as well as induction of many secreted mediators, prominently IFNs, TNF- α , IL-1, IL-6, IL-10, IL-12, and many different chemokines. The responses produced by activation through a TLR are determined by many factors specific to individual cell types, as well as to quantitative and qualitative parameters of the TLR-ligand interaction itself.

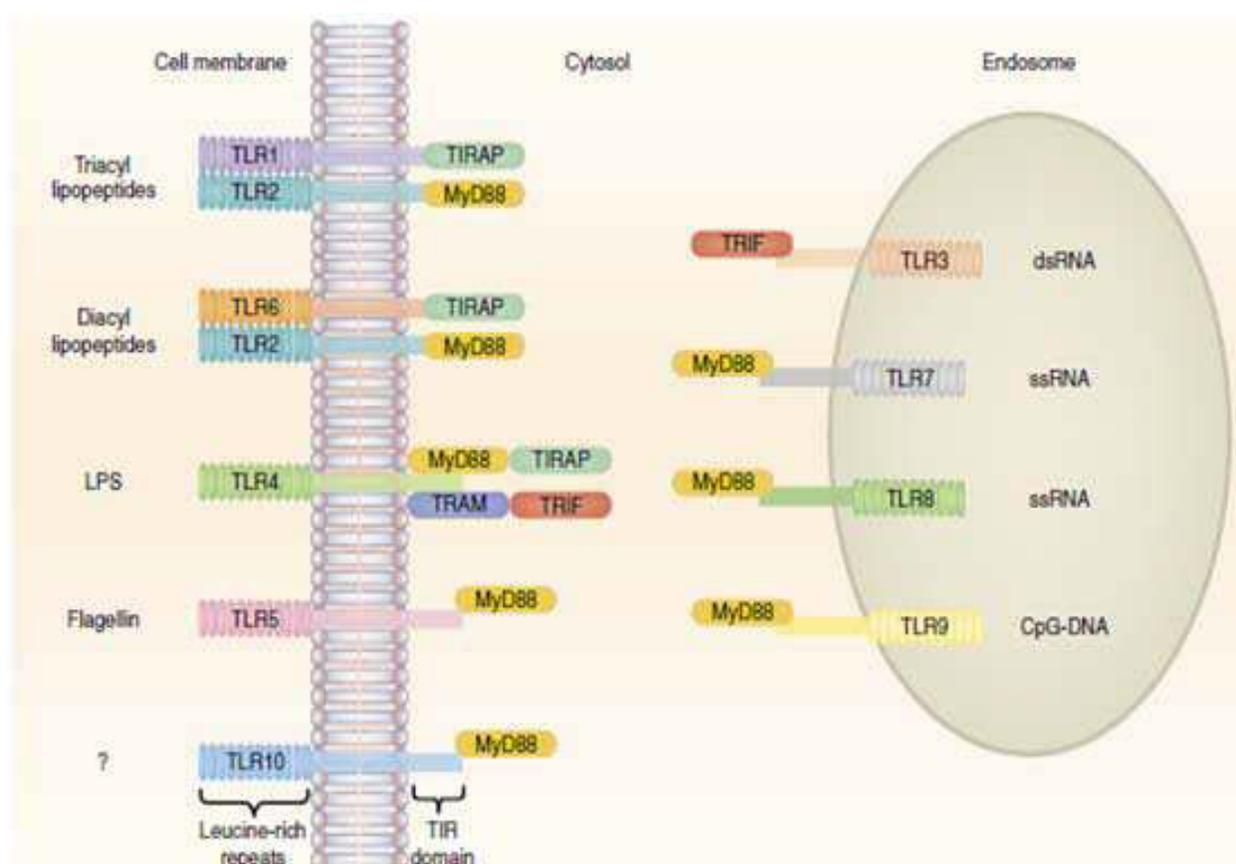


Fig. 1. Schematic diagram of human Toll-like receptors showing adaptors, cellular orientation and complimentary ligands. Source: Holger Kanzler *et al. Nature Medicine* 2007, 13, 552.

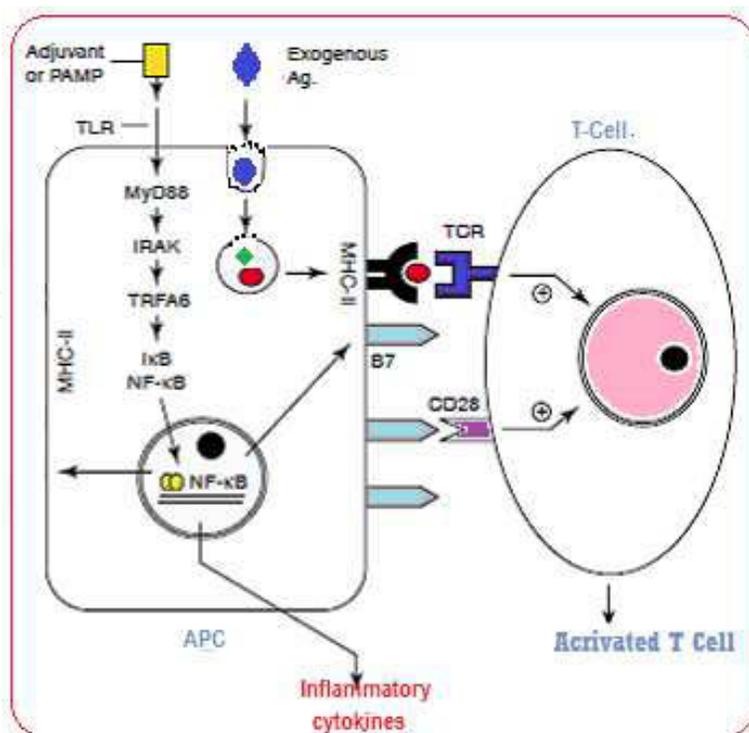


Fig. 2. Stimulation of innate immunity and induction of adaptive immunity by PAMPs or PAMP-related adjuvants.

Redundant and non-redundant functions of TLRs are responsible for the adjuvant activity required to elicit immune responses both in natural infection and in vaccine responses (Lien and Golenbock 2003). They have a distinct function in pathogen recognition and constitute good targets for rational adjuvant development. Table 1 shows, TLRs recognize groups of widely distributed and structurally similar molecules, in contrast to the highly selective molecular-level recognition of T- and B-cell receptors. Synthetic ligands with varying degrees of similarity to natural ligands have been described for most of the TLR. Therapeutic applications to date have used either native or synthetic versions of natural TLR ligands with optimized pharmacologic properties (Kanzler, Barrat et al. 2007; Makkouk and Abdelnoor 2009; Basith, Manavalan et al. 2011).

4.2 Small molecules TLR agonists and antagonists derived from PAMPs

A number of TLR agonist and antagonists currently under investigation are either PAMPs or PAMPs derived molecules such as DNA motifs analogs, monophosphoryl lipid A analogs, muramyl dipeptide analogs, nucleic acid analogs etc. Here, we have presented a brief description of representative clinically potential agonists and antagonists and their pharmacophores responsible for stimulating immunological response.

4.2.1 Lipopolysaccharide, lipid A and monophosphoryl lipid A

The adjuvant effect of lipopolysaccharide (LPS) or endotoxin was described in 1956 (Johnson, Gaines et al. 1956). LPS or endotoxin component of gram negative bacteria has a hydrophilic polysaccharide and lipophilic phospholipids which is responsible for adjuvant

activity (Gupta, Relyveld et al. 1993). The active agent of LPS has been shown to be lipid A - a disaccharide composed of two glucosamine units, two phosphate groups and five or six fatty acid chains (generally C12 to C16 in length). Lipid A 1 is a potent adjuvant for both protein and carbohydrate antigens, and can lead to marked increases in both humoral and cell-mediated immunity (Azuma 1992). Although LPS as a component of whole cell vaccines against pertussis, cholera and typhoid has been used in humans for many years, its extreme toxicity precludes its use as an adjuvant in humans (Johnson, Keegan et al. 1999). Attempts have been made to detoxify LPS and lipid A without affecting its adjuvanticity. The most promising derivative of lipid A is monophosphoryl lipid.

Monophosphoryl lipid A (MPLA, Fig 3) 1a has been shown to exhibit potent adjuvanticity, without exhibiting significant toxicity. Structural activity relationship of the MPL shown that a hexaacylated $\beta(1\rightarrow6)$ -diglucosamine having three 3-n-alkanoyloxytetradecanoyl residues or six fatty acid groups is required for adjuvanticity. Careful structure examination of lipid A analogs suggests that the type and length lipid play a very crucial role in determining the activity towards stimulation (agonist) or inhibition (antagonist). Lipid A analogs having β -alkanoyl lipid having longer chain length shown agonist activity and lipid A analogs with shorter chain length shown antagonist activity. Both LPS and MPL exhibited adjuvant activity by triggering a signaling through TLR4 (Kaisho and Akira 2002; Re and Strominger 2002), but MPLA leads to downstream signaling only through the TRIF adaptor, whereas the LPS leads to TLR4 activation through both the TRIF and MyD88 pathways, the latter pathway resulting in the high level of inflammatory cytokines, prominently TNF- α . On the other hand, MPLA activation leads to the induction of IFN- β and regulation of CD80/CD86, which is a key aspect of adjuvanticity. Three MPLA and its analog containing vaccine formulations have already been approved (Kanzler, Barrat et al. 2007; Makkouk and Abdelnoor 2009; Basith, Manavalan et al. 2011) for various diseases such as *Fendrix* (by GSK) for Hepatitis B, *Cervarix* (by GSK) for cervical cancer and *Pollinex quattro* (by Allergy Therapeutics) for allergic rhinitis and have proven to be both safe and effective. Similarly another synthetic lipid A mimetics structure known as aminoalkyl glucosaminide phosphates (AGPs) also entered clinical studies and one of the AGPs known as RC-529 (1c, structure shown in Fig 3) developed by Dynavax Technologies has been approved for hepatitis B vaccine *Supervax*. Similarly, CRX-675 (Aminoalkyl-glucosamine-4- phosphate of unknown structure; may be identical or similar to RC529, Table 4) developed by Corixa also find clinical application and currently in phase-I for allergen rhinitis. Other lipid A analogs as TLR4 antagonists such as CRX-526 and others are in preclinical studies for inflammatory diseases. Many lipid A analogs containing vaccine formulations are in preclinical and different stage of clinical trial for cancer, infectious and allergic diseases as given in Table 2, 3, 4. Merck has developed an innovative cancer vaccine known as *Stimuvax* containing MLP as adjuvant along with MUC1 a protein antigen to treat cancer because it is widely expressed in common cancer, and is currently undergoing phase-III clinical trial. Researcher also developed lipid A analog as TLR4 antagonists which find important application for the treatment of various autoimmune and inflammatory diseases. E-5564 (*Eritoran*) is a lipid A mimics developed by Eisai Pharmaceuticals and currently in phase-III trial for severe sepsis. From this discussion, it is evident that different lipid A analogs act differently and find useful in the treatment of hepatitis B, cancer, allergic and inflammation diseases (Kanzler, Barrat et al. 2007; Makkouk and Abdelnoor 2009; Basith, Manavalan et al. 2011).

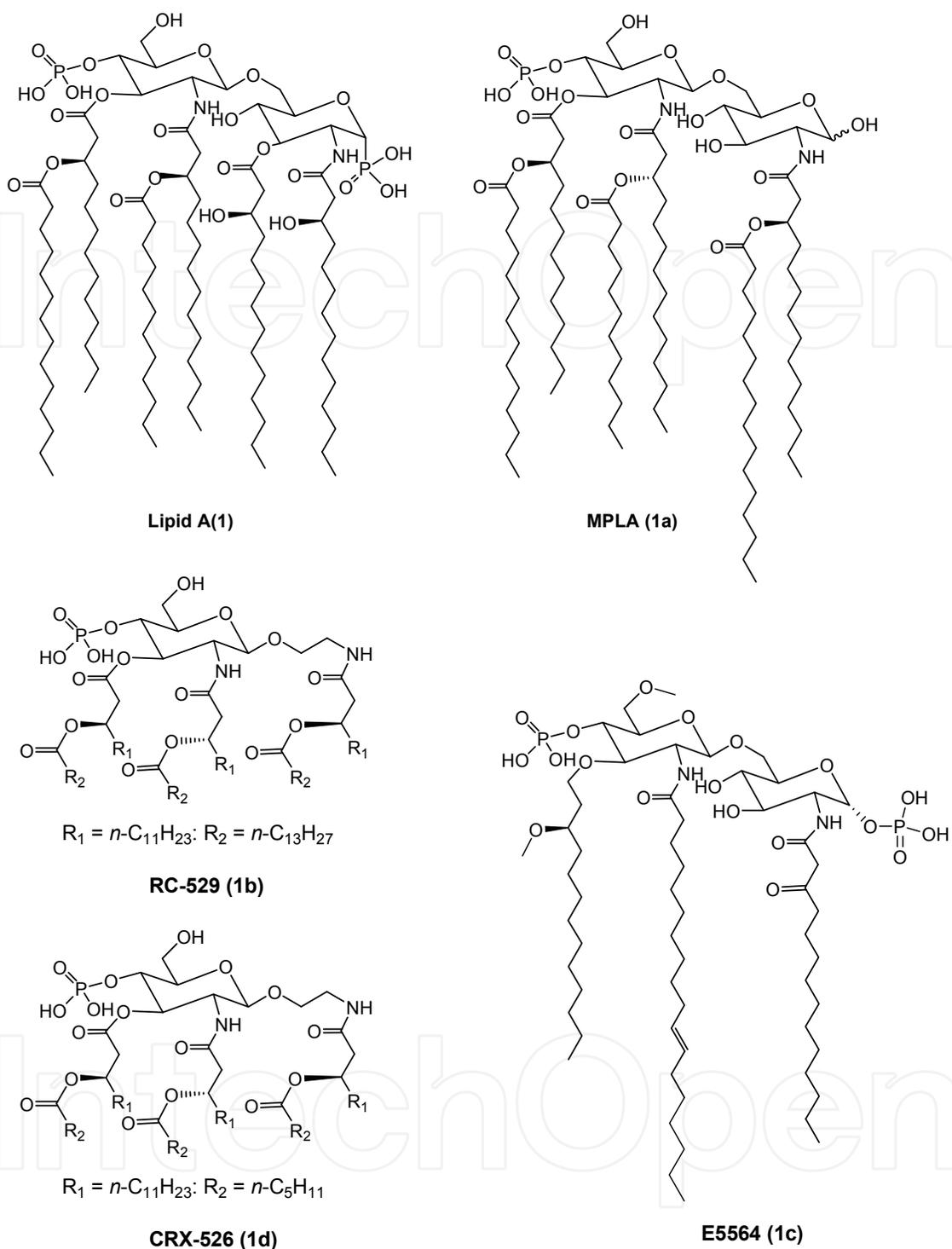


Fig. 3. Structure of Lipid A and their synthetic analogs as TLR4 agonist and antagonists

4.2.2 Imidazoquinolines and guanosine containing compounds

Guanosine- and uridine-rich ssRNA were first identified as natural agonists for TLR7 and 8 and because of their degradation by RNases limited their uses as immune adjuvants. In search of stable and robust small molecule TLR7 and 8 agonists lead to the discovery of imidazoquinolines and guanosine and adenine analogs (Fig 4). Imidazoquinolines such as

Imiquimod **2a**, Resiquimod **2b** are synthetic low-molecular weight TLR7/8 agonists are structural mimics of DNA or RNA oligonucleotides (Gibson, Lindh et al. 2002; Stanley 2002; Lee, Chuang et al. 2003). Imiquimod activate TLR7 while Resiquimod activate either TLR7, TLR8 or both. Imiquimod represent the most promising TLR7 agonist and 3M Pharma developed formulation containing Imiquimod as 5% cream (Aldara™) approved for the treatment of genital warts, superficial basal cell carcinoma, actinic keratoses and lentigo maligna and also been used for the treatment of human papilloma virus (HPV) associated lesions and cutaneous melanoma. Another structurally related compound, R-848 (Resiquimod, 3M Pharma), is currently in Phase II (Bishop, Hsing et al. 2000) clinical study for the treatment of hepatitis C virus (HCV) and other viral infections (Pockros, Guyader et al. 2007). Similarly guanosine containing compound **2c** and other nucleoside analogs also find promising application for the number of diseases e.g., ANA975 (oral prodrug of isatoribine) was developed as an antiviral HCV treatment, shown promising activity in preliminary level but clinical studies for this were discontinued by Anadys Pharma due to indicated toxicity in the long-term animal studies (Pockros, Guyader et al. 2007).

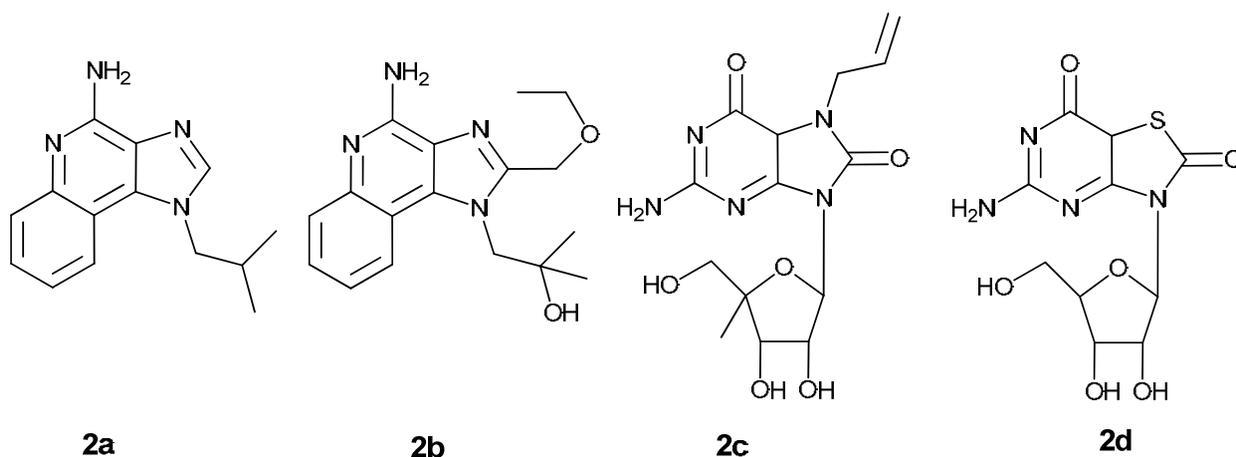


Fig. 4. Structure of imidazoquinolines and other small synthetic compounds

4.2.3 Lipoproteins and lipopeptides

Lipoproteins are part of the outer membrane of gram negative bacteria, gram positive bacteria, *Rhodopseudomonas viridis* and mycoplasma. Bacterial lipoproteins have no shared sequence homology but are characterized by the N-terminal unusual amino acid S-(2,3-dihydroxypropyl)-cysteine acylated by three fatty acids. Synthetic analogues of the N-terminal lipopentapeptide (sLP) **3** of the lipoprotein of *E. coli* proved to be as active as the native lipoprotein (Fig 5). They activate B-cells, monocytes, neutrophils and platelets and act as potent immunoadjuvants *in-vivo* and *in-vitro* (Seifert, Schultz et al. 1990; Wiesmüller, Bessler et al. 1992; Berg, Offermanns et al. 1994; Bessler, Cox et al. 1998; Hoffmann, Heinle et al. 1998). Synthetic lipopeptides with the RR stereoisomer (N-palmitoyl-S-[2,3-bis(palmitoyloxy)-(2R)-propyl]-[R]-cysteine), showed higher B-cell mitogenicity and protective activity when introduced into vaccines than the mixture of other stereoisomers (Wiesmüller, Jung et al. 1989; Wiesmüller, Bessler et al. 1992).

Lipoproteins and lipopeptides induce signaling in immune cells through toll-like receptor-TLR2/TLR1 and TLR2/TLR6 heterodimers. Diacyl lipopeptides like macrophage activating

lipopeptide from *Mycoplasma fermentans* (MALP-2, Pam2Cys-GNNDESNIKFKEK) contain the diacylated lipoamino acid *S*-[2,3-bis(palmitoyloxy)-(2*R*)-propyl]-[*R*]-cysteine require TLR2 and TLR6 for signalling, whereas the triacylated synthetic compound like Pam3Cys-SK4 require TLR2/TLR1 heterodimers for signalling. Structure-activity relationship study supports the fact that the immune modulating activity of lipopeptides is strongly dependent on the fatty acid length and the presence of the natural amino acid *S*-2(*R*)-dihydroxypropyl-(*R*)-cysteine.

Lipopeptide vaccinations have been carried out in all relevant animal models and so far no toxic side effects have been observed. The safety, reproducible production and ease of storage and handling of lipopeptide vaccines suggest that they have significant potential for the development of vaccines for humans and domestic animals. Moreover, several researcher conjugated MHC class-I restricted peptides with Pam3Cys-Ser-Ser resulting in efficient priming of virus-specific cytotoxic T-cells and Tn antigen epitopes.

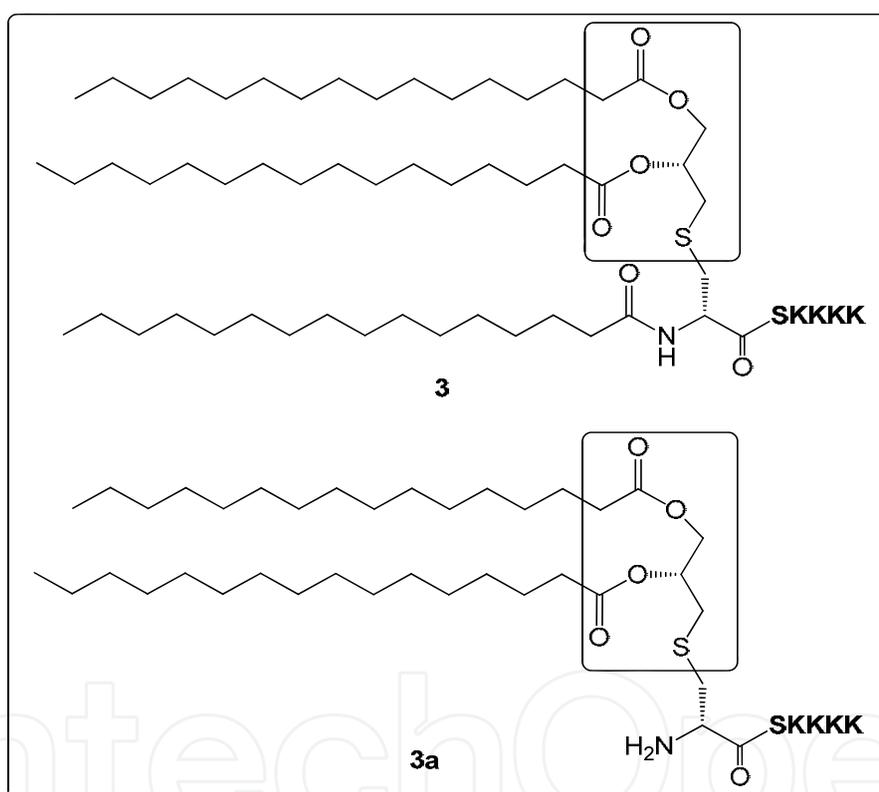


Fig. 5. Structure of lipopetides

4.3 Nucleotide-binding and oligomerization domain (NOD)-like receptor (NLR) proteins

Nucleotide-binding and oligomerization domain (NOD)-like receptor (NLRs) (Fritz, Ferrero et al. 2006; Werts, Girardin et al. 2006; Franchi, Park et al. 2008) represent another family of PRR that received great attention in recent decade and their role in linking host innate immunity to microbes and regulation of inflammatory pathways (Carneiro, Magalhaes et al. 2008) has been extensively studied. In humans the NLR family is composed of 22 intracellular pattern recognition molecules and composed of three different types of domains, a C-terminal LRR domain for ligand binding, a nucleotide binding oligomerization

domain (NOD domain) and an N-terminal effector binding domain for the initiation of signaling. Among all the NLRs, NLRP, NOD1 and NOD2 were extensively studied towards their role in the treatment of inflammatory diseases and in the development of improved vaccine. PAMPs, PAMPs-derived and synthetic ligands that recognize these receptors are presented in Table 1. NOD1 recognizes a molecule called meso-DAP, that is a peptidoglycan constituent of the only gram negative bacteria (Chamaillard 2003; Girardin 2003). NOD2 proteins recognize intracellular MDP (muramyl dipeptide), a peptidoglycan constituent of both gram positive and gram negative bacteria. Whereas NALPRs have been known to detect a range of PAMPs (Hsu; Martinon, Agostini et al. 2004; Boyden and Dietrich 2006; Kanneganti 2006; Kanneganti 2006; Mariathasan 2006; Martinon, Petrilli et al. 2006; Petrilli 2007; Franchi and Nunez 2008; Li, Willingham et al. 2008)

NLR Families

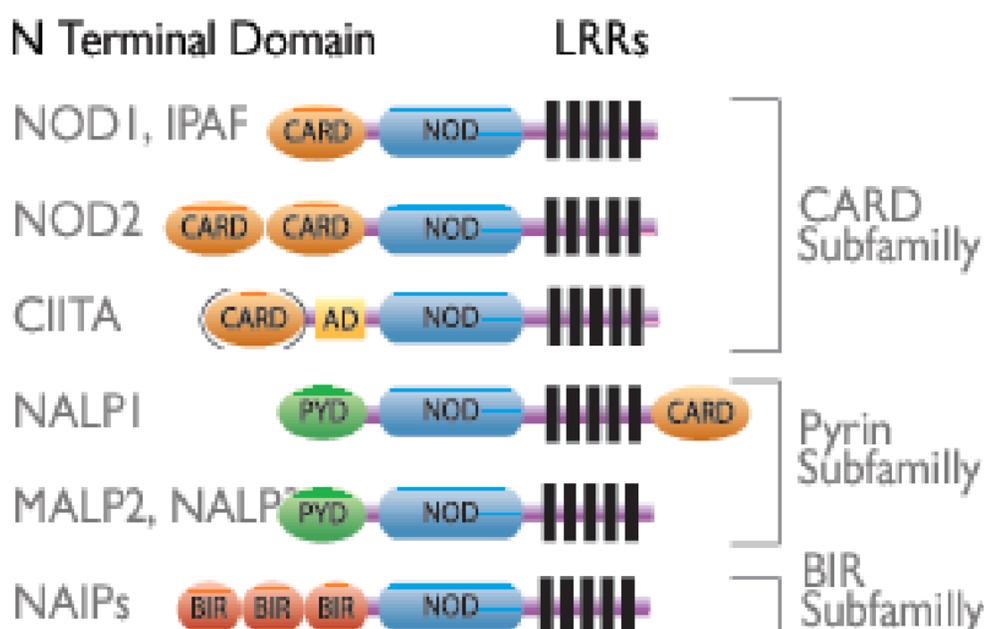


Fig. 6. Nucleotide-binding oligomerization domain (NOD) proteins receptors

4.3.1 Natural and unnatural NOD agonists/antagonists: Muramyl dipeptides

Two major class of compounds *viz.*, bacterial cell wall preparations containing peptidoglycan and inorganic crystals such as aluminium hydroxide (now identified as ligands of NLRs pathway) were extensively used for vaccination strategies throughout the twentieth century represented the strength of this pathway for vaccine and adjuvant development. Furthermore, in recent studies, it has been found that the interaction of NLRs and TLRs are crucial for the adaptive immunity and therefore researchers are looking for the combination strategy by using the ligands of two pathways for the designing of more potent and efficacious immune adjuvants for poor immunogenic vaccines. Although this area is relatively new, but many PAMPs, PAMPs derived and synthetic ligands as well as the role of their receptors in various diseases condition have been identified that will provide very useful inputs for vaccine and adjuvant development.

Eversince the identification of monomeric peptidoglycan subunits in 1970s as minimal structural responsible for the adjuvanticity of complete Freund's adjuvant (CFA) a mixture of NOD1, NOD2 and TLR ligands, which play a central role in the adjuvant action of CFA and therefore their ligands will be explored for the development of effective vaccines. Similarly, *N*-Acetyl muramyl-L-alanine-D-isoglutamine (muramyl dipeptide, MDP) **4** is another component of a peptidoglycan extracted from *Mycobacteria* possessing promising immunostimulatory properties and recently has been found to activate NOD2 (Adam, Ciorbaru et al. 1974). Muramyl dipeptide (MDP) is the minimal unit of the mycobacterial cell wall complex that generates the adjuvant activity of complete Freund's adjuvant (CFA). MDP has a variety of physiological effects, including adjuvanticity, pyrogenicity and leucocytopoietic activity and extensive research has been done on these molecules to understand their role and activation pathway. Despite extensive research on MDP, the molecules was found to be pyrogenic and autoimmunogenic to be used as adjuvants in human. Furthermore, MDP have potent *in-vivo* adjuvant activity when administered as water-in oil emulsions, but MDP itself is a poor adjuvant, due to its rapid excretion in the urine when administered as an aqueous solution. Therefore, efforts towards the synthesis of less pyrogenic derivatives without compromise on their immune stimulatory activity has been attempted. . And as a result, a number of lipophilic derivatives of MDP have been prepared, and their bioactivities have been reviewed (Azuma and Seya 2001). Several MDP derivatives and related compounds such as murametide **4a**, murabutide **4b**, threonyl-MDP **4c**, murapalmitine **4d** and glycoyl-MDP **4e** have host-stimulating activities against bacterial infections in experimental models. Moreover, MDP as well as other muropeptides (tripeptides and disaccharide tripeptides and tetrapeptides) have been found to work in synergy with TLRs and enhance the effect of immunomodulatory factors such as IFN γ , IL-

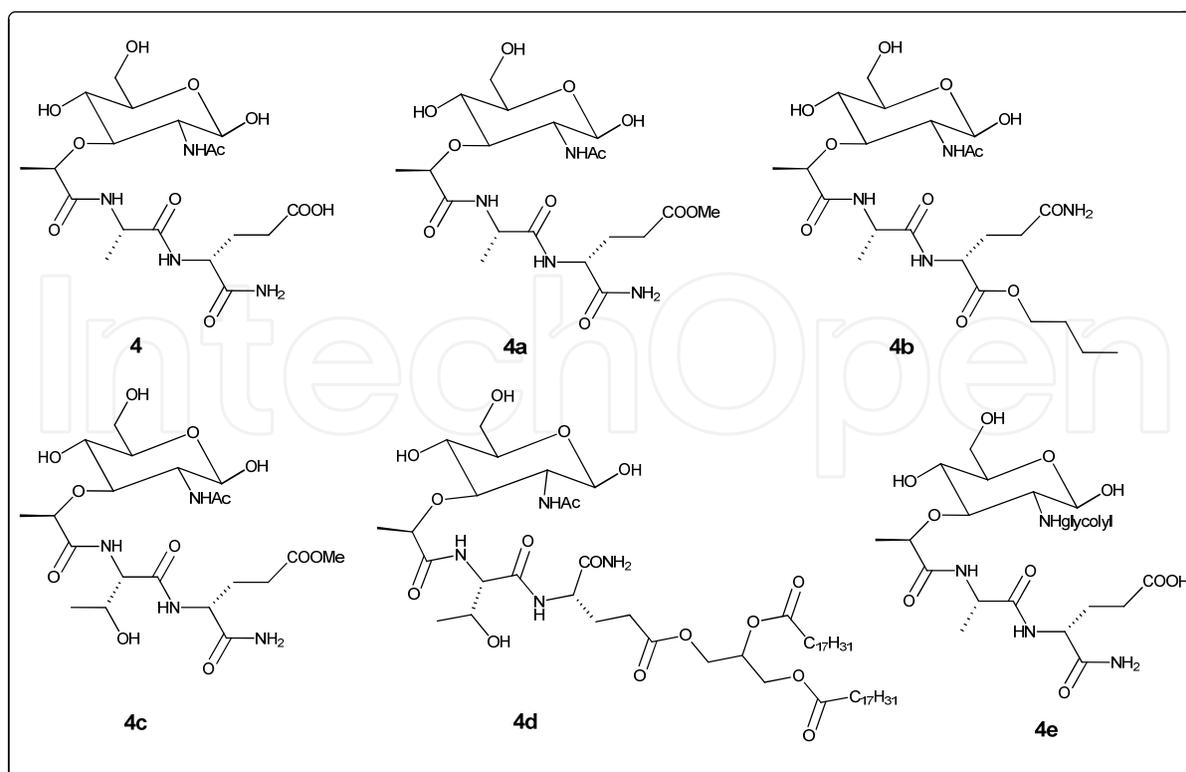


Fig. 7. Structure of MDP and its analogs

1 β , IL-32 and GM-CSF. All these factors are crucial for the recruitment and activation of effector cells as well as for evoking the inflammatory processes which eventually lead to the establishment of an appropriate adaptive immune response, that leads to the increase in the therapeutic potential of NOD2 molecules(Geddes, Magalhaes et al. 2009).

On the other hand, NLRPs recognize wide range of ligands of natural sources but some recent studies shown that some NLRP particularly NLRP3 is an essential component of the inflammasome, it is possible that the activation of NLRP3 as part of the inflammasome is a common event in response to several adjuvants or more generally to particulate compounds such as chitosan (a polysaccharide derived from chitin) and Quil A (a saponin extracted from the bark of *Quillaja saponaria*) as well as silica and asbestos. However, how NLRP3 activation contributes to adjuvanticity is not fully understood. The steadily growing knowledge on NLRs will have a crucial impact on our understanding of the mechanisms of action of immune adjuvants, as well as the pathogenesis and will help direct the development of potent and efficacious immune adjuvants in the near future(Geddes, Magalhaes et al. 2009).

4.4 Endocytic pattern-recognition receptors: Mannose receptors

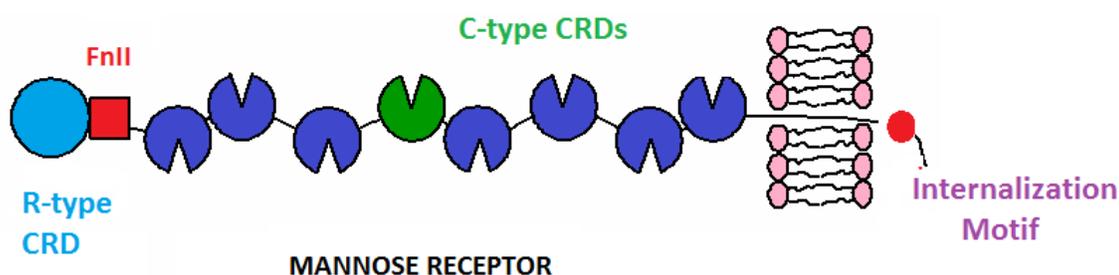


Fig. 8. Mannose receptor-A type 1 transmembrane protein

The mannose receptor (MR) is a PRR primarily present on the surface of macrophages and dendritic cells (Stahl and Ezekowitz 1998) which belongs to the multilectin receptor protein group and, provides a link between innate and adaptive immunity like the TLRs, It is a type I C-type lectin receptor with a long extracellular portion including a N-terminal cysteine-rich domain, a fibronectin type II (FNII) domain, a series of eight C-type lectin-like domains and the carbohydrate-recognition domains (CRDs), which is endowed with the capability to recognize mannosyl-, fucosyl- or N- acetylglucosamidyl-terminal glycoconjugates and sulfated sugars(Taylor and Drickamer 1993). Mannose receptor endocytoses mannosylated motifs for processing and presentation to T cells by MHC class II. Mannosylation of antigen enhances the efficiency of its presentation to T cells. In a variety of antigen delivery approaches, the MR has demonstrated effective induction of potent cellular and humoral immune responses. Therefore, MR-targeted vaccines are likely to be most efficacious *in-vivo* when combined with agents that elicit complementary activation signals.

5. Importance of Th1 immune modulators

The basic knowledge of adjuvant action is very important for developing suitable vaccines for newly emerging cancer and infectious diseases. In the last one decade, much progress has been made on understanding the molecular basis for action of adjuvants, the role of

cytokines and different types of cells involved in immune response and a better understanding of the correlates of immunity to various diseases (Moingeon, Haensler et al. 2001). The induction of Th1 responses is highly desirable for vaccines (Moingeon, Haensler et al. 2001) against chronic viral diseases, infections linked to intracellular pathogens such as *M.TB*, Malaria or cancer (therapeutic vaccines). This leads to the development of adjuvants, which can selectively modulate the immune response and even evoke selective T-cell response alone. Due to limitations of potential adjuvants to elicit cell mediated immune responses such as cytotoxic T-cell responses, there is a need for alternative adjuvants, particularly for diseases in which cell mediated immune responses are important for eliminating intracellular pathogens.

6. Plant based immune adjuvants

The toxicity, adverse reactions, pyrogenicity and reactogenicity associated with synthetic as well as bacterial products limited their development as immunoadjuvants and therefore, in this direction, plants can provide potent, safer and efficacious alternatives. Crude extracts derived from plants have been used as immune-potentiators from the time immemorial in various traditional medicines (Alamgir and Uddin 2010). A traditional Indian system of medicines like *Siddha* and *Ayurveda* suggested that various plants derived *Rasayanas* possess potential immunostimulatory activity (Thatte and Dahanukar 1997). The extracts and formulations prepared from Ayurvedic medicinal plants such as *Withania somnifera*, *Tinospora cordifolia*, *Actinidia macrosperma*, *Picrorhiza kurroa*, *Aloe vera*, *Andrographis paniculata*, *Asparagus racemosus*, *Azadirachta indica*, *Boswellia carterii*, *Chlorella vulgaris*, *Embllica officinalis*, *Morinda citrifolia*, *Piper longum*, *Ocimum sanctum* etc demonstrated significant immunostimulatory activity particularly at humoral level in experimental systems (Patwardhan 2000; Kumar, Gupta et al. (2011). QS-21 a plant based saponin present the finest example of alternate immunoadjuvants isolated from the crude extracts of *Quillaja saponaria* which was known for immunostimulatory properties (Jacobsen, Fairbrother et al. 1996). Similarly, several others single molecules based immune potentiators have been isolated and characterized from the plant sources. Even though these molecules may not operate through the similar mechanism as various PAMPS, the adjuvant effect owing to such amphiphilic natural products is undisputed possibly with low toxicity unlike those derived from PAMPS. There is a major unmet need for a safe and efficacious adjuvant capable of boosting both cellular and humoral immunity. The evaluation and development of plant based immunomodulators, as the alternate adjuvants for providing maximum and lasting protective immune responses with existing vaccines, is justified due to proven safety aspects in comparison with their synthetic counterparts along with excellent tolerability, ease of manufacture and formulation.

6.1 Plant based products currently under investigation

In the last few decades, improved understanding of the mechanism of action of traditional plant based crude extracts and formulations, lead to the discovery of various class of compounds as potential immunostimulators viz., alkaloids, saponins, polysaccharide, triterpenoids, iridoids, organic acids, etc (Alamgir and Uddin 2010). Several plant based products are currently under investigation for use as vaccine adjuvants. Enriched fractions of iridoid glycosides has been isolated from *Picrorhiza kurroa* (a high altitude Himalayan

perennial herb) employed for medicinal purpose from time immemorial to relieve immune related diseases(Puri, Saxena et al. 1992). Several polysaccharides such as mannan and β 1-3 glucan **5** isolated from many plant sources such as *Chlorella sp*, *Tinospora cordifolia* etc. have been known for stimulating and targeting APCs to up-regulate Th1 responses and used as vaccine adjuvants either mixed with or conjugated to immunogen. Picrosides **6** isolated from *Picrorhiza kurroa*, Cardioside **7**isolated from *Tinospora cordifolia* possesses potential immunostimulatory activity(Panchabhai, Kulkarni et al. 2008). Cannabidiol **8** and tetrahydrocannabinol **9** isolated from *Cannabis sativa* significantly attenuated the elevation of IL-2, IL-4, IL-5, and IL-13 and represent the potential therapeutic utility.

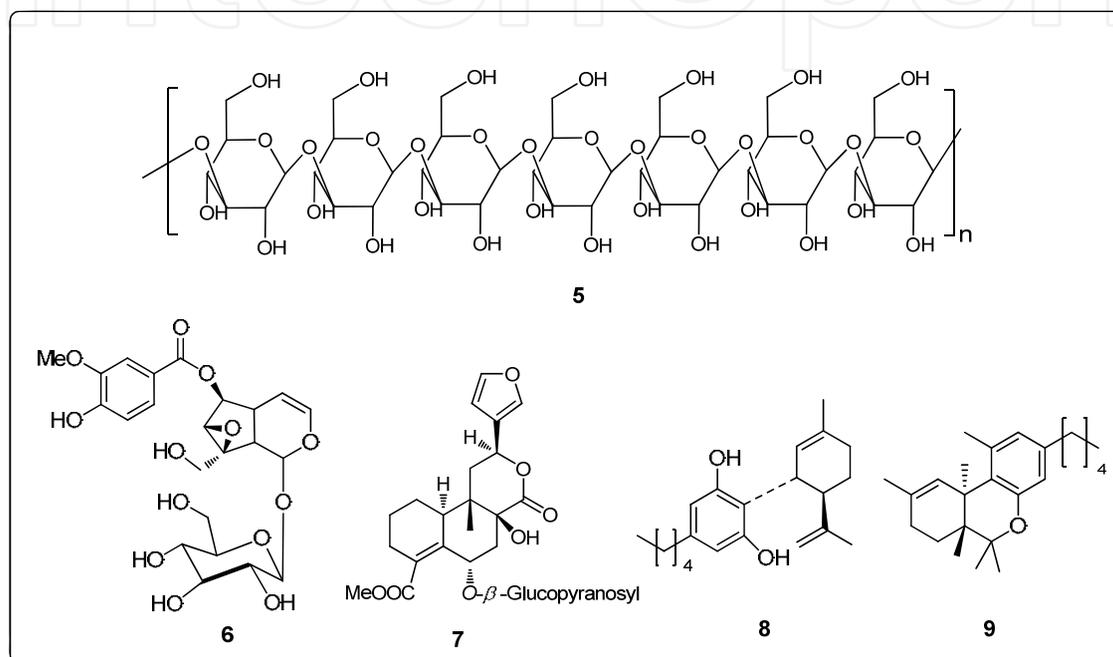


Fig. 9. Structure of plant based immunopotentiators

Despite the long term human use of secondary metabolite enriched fractions of *Picrorhiza kurroa* as potential immunomodulator in traditional medicines, there had been no report regarding the adjuvant activity of the molecular constituents of this valuable plant. While exploring the novel immunoadjuvants derived from *Picrorhiza kurroa*, it was found that many glycoconjugates such as picroside-I, picroside-II and catalpol, possess promising dose dependant immune potentiation ability as indicated by B and T cell proliferation. The single molecules derived from these fractions revealed varying degrees of adjuvant activity. The enriched fractions [RLJ-NE-299A, a mixture of picroside-I (PK-I) and picroside-II (PK-II)] derived from this plant exhibited promising adjuvant activity(Khajuria, Gupta et al. 2007) without significant sustained immune memory or depot formation properties, which restricted their use as plant based immune-adjuvants. In order to develop more potent, efficacious and alternate plant based immune adjuvant, recently acylated analogs of picroside-II viz. PK-II-2, PK-II-3 and PK-II-4 were synthesized and tested for immune-adjuvant activity in the presence of weak antigen ovalbumin. Among the acylated analogs PK-II-3 and PK-II-4 were found to stimulate anti-OVA IgG titer, neutralizing antibody (IgG1 and IgG2a) titer as well as the production of soluble mediators of a Th1 response (IL-2 and IFN- γ) and Th2 response (IL-4) and proliferation of T lymphocytes sub-sets (CD4/CD8).

These results support the use of acylated analogs particularly PK-II-3 and PK-II-4 as potent enhancer of antigen-specific Th1 and Th2 immune responses and thus are promising immune-adjuvant candidate for vaccines (Kumar and Singh 2010)

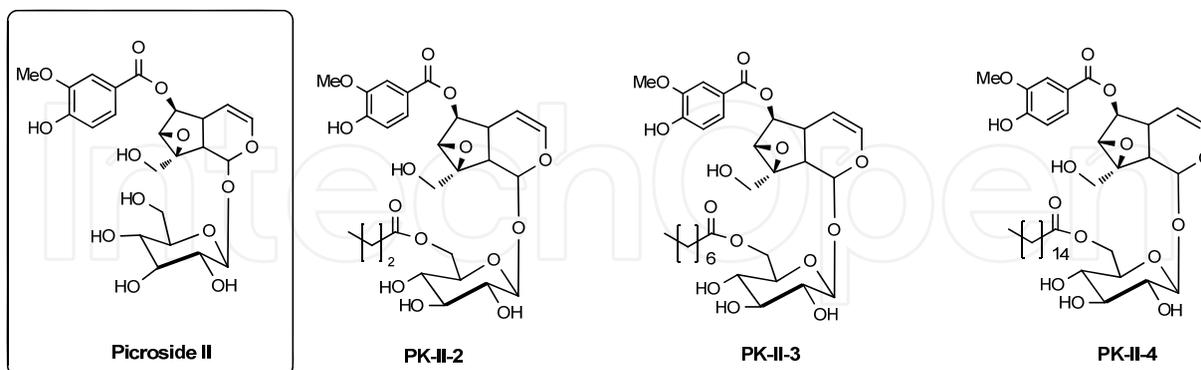


Fig. 10. Picroside II and its lipidated derivatives as immune adjuvants

Based on the traditional system of medicine, plant based products can be attractive candidates for use as safe vaccine adjuvants.

6.2 Saponins, Quil-A and QS-21

Saponins are triterpene glycosides isolated various plant sources. Crude extracts of *Quillaja saponoria* –a bark tree native of Chile, have long been known as an immunostimulator (Dalsgaard 1974). Crude extracts of plants containing saponin enhanced potency of foot and mouth disease vaccines. However, these crude extracts were associated with adverse side effects when used as vaccine adjuvants. Dalsgaard et al. partially purified the adjuvant active component from crude extracts by dialysis, ion exchange and gel filtration chromatography. The active component known as Quil A exhibited enhanced potency and reduced local reactions when compared to crude extracts.

Quil A is widely used in veterinary vaccines but its hemolytic activity and local reactions made it unsuitable for human vaccines. Further analysis and refining of Quil A by high pressure liquid chromatography (HPLC) revealed a heterogenous mixture of closely related saponins and led to discovery of QS-21 (10) a potent adjuvant with reduced or minimal toxicity. QS-21 is a quillaic acid-based triterpene with a complex acylated 3, 28-O-bisglycoside structure (Jacobsen, Fairbrother et al. 1996). Unlike most other immunostimulators, QS-21 (Fig 8) is water-soluble and can be used in vaccines with or without emulsion type formulations. In a variety of animal models, QS-21 has augmented the immunogenicity of protein, glycoprotein and polysaccharide antigens (Singh and O'Hagan 1999). QS-21 has been shown to stimulate both humoral and cell-mediated Th1 and CTL responses to subunit antigens. Clinical trials are in progress with QS-21, alone or in combination with carriers and other immunostimulants for vaccines against infections including influenza, HSV, HIV, HBV and malaria and cancers including melanoma, colon and B-cell lymphoma. Several structural analogs of QS21 derived from wholly synthetic or semi synthetic route have resulted in improved understanding of the mechanism of action of this saponin molecule. Now it is imperative that the mode of action of this molecule is through the action of formyl group on the triterinoid moiety of the saponon with the T-cell

receptor that leads to to strong TH₁ response. Thus it hardly needs to emphasize that development of more plant based adjuvants are highly desirable for developing vaccines against today's societal dreadful diseases like cancer and other infectious diseases.

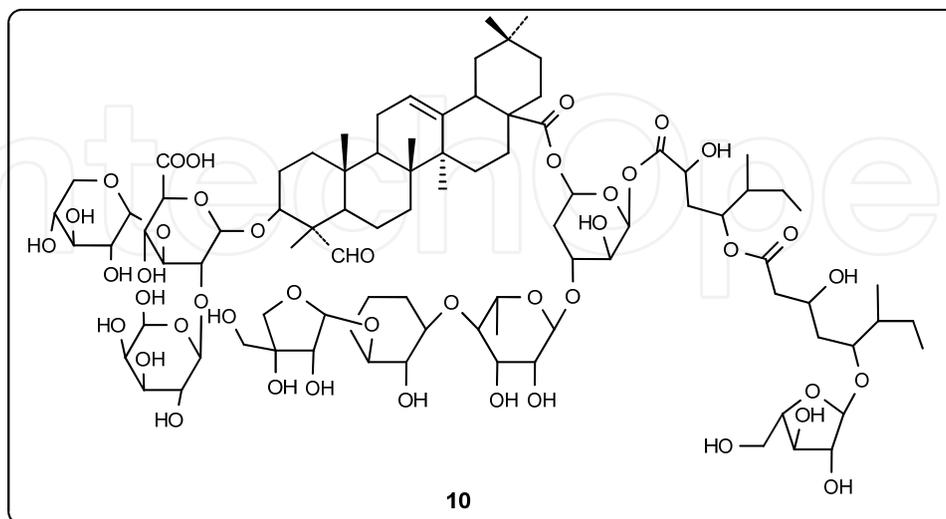


Fig. 11. QS-21, a saponin isolated from *Quillaja saponaria*

7. Current clinical status of potent immune adjuvants

As reviewed in the above sections, the use of PRR ligands particularly TLR and NLR agonists as vaccine adjuvants has been extensively explored with the new generation vaccines which contains defined antigens, These PRR ligands function as immune adjuvants and provide safe and even more effective alternate to live attenuated/dead whole organism based vaccines which induces strong Th1 and T-cytotoxic responses need to treat various cancer as well as infectious and allergic agents. In-fact, these PRR based immune adjuvants not only enhances the immunological response of vaccine candidates/formulations but perform many functions. A number of ongoing clinical trials with PRR ligands in prophylactic as well as therapeutic vaccines against infectious agents, cancer and allergic agents are presented in Table 2, 3 and 4.

Various small molecules derived from lipid A and RNA/DNA oligonucleotides activate TLR4 and TLR7/8 respectively represents potential class of immune adjuvants and vaccine encompassing these agonists finds application in the area of infectious and allergic diseases including cancer as shown in **Table 2, 3 and 4**. As discussed in section 4.3 and 4.3.1, the discovery of and understanding of functioning of NLRs as well as identification of their ligands such as DAP-containing peptidoglycan (FK156 and FK565), MDP and its lipidated and less pyrogenic analogs, chitosan, Quil A etc. are in preclinical studies also represent important classes of future's immune adjuvants and might find clinical applications along either alone or in combination with TLRs agonist against infectious and other disease conditions. Apart from the small molecules, several proteins such as flagellin (TLR5 agonists), oligonucleotide such as polyI:poly C RNA (TLR3 agonist) and several CpG (or CpG ODN) based ligands (TLR9 agonists) also find very promising results in various phase of clinical trials. Among these three, only unmethylated cytosine-phosphate-guanine-oligodeoxynucleotides (CpG ODN) and its analogs find applications against infectious, cancer and allergic diseases as shown in **Table 2, 3 and 4**. The analogs of CPG stabilized by a phosphorothioate backbone and based on

nucleotide sequence and length, CpGs are classified into class A, class B, and class C, and activate a predominantly strong Th1 response, a property which has been harnessed for oncological clinical trials (shown in Table 3). CpG ODNs can also be used as an adjuvant in vaccines and could be considered for the treatment of Th2-mediated Type I allergic disorders (Kanzler, Barrat et al. 2007; Basith, Manavalan et al. 2011) (Table 4).

Target	TLR agonist as adjuvants	Vaccines/antigens	Indication	Status (Company)*
TLR3	Synthetic, mismatched double-stranded poly I:poly C RNA	Ampligen	HIV	P-II (H)
TLR4	MPL adjuvant	Fendrix	Hepatitis B	Approved in EU(GSK)
	MPL adjuvant	Cervarix	Human papillomavirus	Approved (GSK)
	Synthetic MPL RC-529	Supervax	Hepatitis B	Approved in Argentina (DT)
TLR5	Fusion proteins of flagellin to hemagglutinin	Matrix Protein-2	Influenza	P-I (V)
TLR7/8	Imiquimod cream 5%	Aldara	Papilloma-induced genital warts	Approved (3MP)
	ANA975; oral prodrug of isatoribine (nucleotide analog)		Hepatitis C	P-I on hold (A/N)
	Resiquimod (R-848) (TLR7/8)		HCV Herpes simplex virus	P-II (3MP) P-III suspended (3MP)
	R851 (topical treatment)		Human papillomavirus	P-II (3MP/T)
TLR9	CpG-ODN 1018 ISS	Heplisav	Hepatitis B	P-III (DT) on hold
	CpG C class ODN: CpG10101,		Hepatitis C	P-II discontinued (CP)
	CpG B class ODN: CpG7909,	VaxImmune	Anthrax	P-I (CP)
	CpG-ODN	Influenza antigens	Influenza	Preclinical (DT)
	CpG-ODN	Remune (inactivated HIV-1 virus) with YB2055	Human immunodeficiency virus	P-I/II (IP/IRC)

*Full name of developing company/institutes: H - Hemispherx; GSK - GlaxoSmithKline; DT-Dynavax Technologies; V - Vaxinate; 3MP - 3M Phrama; A - Anadys; N - Novartis; CP - Coley Pharmaceuticals; IP - Idera Pharmaceuticals; IRC - Immune Response Corporation.

Table 2. TLR agonists in clinical development for infectious diseases

Target	TLR agonists as adjuvants	Vaccine/antigens	Indication	Status (Company)
TLR3	IPH 31XX (structure not disclosed)		Breast cancer	Preclinical (InP)
TLR4	MPL (enclosed in liposomal vehicle)	Stimuvax/BLP25 (Synthetic cancer-associated MUC1 protein)	Non-small-cell lung cancer	P-II (B/M)
TLR5				
TLR7/8	Imiquimod (used as 5% cream)	Aldara	Basal cell carcinoma	Approved (3MP)
	Imidazoquinoline 852A		Melanoma	P-II (3MP)
TLR9	CpG B class ODN CpG7909 or PF3512676	Along with chemotherapy	Non-small-cell lung cancer	P-III (CP/P)
	CpG B class ODN :1018ISS	Along with Rituxan	Non-Hodgkin's lymphoma	P-II (DT)
	CpG-ODN : HYB2055 or IMO-2055 or IMOXine		Renal cell carcinoma	P-II (IP)
	CpG motif containing circular ODN	dSLIM	Metastatic colorectal cancer	P-I/II (M)
	CpG B class ODN	Along with chemotherapy	Colorectal cancer	P-I (DT)
	CpG-ODN 7909 in incomplete Freund adjuvant	Melan-A peptide	Melanoma	P-I (CP/GSK)
	Immunodrug carrier QbG10	CYT004-MelQbG10 vaccine containing Melan-A/MART-1 protein	Melanoma	P-II (CB)

*Full name of developing company/institutes: InP - Innate Pharma; B - Biomera; M - Merck; P - Pfizer; M - Mologen; GSK - GlaxoSmithKline; DT-Dynavax Technologies; CB - Cytos Biotechnology; 3MP - 3M Phrama; CP - Coley Pharmaceuticals; IP - Idera Pharmaceuticals.

Table 3. TLR agonists in Clinical development for cancer

Like agonists, TLR antagonist showing promiscuous results in the clinical trial for the treatment of number of inflammatory and auto-immune diseases. These TLR antagonists have been mostly developed as structural analogs of agonists which bind to the receptor but fail to induce signal transduction, thus preventing the agonistic action of TLR ligands responsible for the induction of the inflammatory/autoimmune cascade. Two lipid A analogs such as E5564 and Tak 242 developed by Eisai and Takeda Pharma (derived from SAR studies during design of agonists) acts as potent antagonists of TLR4 and currently are in clinical trials for the treatment of sepsis or septic shock (inflammatory disorder)(Kanzler,

Barrat et al. 2007; Basith, Manavalan et al. 2011). Similarly, CpG ODN analog such as DV1079 (developed by collaborative efforts of Dynavax and GSK) act as potent TLR7/9 antagonists and is currently in preclinical study for the treatment of autoimmune disorder. Despite the development of many small immune adjuvants, still there is a need for more efficacious and potent immune adjuvants for poorly immunogenic antigenic based vaccine, while going through the structural features of many PAMPs based and synthetic adjuvants as well as understanding their role in vaccine formulation, the molecules become ideal immune adjuvants when they qualifies the parameters as discussed in section 9. Furthermore, while designing immune adjuvant, we should keep certain structural features in mind as discussed in section 10 which might give direction for the generation of potent, efficacious and ideal immune adjuvants. Moreover, in recent years, the co-crystal structure of PRRs(Kanzler, Barrat et al. 2007; Basith, Manavalan et al. 2011) particularly TLRs with their ligands have been identified, therefore their bio-informatics model system would be developed which further provide very useful inputs towards the designing of potent and ideal immune adjuvants.

Target	TLR agonists as adjuvants	Vaccines/antigens	Indication	Status (Company)
TLR4	MPL	Pollinex Quattro (modified allergens)	Allergic rhinitis (multiple allergens)	Marketed (EU)
	MPL	Ragweed SC/Pollinex Quattro; Ragweed/Pollinex R (ragweed pollen extract)	Allergic rhinitis (ragweed)	P-II (AP)
	CRX-675	Ragweed allergen	Allergic rhinitis (ragweed)	P-I (GSK(C))
TLR9	Covalently linked CpG B class ODN: 1018 ISS	Tolamba (Amb a 1 ragweed allergen)	Allergic rhinitis (ragweed)	P-II/III (DT)
	Immunodrug carrier QbG10	Allergen extract (CYT005-AllQbG10)	Allergic rhinitis (dust mite)	P-II (CB)
	CpG B ODN	Amba 1	Asthma	P-II (DT)
	Second generation CpG-ODN		Asthma	P-I (DT/AZ)
	CpG-ODN	AVE0675	Asthma	P-I (CP/SA)

*Full name of developing company/institutes: AP – Allergy Therapeutics; GSK – GlaxoSmithKline; DT – Dynavax Technologies; N – Novartis; CP – Coley Pharmaceuticals; IP – Idera Pharmaceuticals; CB – Cytos Biotechnology; AZ – Astra Zeneca; SA – Sonafi-Aventis.

Table 4. TLR agonists in clinical development for allergic diseases

Target	TLR antagonists	Indication	Status (Company)
TLR4	TAK-242	Severe sepsis	P-III (TPC)
	E5564 or Eritoran: a lipid A derivative	Severe sepsis	P-III (E)
TLR7 and TLR 9	Immunoregulatory sequence IRS 954	Lupus	Preclinical (DT)

Full name of developing company/institutes: TPC - Takeda Pharmaceutical Company; E- Eisai.

Table 5. TLR antagonists in clinical development for anti-inflammatory and auto-immune diseases

8. Role of adjuvants in the immune responses

Precisely, how adjuvants enhance the immune response is yet unknown, but they appear to exert different effects to improve the immune response to vaccine antigens, as such they:

- i. *Immunomodulation*- This refers to the ability of adjuvants to activate the immune response either to Th1 or Th2.
- ii. *Targeting*- Improve antigen delivery to antigen presenting cells (APCs), increase cellular infiltration, inflammation, and trafficking to the injection site.
- iii. Activation of APCs by up-regulating co-stimulatory signals, major histo-compatibility complexes (MHC) expression and inducing cytokine release.
- iv. *Antigen Presentation*- Enhance antigen processing and presentation by APCs and increase the speed, magnitude and duration of the immune response.
- v. Antigen Depot formation
- vi. *Induction of antibody*-modulation of antibody avidity, affinity as well as the magnitude, isotype or subclass induction.
- vii. *Stimulate cell mediated immunity* and lymphocyte proliferation nonspecifically.

9. Characteristics of an ideal adjuvant

It is likely that the "ideal" adjuvant does not and will not exist, because each adjuvant and its targeted antigen will have their unique requirements. Nevertheless, the generic characteristics summarized below would be desirable. To date, no adjuvant meets all of these goals.

- i. It must be safe, including freedom from immediate and long-term side effects.
- ii. It should be biodegradable or easily removed from the body after its adjuvant effect is exhausted to decrease the risk of late adverse effects.
- iii. It should elicit a more robust protective or therapeutic immune response combined with the antigen than when the antigen is administered alone.
- iv. It must be defined chemically and biologically, so that there is no lot-to-lot variation in the manufactured product, thereby assuring consistent responses in vaccines between studies and over time.
- v. Efficacy should be achieved using fewer doses and/or lower concentrations of the antigen.

10. Requirement for adjuvants

While going through the structure of various immune adjuvants of diverse classes, we conclude that following are few chemical traits molecules should possess to become more efficacious and potent immune adjuvants. Furthermore, following chemical traits should also be considered for the chemical modifications of PAMPS or other natural derived molecules.

Following are the structural requirements for a molecule to act as an efficient adjuvant:

- i. Hydrophilic-lipophilic balance.
- ii. Presence of micellar structures to facilitate depot formation.
- iii. Lipophilic structure enveloping the antigen to preserve the structure required for its immunogenicity.
- iv. Lipophilic structures capable of effective cytosol trafficking.
- v. Presence of functional groups to activate/substitute co-stimulatory signals for effective Th1 immunity.
- vi. Overall structural design to stimulate Th1 and Th2 balance.
- vii. Structure to be ligand for T cell or DC surface receptors.

11. Conclusion and future prospects regarding the use of immune adjuvants towards design of new breed potent vaccines

The literature discussed here present a wide variety of pathogen derived natural and synthetic PRR agonist and antagonists, among these some of these molecules already which find potential application as immune adjuvants in various vaccine formulations to treat dreadful cancer, infectious and allergic diseases. Furthermore, various plant derived immune are also discussed here which are now in very preliminary stages and also present the potential starting points and need serious efforts first towards the understanding of their mechanism of action and further development. For TLR agonists to achieve further recognition in the clinic it will be critical to undertake side-by-side comparisons against the same antigen using selected immune monitoring assays that measure the quantity and quality of responses (e.g. avidity, memory cell generation, durability) as well as further refinement in their chemical structure (wherever need) keeping in mind the above motioned points required for molecules to become ideal immune adjuvants. Furthermore, the potential immune adjuvant candidates either in the preclinical or in various clinical phases discussed here represent only a fraction of the current efforts to clinically translate our current understanding of some of the exploited PRR and innate immunity. Many other strategies and tactics to stimulate or inhibit others PRR are being developed and these studies are just beginning. Understanding the role of different PRR for different pathological conditions are growing rapidly and this will surely continue to be more productive and fruitful field for the development of more efficacious and potent vaccine candidates for various unmet cancer and infectious diseases.

12. References

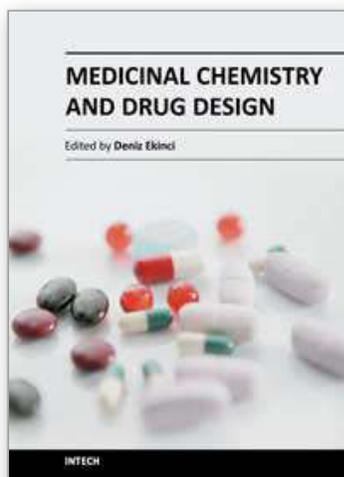
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Over the recent years, medicinal chemistry has become responsible for explaining interactions of chemical molecules processes such that many scientists in the life sciences from agronomy to medicine are engaged in medicinal research. This book contains an overview focusing on the research area of enzyme inhibitors, molecular aspects of drug metabolism, organic synthesis, prodrug synthesis, in silico studies and chemical compounds used in relevant approaches. The book deals with basic issues and some of the recent developments in medicinal chemistry and drug design. Particular emphasis is devoted to both theoretical and experimental aspect of modern drug design. The primary target audience for the book includes students, researchers, biologists, chemists, chemical engineers and professionals who are interested in associated areas. The textbook is written by international scientists with expertise in chemistry, protein biochemistry, enzymology, molecular biology and genetics many of which are active in biochemical and biomedical research. We hope that the textbook will enhance the knowledge of scientists in the complexities of some medicinal approaches; it will stimulate both professionals and students to dedicate part of their future research in understanding relevant mechanisms and applications of medicinal chemistry and drug design.

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