Review

Polysaccharides: Candidates of promising vaccine adjuvants

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Summary **Aluminium-based adjuvants remain the only adjuvants approved for human use in the USA for over 80 years because of alum's simplicity, tolerability, safety and cost-efficiency. Recent development of vaccines, especially the increasing applications of recombinant subunit and synthetic vaccines, makes aluminium adjuvants cannot stimulate enough immunity to the antigens, since aluminium adjuvants can only induce Th2 type immune responses. So, novel adjuvants are urgent to make up the disadvantages of aluminium adjuvants. However, some major hurdles need to be overcome, not only the scientific knowledge of adjuvants but also unacceptable side-effects and toxicity. A number of carbohydrate-based polysaccharides from plant, bacterial, yeast and synthetic sources can act as pathogen-associated molecular patterns (PAMPs) and recognize pattern recognition receptors (PRRs) on immune cells, followed by triggering innate immunity and regulating adaptive immunity. What is more, polysaccharides are safe and biodegradable without tissue deposits as observed in aluminium adjuvants. Therefore, polysaccharide-based compounds and formulations are potential vaccine adjuvant candidates. Here, we mainly review polysaccharide-based adjuvants investigated in recent years.**

Keywords: Polysaccharide, vaccine, adjuvant, immunity

1. Introduction

Vaccination is the most effective strategy to prevent and control the spread of infectious diseases through generation of sufficient protective immune response. Vaccines based on inactivated pathogens, live attenuated pathogens, surface molecules such as proteins, carbohydrates and lipids, or recombinant antigens can induce neutralizing antibodies against the particular pathogen by intradermal, oral, or intranasal administration route. In many cases, the vaccine consisting of antigen alone can only stimulate too weak immunogenicity to prevent infection, therefore, an adjuvant is needed to strengthen the immune response (*1*). At present, aluminum-based adjuvant (aluminum hydroxide) is the only one approved for human use

by FDA. Although it has been used for more than 80 years, it only contributes to the induction of a good Th2 immune response but has little capacity to induce cellular (Th1) response, which limits its applications (*2*). Besides, high aluminum levels that may be accumulated in persons with reduced renal function, may affect the brain and bone tissues resulting in neurological syndrome and dialysis-associated dementia (*3*). MF59 is another adjuvant approved for human use in Europe, which is added to influenza vaccines to stimulate the immune response (*4*). MF59 can induce the body to generate both Th1 and Th2 type immune response (*5*), however, as an oil emulsion adjuvant, it also has the problem of local injection site reactogenicity. To enhance the immunogenicity and safety of vaccines, novel adjuvants and formulations are needed to improve the cell mediated immune response to antigens.

So far, the licensed vaccine adjuvants and formulations are rare and precious, which include aluminum with no special indication, MF59 used in influenza (Fluad), liposomes in HAV/influenza, AS03 in pandemic flu (Pandemrix), AS04 in HBV (Fendrix)

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and HPV (Cervarix) (*2,6*). These so-called generation 1 adjuvants share common mechanism of action based on their particulate structure and dimensions: as a depot of antigen to increase uptake and stability of the antigen at the site of injection and as a carrier to deliver antigens to the appropriate immune cells. These actions are effective in some cases, but in poorly immunogenic antigens, are not able to induce sufficient protective immune response, therefore, an immune potentiator may be needed to assist the antigens (*6*). A number of carbohydrate polymers from plants, microbes, and synthetic sources have been studied to possess immunomodulating activities, as well as the performance of high biocompatibility and low toxicity (*7*). These characteristics of carbohydrates, especially polysaccharides, have drawn the attention of many experts to explore the possibility to develop polysaccharides as successful vaccine adjuvants (*7,8*).

2. Polysaccharide-based adjuvants

2.1. *Inulin-based adjuvants*

Inulin is found in the roots of Compositae, consisting of a linear β-D-(2→1)polyfructofuranosyl α-D-glucoses, with up to 100 fructose moieties linked to a single terminal glucose (*9*). Inulin contains four forms distinguished by solubility: alpha, beta, gamma and delta, in which gamma and delta forms have been demonstrated to exhibit adjuvanticity with enhancement of both cellular and humoral immunity, whereas no toxicity. Petrovsky group (Flinders) has done a lot of work to discover and verify adjuvant activities of inulin against different infectious pathogens (*10-16*). Cooper *et al*. (*10*) found that delta inulin showed stronger immune enhancement effect when combined with recombinant hepatitis B surface antigen (HBsAg) than gamma inulin. Advax is the latest generation of adjuvants made from delta inulin developed through the National Institutes of Health's Adjuvant Development Program (*11*). Advax has been demonstrated to enhance immunogenicity as an adjuvant in many vaccines in animal models and human clinical trials. Based on preclinical studies (*10,12*), Gordon *et al*. (*13*) designed a Phase I trial to evaluate immunogenicity and safety of Advax when formulated with HBsAg. The results revealed that Advax was safe and well tolerated in healthy adult subjects, and also enhanced HBsAb titers and CD4^{+} T-cell proliferation compared to HBsAg containing aluminium adjuvant. Lobigs *et al*. (*14*) reported the Advax-adjuvant Vero cell culture vaccine could elicit strong neutralizing immunity against challenge of live Japanese encephalitis virus (JEV) both in mice and horse models. The Advax-adjuvanted vaccine stimulated a balanced Th1/Th2 immune response and produced neutralizing antibody not only against JEV, but also Murray Valley encephalitis virus

(MVEV), another virus belonging to JEV serocomplex. These results indicated that Advax as an adjuvant was able to improve the efficiency and application range of JEV vaccine compared to aluminium adjuvants.

Advax has also been shown to be effective in influenza vaccines when challenged by the particular influenza virus. Vaccination of ferrets with split-virion H5N1 vaccine combined with Advax improved protection effect against H5N1 virus significantly by enhanced immunogenicity, survival, as well as reduced morbidity, compared with vaccine alone (*15*). Similarly, Advax increased both humoral and cellular responses of mice immunized intramuscularly with trivalent human influenza vaccine (TIV), and provided significant antigen-sparing effect, in contrast to influenza antigen alone (*11*). A randomized clinical trial confirmed that Advax adjuvant used in H1N1/2009 vaccine could enhance recombinant hemagglutinin immunogenicity, and this vaccine system may be effective in acute influenza pandemic (*16*).

The possible mechanism of Advax working as an adjuvant in aforementioned vaccines is that Advax can bind to antigen-presenting cells (APCs), such as mononuclear cells and dendritic cells (DCs), resulting in antigen presentation upregulation and antigen-specific T- and B-cell activation (*11*). Advax administered with vaccines induces the generation of both Th1 (IgG2a) and Th2 (IgG1, IgA) antibody responses, and the production of Th1 (IL-2, IFN-γ) and Th2 (IL-5, IL-6) cytokines, which makes up the limitation of aluminum adjuvant. Advax itself does not stimulate innate immune response, therefore, there are no obvious inflammation reactions in tested subjects. The properties of safety and tolerability of Advax are superior to emulsion formulation adjuvants.

2.2. *Chitosan-based adjuvant*

Chitosan is a linear β-(1→4)-linked copolymer of D-glucosamine and *N*-acetyl-D-glucosamine (GlcNAc), prepared by alkaline partial deacetylation of chitin (*17*). Chitosan possesses good biological characteristics *in vivo*, such as favorable biocompatibility and biodegradability, no toxicity and no reactogenicity. Studies have found that chitosan is capable of inducing immunity, and chitosan-based powders and micro/nanoparticles used in parenteral and mucosal delivery of antigen vaccines can enhance antigen-presenting functions, which exhibits its vaccine immunoadjuvant property (*18*). An *in vitro* assay indicated that BSA- or OVA-encapsulated chitosan particles could stimulate RAW264.7 macrophages and BMDCs activation compared to soluble antigens. The expressions of surface activation markers MHCI, MHCII and co-stimulatory cytokines CD40, CD80, CD86, and CD54, were up-regulated significantly than those of soluble antigens in the two APCs. Meanwhile, chitosan

particles also enhanced the release of proinflammation cytokines, IL-1β, IL-6, TNF-α, MCP-1 and MIP-1α, as well as the proliferation of CD4$^+$ and CD8$^+$ T cells (*19*). Dry-powder chitosan nanospheres encapsulated with influenza virus, CpG oligodexynucleotide (CpG ODN) and *Quillaja saponins* (QS) induced humoral and cellular immune responses after nasal administration to rabbits (*20*). It is thought that dry powder particulate system provides long lasting protective immunity, and offers physical and chemical stability for antigens (*21,22*). Chitosan nanospheres delivery system elevated both local sIgA and systemic IgG titers against the virus, suggesting this nanoparticulate chitosan may be an efficient adjuvant delivery system for immunization against influenza virus (*20*). Similar to chitosan, its trimethylated derivative (TMC) nanoparticles loaded with HBsAg given to mice intranasally and intramuscularly induced higher nasal and serum antibody titers than HBsAg solution. Both chitosan and TMC nanoparticles induced higher level of IgG1 and IgG2a after intramuscular injection of mice than those of aluminum absorbed HBsAg (*23*).

Neimert-Andersson and his colleagues (*24,25*) have prepared a hydrogel named ViscoGel from one kind of soluble chitosan and investigated its immune adjuvant activity. ViscoGel together with vaccine against Haemophilus influenza type b (Act-HIB) could induce stronger humoral and cellular response to antigen than those induced by vaccine alone. The titers of IgG1 and IgG2a in serum were significantly enhanced, and the productions of Th1-, Th2-, and Th17-type cytokines tested were also elevated. ViscoGel recruited neutrophils at the injected site and generated a pro-inflammatory environment which is important for early immune response (*24*). ViscoGel may trigger immune response in the similar way as chitin, since chitin particles were reported to function as PAMPs and recognize TLR-2 receptor on macrophages to induce innate immune response (*26*). Furthermore, the safety and efficacy of ViscoGel as an adjuvant with Act-HIB vaccine has been evaluated in the Phase I/IIa clinical trial (*25*). No serious adverse events were observed in all of the subjects, and a larger percentage of subjects appeared local, transient reactions at the injection site in groups received ViscoGel than those vaccinated with Act-HIB alone. These side effects were mild and resolved within short time, therefore, it was thought ViscoGel was safe and well tolerated in combination with Act-HIB. The efficacy study of ViscoGel showed that ViscoGel stimulated cell-mediated response through enhancement of interferon-γ (IFN-γ) response to Act-HIB in peripheral blood monuclear cells (PBMCs). However, it did not significantly elevate anti-HIB antibody level and this may be somewhat related to inter-individual variation. This clinical trial revealed that ViscoGel was safe as a vehicle for vaccine delivery, but the adjuvant effect was not so ideal in human and may need further study afterwards.

The immunomodulating activity of chitosan endows its application not only in the pathogen antigen vaccine adjuvant, but also anti-tumor adjuvant. Chitosan substrate was able to induce mouse bone marrow-derived monocytes differentiation into DCs, and vaccination of mice with tumor lysate-pulsed DCs cultured on chitosan system increased cytotoxic T lymphocyte (CTL) activity against inoculated tumor, which indicated chitosan may be useful for DC vaccines in the treatment of tumor (*27*). Highton *et al*. (*28*) applied vaccine consisted of hydrogel chitosan with OVA antigen and Quil-A adjuvant to against melanoma challenge in mice. Chitosan exhibited protection effect by stimulating stronger OVA-specific CD8$^+$ T cell memory response than those receiving DC vaccination instead of chitosan hydrogel. Cytotoxic CD8$^+$ T cells are critical for cancer therapy and prevention. Ma *et al*. (*29*) investigated the adjuvant effect of water soluble chitosan (WSC) on the immunity of radiotherapy patients suffered from lung cancer. The study found that oral administration of WSC to radiotherapy patients increased CD3, CD4, NK cells, and CD4/CD8 ratio, as well as IL-6, TNF-α levels remarkably compared with control group. These results indicated the possibility of chitosan and its formulation action as an immune adjuvant in anti-tumor therapy in future.

2.3. Glucan-based adjuvant

Glucans are polysaccharides from plants and microorganisms made up of repeating D-glucose units linked by glycosidic bonds, and can be divided into α- and β-glucans according to conformations (*7*). β-Glucans are one of the most abundant polysaccharides in bacteria, fungi, and plants, such as zymosan, lentinan, and algal glucan. They have been reported to possess immunological, anti-tumor, anti-infection activities, and also enhance the immune response of vaccines. β-Glucans can be recognized as PAMPs by the innate immune system through binding of immune cell receptors on neutrophils, macrophages, and DCs, such as toll-like receptors (TLRs), dectin-1, CR3 and CD5. The interactions between β-glucans and receptors trigger intracellular signalings activation followed by expression of immune-related molecules and regulate innate and adaptive immune responses (*30*).

The adjuvant and immunomodulatory effect of β-glucan in fish has been studied when β-glucan was injected intramuscularly with recombinant glyceraldehyde-3- phosphate dehydrogenase (rGAPDH) vaccine (*31*). The results showed that fish immunized with rGAPDH combined with β-glucan produced high level antibody, and up-regulated transcription levels of immunomodulatory molecules involved in innate and adaptive immune responses significantly, compared to rGAPDH immunization group. When challenged with *E. tarda* after immunization, fish in the group of rGAPDH containing β-glucan showed the

highest relative percentage survival compared to the other groups. These results indicated the adjuvant and protective effects of β-glucan when vaccinated fish with rGAPDH. Sulfated glucan from *Saccharomyces cerevisiae* could induce chicken splenic lymphocyte proliferation *in vitro*, and when injected to chickens as the adjuvant with Newcastle disease (ND) vaccine, sulfated glucan enhanced serum antibody titers, as well as improved serum IL-2 and IFN-γ concentrations (*32*). Another kind of β-glucan, curdlan, the extracellular polysaccharide from the bacteria of *Alcaligenes faecalis* var. *myxogenes* 103K with the structure of β-(1→3)-D-glucan, has been demonstrated to have good immunomodulating activity by recognition of dectin-1 receptor on immune cell surface (*33*). Our study found that its sulfation product, curdlan sulfate, could stimulate splenic lymphocyte proliferation, RAW264.7 cell activation, DCs maturation, and increase TNF-α, IL-6, and IL-1β cytokines secretion (*34*). Compared to aluminum adjuvant, curdlan sulfate enhanced recombinant HBsAg vaccine immunogenicity, and increased both cellular and humoral responses in mice. A higher IgG2a/IgG1 ratio within anti-HBs antibodies was produced in mice received HBsAg plus curdlan sulfate than those in mice immunized with HBsAg and an aluminum adjuvant, which indicated that curdlan sulfate induced a shift toward a Th1-biased immune response (*35*). β-Glucan particles (GP) from *Sacharomyces cerevisiae* has been shown to regulate innate immunity and can be phagocytized by DCs *via* dectin-1 receptor. The hollow structure of GP allows encapsulation of antigen, and OVA can be electrostatically complexed inside the hollow GP shells formed GP-OVA antigen. Mice immunized with GP-OVA produced substantially higher antigen-specific CD4^{+} T cell proliferation than those with alum/OVA. Moreover, GP-OVA induced strong secretions of IgG1 and IgG2c anti-OVA antibodies, and Th1 and Th17 biased CD4^{+} T cell immune responses (*36*). Further study found that oral administration of GP-OVA by mice stimulated IL-17 expression significantly in the spleen, and increased OVA-specific IgA, secretory-IgA and secretory component production in intestinal fluids, suggesting GPs were potent delivery vehicles to deliver OVA *via* an oral route and resulted in Th17-biased cellular and humoral responses (*37*).

In addition to β-glucans, some α-glucans also exhibit immune adjuvant functions. Lu *et al.* (*38*) found that a dendrimer-like α-D-glucan nanoparticle made from chemical modification of phytoglycogen, Nano-11, could adsorb negatively charged protein antigens by electrostatic interaction. The Nano-11 as the antigen delivery vehicle could enhance antigen-uptake by DCs and stimulate activation of DCs *in vitro*. Intramuscular injection of Nano-11-antigen formulations increased the immune responses to antigens obviously, since Nano-11 not only enhanced the antibody titers to

antigen, but also recruited large number of monocytes at the injection sites. Compared to aluminum adjuvant, Nano-11 induced fewer neutrophils at the injection sites, which indicated Nano-11 as a vaccine delivery vehicle was safe to be an effective immune adjuvant. Acetalated-dextran (Ac-DEX) microparticles were used to encapsulate the TLR7 ligand, imiquimod, to stimulate immune cells. Compared to the free form, encapsulated imiquimod significantly increased IL-1β, IL-6, TNF-α, inducible nitric oxide synthase (iNOS) and PD1-L1 expression and nitric oxide (NO) level of macrophages, as well as IL-1β, IL-6, IL-12p70, and MIP-1α of BMDCs. These results suggested that Ac-DEX microparticles encapsulation could increase the potency of TLR ligands, and may be an effective delivery vehicle in vaccine formulations for future *in vivo* study (*39*).

2.4. Other polysaccharide adjuvants

The actions of polysaccharides based on mannose possessing adjuvanticity may be dependent on the binding of polysaccharides by mannose-binding lectin (MBL) and other C-type lectins of the mannose receptor family on macrophages and DCs, since the binding interactions can induce complement activation, opsonization and phagocytosis, which play important roles in innate immunity (*40*). Vaccination with mannan or mannan-BSA conjugate could protect mice against systemic aspergillosis (*41*). In mice, oxidized mannan-MUC1 stimulated Th1 type responses mediated by CD8^{+} T cells with IFN-γ secretion and mainly IgG2a antibody response, whereas reduced mannan-MUC1 stimulated Th2 type responses with IL-4 production and a high IgG1 antibody response (*42*). A phase III clinical trial revealed that oxidized mannan-MUC1 as the immunotherapy adjuvant in the treatment for breast cancer patient could decrease the cancer recurrence rate and prolong recurrence time compared to the placebo group during the 12-15-year follow-up, without any evidence toxic or autoimmune adverse effects (*43*). Moreover, oligomannose-coated liposomes were able to deliver antigens to peripheral phagocytic cells and induce antigen-specific Th1 immune responses and CTLs (*44*). Besides mannan, fructooligosaccharide (FOS) was also reported to recognize MBL receptor. The chickens with high serum MBL concentrations (L10H) immunized with infectious bronchitis vaccine (IBV) plus FOS enhanced higher IBV-specific IgG antibody than that of IBV alone, indicating adjuvant effect of FOS in IBV vaccine (*45*). Lipopolysaccharide (LPS) derivative, monophosphoryl lipid A (MPL), has been demonstrated to be a non-toxic and immunoactive adjuvant *via* binding to TLR4 complex (*46*). AS04 adjuvant, consisting of MPL and aluminum phosphate, produced by GlaxoSmithKline (GSK), has been licensed in the vaccines of HBV and HPV as an adjuvant (*6*). The adjuvant system composed

of MPL and QS-21 applied in HBV vaccine has also been studied in clinical trials in recent years (*47*).

3. Conclusions

The potent immune adjuvants derived from polysaccharides have been the hot topic in the pathway to a successful vaccine adjuvant, due to the advantages of non-toxic, biodegradation, good biocompatibility, strong immune enhancement and low reactogenicity. Polysaccharide-based compounds and formulations are promising candidates for vaccine adjuvants in prevention and treatment of infectious pathogen diseases or cancers. Although a large number of studies on novel adjuvants have been carried out, further efforts are needed to select one adjuvant to challenge the monopoly of aluminum adjuvants in human vaccine adjuvant usage.

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