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REVIEW

Polysaccharide nanoparticles as potential immune adjuvants: Mechanism and function

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Abstract Adjuvants as essential ingredients amplify the magnitude and durability of immune responses in various vaccine strategies. Polysaccharides with potent immunoenhancing effects are widely applied as promising vaccine adjuvants, however, they have rarely been licensed for use in human vaccines due to the limitation of their efficacy and safety. Moreover, nanoparticles not only act as antigen drug delivery vectors but also possess intrinsic adjuvant functions, revealing the dual effects of nanoparticles in augmenting antigen-specific immune responses. Intriguingly, nanoparticle forms can enhance the immunostimulatory potency of polysaccharide adjuvants, since polysaccharide nanoparticles exert more excellent adjuvant effects than polysaccharides in initiating humoral, cellular and mucosal immune responses. Emerging evidence has also suggested that multiple immune-related signaling pathways including cGAS−STING, NLRP3, TLRs, cell death or metabolism signaling probably participate in the immunomodulation of polysaccharide nanoparticles, but systemic investigations into the adjuvant mechanism are still inadequate. This review aims to give an updated summary and discussion on the adjuvant function and mechanism of polysaccharide nanoparticles for understanding their superior adjuvant property and effectively utilizing them as potent immune adjuvants in vaccine development.

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1. Introduction

While infectious diseases have threatened humanity over centuries, vaccines as one of the most powerful strategies have been developed for preventing the outbreak and progression of pandemics[1]. Since traditional whole-pathogen vaccines always possess serious safety problems, less immunogenic vaccines are newly developed, such as subunit, inactivated, or modified mRNA vaccines, which usually need the assistance of immune adjuvants[2,3]. Adjuvants as essential substances are widely applied in vaccine strategies to potentiate the immunogenicity of antigens as well as immune responses. In 1926, Alexander Glenny discovered that aluminum salts triggered potent adjuvant effects, and were further approved by US Food and Drug Administration (FDA) for human vaccines[2]. Aluminum has been one of the first-line adjuvants used in vaccines until now, for instance, it was added as an adjuvant in inactivated COVID-19 vaccines for enhancing immune responses of inactivated virus, although it has been discovered that aluminum is not quite effective in Th1-based immunity[2,4]. Apart from aluminum, some adjuvants, including oil-in-water emulsion MF59, AS04, AS03, AS01, CpG ODN 1018, and lipid nanoparticles (LNPs), have been continuously licensed for use in human vaccines over a few decades, but the category of adjuvants is limited. Obviously, there is an urgent need for exploring novel adjuvants for human vaccines.

Lipopolysaccharide (LPS) can function as a TLR4 agonist adjuvant to augment immune responses of vaccines, but it may trigger excessive immune responses, such as sepsis and septic shock, and therefore it has been not put into the market due to its local and systemic toxicity[2,5]. More importantly, as a detoxified derivative from LPS, monophosphoryl lipid A (MPLA) can activate TLR4 and trigger potent Th1-mediated cellular immune responses, which can remedy the weaknesses of aluminum adjuvant. Unsurprisingly, AS04 adjuvant containing MPLA and aluminum possessed stronger immunoenhancing effects than aluminum alone and have been approved for use in HPV and HBV vaccines[6]. Alternatively, saponin-based adjuvant QS-21 has been applied as an essential component in licensed AS01 adjuvant for human vaccines, whereas the inherent liabilities of QS-21, including scarcity, heterogeneity, hydrolytic instability and dose-limiting toxicity, restricted its clinical application[3,7]. These investigations and applications suggested the potential of polysaccharides and polysaccharide derivatives as immune adjuvants in vaccine strategies. Intriguingly, it has been reported that polysaccharides, such as chitosan, dextran, can assist antigens to facilitate humoral and cellular immune responses *via* boosting functions of antigen-presenting cells, T cells as well as antibody productions[3,8,9]. For instance, chitosan as a cationic polysaccharide promoted DC maturation and Th1-based cellular immune responses through regulating STING signaling, supporting the excellent immunostimulatory functions of chitosan[8]. Meanwhile, the Norwalk VLP vaccine (NCT00806962), which used chitosan as an adjuvant, could effectively prolong the adhesion and retention of antigens in nasal mucus and epithelial cells, and was involved in acute epithelial gastroenteritis with good tolerance and high immunogenicity, successfully passing phase one clinical trials. Other types of polysaccharides have been also conducted in clinical trials, for instance, β-glucan can interact with specific receptors (dectin-1, TLR 2/6 or CR3) in immune cells to stimulate antibody production or phagocytosis to strengthen defence against infection or cancer cells (NCT01829373, NCT04798677, NCT06057948, NCT04936529, and NCT00911560), while lentinan can increase the expression of CD4 cells to improve the body's immunity to against HIV infection (NCT00002098 and NCT00002099). However, it is not hard to find that the approval of polysaccharides as adjuvants for clinical use still faces significant challenges. While polysaccharides are generally considered safe, their potential to induce unnecessary immune responses needs thorough evaluation, and further comprehensive investigations are required on the adjuvant potency, safety and mechanism of polysaccharides.

Alternatively, numerous studies have nanosized and fabricated polysaccharides as drug delivery vectors, which can efficiently transport drugs, including vaccine antigens, to specific sites to improve the effectiveness and safety of drugs[9,10]. Polysaccharide nanoparticles possessed the function or mechanism of nanoparticle platforms in antigen protection, antigen retardation and antigen delivery. For instance, nanoparticles including polysaccharide nanoparticles can encapsulate antigens and protect them from degradation by enzymes or acids in physiological environments[9,11]. Polysaccharide nanoparticles can also provide a controlled release of antigens and prolong the exposure time, consequently enhancing immune responses[12]. Moreover, the functionalization of polysaccharide nanoparticles can facilitate targeted delivery of antigens to specific cells or tissues. It has been reported that dextran-based nanoparticles successfully encapsulated paclitaxel and silybin and assisted these drugs to accumulate in tumor sites, and consequently triggered superior anti-tumor effects[13], suggesting polysaccharide-based nanoparticles as excellent drug delivery systems. Interestingly, since polysaccharides, such as chitosan, possess natural mucoadhesive properties, polysaccharide nanoparticles can adhere to mucosal surfaces to promote retention at the site of administration (*e.g.*, oral, nasal, or vaginal), improving mucosal immune responses[14,15]. Apart from antigen drug carriers, nanoparticles exhibited intrinsic adjuvant properties by eliciting immune signaling and modulating immune cells[16,17]. Gold nanoparticles markedly potentiated DC and T cell activation as well as antibody responses, and their immunostimulatory functions were highly dependent on the shape and size of nanostructures[18]. More importantly, Lipid nanoparticles (LNPs) as novel adjuvants have been recently approved for use in Pfizer/BioNTech's BNT162b2 and Moderna's COVID-19 mRNA-1273 vaccines[19], further supporting the adjuvant functions of nanoparticles.

Unsurprisingly, emerging studies have attempted to nanosize adjuvants to enhance their immunostimulating functions and amplify vaccine efficiency[15,20,21]. For instance, aluminum-based nanoparticles could notably facilitate antigen uptake, DC maturation, Th1 and $CD8^+$ T immune responses to stimulate antitumor immunotherapy, which were more potent than aluminum alone[22]. Chitosan nanoparticles also triggered stronger adjuvant effects than chitosan *via* modulating STING and autophagy signaling and subsequently augmenting immune cell functions[9]. There are many attempts to probe polysaccharide nanoparticles as promising adjuvants to augment humoral, cellular and mucosal immune responses, while they suggested a potential key immunoenhancing mechanism of polysaccharide nanoparticles in modulating signal transductions in immune cells, including cGAS−STING signaling, NLRP3 signaling, TLRs signaling, cell death signaling (autophagy, pyroptosis, apoptosis, necroptosis) and metabolism signaling (Fig. 1). However, the adjuvant effects and signal mechanisms of polysaccharide nanoparticles still require further explorations. Significantly, there are rare systemic summaries on nanosized polysaccharides acting as immune

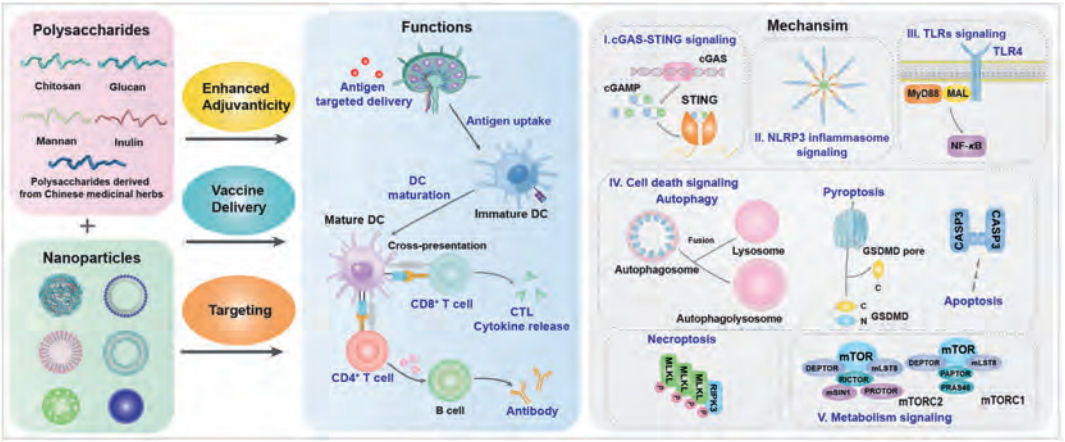

Figure 1 The schematical diagram of polysaccharide nanoparticle-enhanced immune responses and adjuvant mechanism.

adjuvants. This review aims to summarize and discuss the updated discovery of the adjuvant functions and underlying mechanisms of polysaccharide nanoparticles, providing a platform for adequately utilizing polysaccharide nanoparticles as promising adjuvants in future studies.

2. Polysaccharide-based adjuvants

Polysaccharides are large complex carbohydrates composed of long chains of monosaccharide units *via* the linkage of glycosidic bonds, and they are classified into various categories based on their structure and function (Fig. 2). Importantly, various polysaccharides, such as chitosan, glucan, mannan and inulin, has been reported to exhibit the potential of immune adjuvants by amplifying humoral, cellular and mucosal immune responses, and physicochemical property largely affected the immunoenhancing functions of these polysaccharides[23].

2.1. *Chitosan*

Chitosan as a cationic natural macromolecule that consists of D-glucosamine and *N*-acetyl-D-glucosamine connected by β (1→4) glycosidic bonds possesses excellent biodegradable and biocompatible properties, indicating the safety of chitosan in therapeutical applications. Intriguingly, chitosan has become one of the most widely investigated polysaccharides in immune adjuvant development over a few decades[14]. Numerous studies revealed that chitosan as an immunoenhancing substance can markedly elevate antigen uptake, DC maturation, T cell immunity and humoral immunity as well as macrophages and NK cell activations[24]. An exciting study discovered that STING-knockout DCs (*Tmem173*$^{-/-}$ DCs), but not WT, failed to initiate IFN-β and CXCL10 productions as well as DC maturation, and suggested that chitosan mainly activated cGAS−STING-dependent type I interferon signaling to boost DC activation and antigen-specific Th1 immune responses *in vitro* and *in vivo*, uncovering the possible adjuvant mechanism of chitosan (Fig. 3)[8]. Importantly, the physicochemical characterizations, including molecular weight (MW) and the degree of deacetylation (DDA), of chitosan deeply influenced its immunostimulatory functions and mechanisms. It has been reported the effect of MW and DDA on type I IFN responses or inflammasome activation in macrophages through screening a library of chitosans with different structure characterizations, which included twenty chitosans with controlled DDA (60%−98%), MW (1 to >100 kDa), and acetylation pattern (block *vs*. random)[25]. At low dose (<50 μg/mL), 10−110 kDa 80% DDA chitosans induced type 1 IFN response to elevate the CXCL10 and IL-1ra productions, whereas all 98% DDA over 3 kDa chitosans stimulated NLRP3/inflammasome to release IL-1β and PGE2 at higher dose (>50 μg/mL), which was highly dependent on lysosomal rupture. It suggested that more insoluble and deacetylated chitosans with a particle size amenable to phagocytosis would trigger a greater capacity to activate the inflammasome.

Furthermore, since chitosan exhibits the limitation of poor solubility, its water-soluble derivatives, such as *N*,*N*,*N*-trimethyl chitosan (TMC), hydroxypropyltrimethyl ammonium chloride chitosan (HACC), sulfated chitosan (SCS) and chitooligosaccharides, have been investigated as immune adjuvants and they also triggered strong immunostimulatory effects in different vaccine strategies[23,26,27]. Alternatively, chitosan has been widely used as a mucosal adjuvant due to its excellent mucoadhesive properties. Apart from potent systemic immune responses, chitosan and its derivatives could enhance mucosal residence and initiate SIgA antibody production to elicit excellent mucosal immunity[23,26], suggesting the superior adjuvant function of chitosan in cellular, humeral and mucosal immunity.

2.2. *Glucan*

As an anionic polysaccharide, glucan is a complex branched biopolymer composed of multiple glucose molecules. Similar to chitosan, glucans including α-glucan (dextran) and β-glucan also trigger strong immunoenhancing functions without any obvious toxic problems. Early studies have reported that dextran sulphate as a promising adjuvant largely modulated humoral and cell-mediated immunity, and more importantly, MW influenced the adjuvant potency since dextran sulphate with high MW (900 kDa), but not low MW (20 kDa), could trigger notable enhancement of humoral immune responses[28,29]. Dextran can be chemically conjugated with CpD DNA to form a novel potential adjuvant, which notably promoted tumor-specific Th1-based CD4$^+$ T cells and CTL responses and alleviated CD11b$^+$Gr1^{low} MDSCs, consequently triggering excellent anti-tumor activities[30]. Acetalated dextran as a tuneable acid-sensitive biopolymer could also facilitate antigen presentation, CD8$^+$ T cell responses and humoral

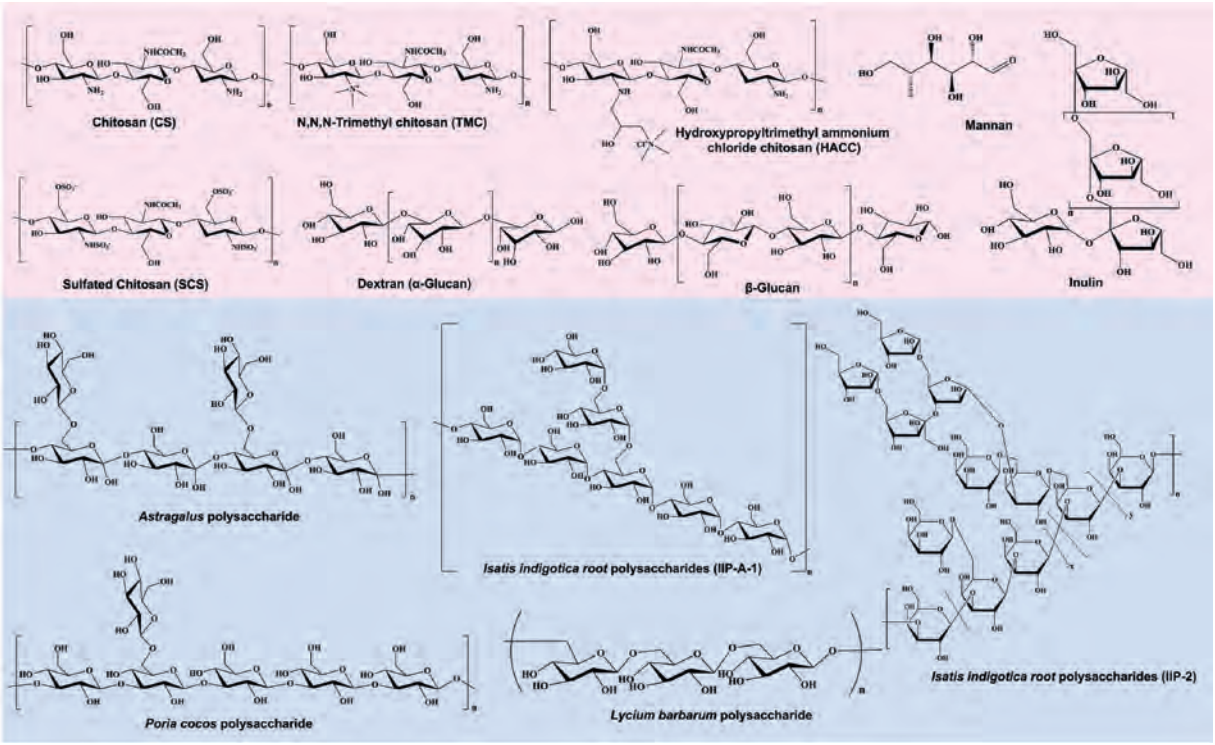

Figure 2 The structures of representative polysaccharides.

immunity[31], exhibiting potential adjuvant properties. Meanwhile, numerous studies revealed the strong immunostimulatory functions of β-glucan. β-glucan was potent in initiating protective trained immunity against *Mycobacterium tuberculosis via* regulating IL-1 signaling[32], while β-glucan from *Grifola frondose* augmented activation and maturation of APCs in a dose-dependent manner, and subsequently enhanced OVA antigen-specific antibody responses[33]. Moreover, β-glucan-containing

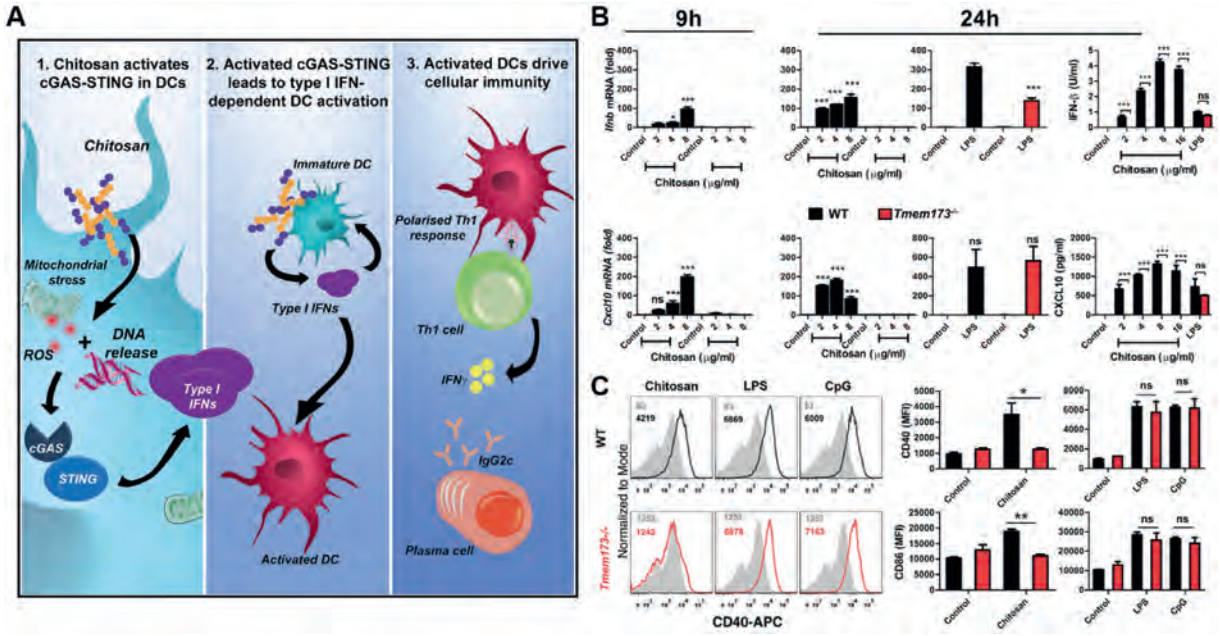

Figure 3 Chitosan activated cellular immunity *via* GAS−STING signaling. (A) Schematic diagram of the adjuvant effects of chitosan. (B) The mRNA expression and protein levels of IFN-β or CXCL10 produced by WT or *Tmem173*$^{-/-}$ DCs stimulated with chitosan or LPS. (C) CD40 and CD86 expression on WT and *Tmem173*$^{-/-}$ DCs which were either untreated or stimulated with chitosan, LPS or CpG. Data are presented as mean ± SEM (two independent experiments). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. ns, not significant. Reprinted with the permission from Ref. 8. Copyright © 2016 Elsevier Inc.

polysaccharides including α (1→3)-glucan, α (1→4)-glucan and β (1→6)-glucan as potent adjuvant markedly assisted the inactivated influenza A virus (IAV) vaccine to trigger IAV-specific antibody production as well as IL-2, IL-5 and IL-6 release[34].

2.3. Mannan

Mannan as another type of polysaccharide consisting of β (1→4)-linked D-mannose monomer units can potentiate antigen presentation and DC maturation, and stimulate the complement pathway *via* binding to mannan-binding lectin and C-type lectin receptors[3]. Fungal mannan could trigger powerful lymph node innate responses which required dectin-2- expressing $CD169^+$ sinus macrophages, non-canonical NF-κB and interferon pathways, while the combination of mannan and alum stimulated anti-SARS-CoV-2 spike type 1 immunity and neutralizing antibodies, and generated protection against viral infections of the lung[35]. D-Galacto-D-mannan also acted as a vaccine adjuvant to regulate Dectin-2 activation to simultaneously generate cellular and humoral immune responses against foot-and-mouth disease[36]. Furthermore, MGCP polysaccharides containing mannan and β-glucan amplified regulatory T (Treg) cell induction to exhibit anti-inflammatory activities *via* a Dectin1−Cox2 signaling and mitigated IFN-γ expression to prevent Th1 differentiation of effector T cells, whereas β-glucan-containing polysaccharides promoted inflammatory responses[37], suggesting the immunomodulatory functions of mannan polysaccharides.

2.4. Inulin

Inulin is a polysaccharide composed of fructose units mainly connected by β (2→1) glycosidic bonds. Delta-inulin named as Advax has been applied as an immune adjuvant in a variety of vaccines, such as hepatitis B, pandemic influenza, *Mycobacterium tuberculosis* and SARS-CoV-2 infection, and even some of them are in human phase 1 clinical trials[38,39]. Intrapulmonary delivery of delta-inulin showed excellent immunomodulatory capacity in augmenting tuberculosis vaccines to elicit the recruitment and activation of a diverse range of innate immune cells and cytokine releases[38]. Moreover, delta-inulin adjuvanted SARS-CoV-2 vaccines trigger robust immune responses and protection from COVID-19, and this immunoenhancing effect highly depended on the delivery site[40]. While both intratracheal and intramuscular vaccination could induce strong neutralizing antibody responses, intratracheal delivery of delta-inulin exhibited more potent and long-lasting lung-resident memory $CD4^+$ and $CD8^+$ T cells than intramuscular administration. Alternatively, it has been reported that TNF-α signaling was of the significance for the adjuvant effect of delta-inulin since $Tnfa^{-/-}$ mice failed to elicit antigen-specific antibody responses[41].

2.5. Polysaccharides derived from Chinese medicinal herbs

A variety of polysaccharides can be extracted from Chinese medicinal herbs, and many of them, such as *Isatis indigotica* root polysaccharides, *Astragalus* polysaccharide, *Poria cocos* polysaccharides, *Lycium barbarum* polysaccharides and lentinan, have been developed as potent adjuvants in vaccine strategies over few decades[42]. Recently, it has been reported that polysaccharide from Danshen which was the dried root of *Salvia miltiorrhiza* significantly magnified dendritic cell maturation in lymph nodes, spleen lymphocyte activation and OVA-specific antibody production, suggesting the potential immunostimulatory effect of *Salvia miltiorrhiza* polysaccharide[43]. Moreover, polysaccharide derived from cultivated *Cistanche deserticola* Y.C. Ma was potent in initiating DC maturation, antibody productions and Th1/Th2 responses *via* TLR4/NF-κB pathway[44], while *Artemisia rupestris* L. polysaccharide also activated NF-κB and MAPK signaling in a dose- or time-dependent manner and exhibited strong immunomodulatory functions in the enhancement of allogeneic T-cell activation and cytokine releases[45]. Importantly, recent studies uncovered the adjuvant mechanism of *Astragalus* polysaccharide in influenza split vaccine and recombinant SARS-CoV-2 vaccine[46]. *Astragalus* polysaccharide elicited lymphocyte activation and cytokine release to amplify immune responses, and also exhibited a positive feedback modulation since it would alleviate cytokine secretions to avoid excessive antibody productions. Furthermore, NF-κB and Fc gamma R-mediated phagocytosis pathways were essential for the potent immunomodulatory properties of *Astragalus* polysaccharide in the influenza split vaccine.

Although polysaccharide-based adjuvants have been widely investigated for many years, they were not ultimately approved for clinical vaccine applications due to their effectiveness and safety. Obviously, it still needs further improvements to make polysaccharides as potent adjuvants suitable for use in vaccines.

3. The immunoenhancing effects of nanoparticles

Nanoparticles have been commonly applied as versatile drug delivery systems for protecting and transporting antigens to immune organs and tissues, which usually endow vaccines with targeting properties[47-49]. More importantly, various nanoparticles, such as lipid nanoparticles, inorganic nanoparticles, polymeric nanoparticles, could regulate immune cells and downstream signaling pathways to possess intrinsic immunomodulatory effects[16], indicating the dual functions of nanoparticles in adjuvant platforms. Since nanoparticles are biologically effective in triggering immune signaling and responses, polysaccharides can be constructed into polysaccharide-based nanoparticles for enhanced adjuvant functions in further applications.

3.1. Lipid-based nanoparticles

Numerous studies have reported that liposome that is a classical type of nanoparticles could facilitate antigens to amplify humoral and cellular immune responses[50-53]. For instance, the stable presentation of recombinant hemagglutinin on immunogenic liposome boosted functional immune responses and elicited protection against the H5N1 influenza virus challenge[50]. Moreover, anionic liposomes exhibited potent adjuvant effects in tuning Th1, Th2, Treg and $CD8^+$ T cell responses depending on MyD88 signaling pathways to augment anti-tumor immunotherapy[52] while cationic liposomes could deliver antigens to draining lymph nodes and also function as depot-forming adjuvants to enhance strong adaptive immune response[53,54]. In recent years, lipid nanoparticles (LNPs) as another lipid-based nanoparticle have attracted more and more attention due to their successful approval for use in COVID-19 mRNA vaccines[19,55-57]. The adjuvant lipidoid-substituted LNPs have also been developed to deliver mRNA and enhance TLR7/8-agonistic activity for stimulating Th1-based cellular immune responses and neutralizing antibodies against multiple SARS-CoV-2 variants[19], while 480 biodegradable ionizable lipids in LNPs have been screened and optimized for enhancing the efficacy, safety

and ease of administration of LNPs-adjuvanted mRNA vaccines[55]. Another study has also reported that the modified LNPs could boost Tfh cell and humoral immune responses, and their immunomodulatory properties mainly depended on IL-6 release and the ionizable lipid[57].

3.2. *Inorganic nanoparticles*

Inorganic nanoparticles, such as gold nanoparticles, mesoporous silica nanoparticles, silver nanoparticles and iron oxide nanoparticles, exhibit potent immunoenhancing effects and function as promising adjuvants in vaccine applications[58-60]. Gold nanoparticles sized at 4.5 nm but not >10 nm activated NLRP3 inflammasome *via* ROS production and LC3 degradation to trigger antigen-specific immune responses, while rod gold nanoparticles preferably initiated NLRP3-mediated IL-1β and IL-18 release, and spherical or cube gold nanoparticles were potent in other inflammatory cytokine production, including TNF-α, IL-6, IL-12 and GM-CSF, suggesting that shape and size were crucial for the adjuvant effect of gold nanoparticles[61,62]. Moreover, mesoporous silica nanoparticles triggered the pore size-dependent immunostimulatory function in anti-tumour efficacy since mesoporous silica nanoparticles with different pore sizes (7.8, 10.3, and 12.9 nm) could markedly initiate the drainage to lymph nodes, antigen uptake and DCs maturation but those with larger pores was more effective in presentation of peptide-MHC I complexes to $CD8^+$ T cells (Fig. 4)[63]. Sliver nanoparticles also enhanced NK cell migration and IFN-γ production *via* interaction with alveolar macrophages to prevent influenza infection, and also adjuvanted vaccines to elicit bronchus-associated lymphoid tissue neogenesis and IgA-mediated mucosal immunity[64,65]. Iron oxide nanoparticles acted as anti-tumour immune adjuvants to suppress breast tumour metastasis and progression, which was probably related to TLR3 and IRF3 signaling[66]. $ZnFe_2O_4$-based nanoparticles promoted dendritic cell maturation, increased cytotoxic T lymphocyte and NK cell infiltration *via* cGAS/STING signaling, eventually alleviating tumor progression[67]. Additionally, metal adjuvants including aluminum and manganese exhibited enhancement of immunomodulatory effects after nanoparticle formation. For instance, aluminum nanoparticles elicited more potent $CD8^+$ T cell responses and anti-tumor immunotherapy than commercial aluminum adjuvant[68]. Manganese-based nanoparticles also possessed remarkable immunotherapeutic efficacy in preventing tumor progression *via* augmenting STING signaling[69].

3.3. *Polymeric nanoparticles*

Apart from lipid-based nanoparticles and inorganic nanoparticles, polymeric nanoparticles have been identified as potential immune adjuvant candidates for vaccine development[5,70]. The fluoropolymer could be mixed with ovalbumin antigen to fabricate F-PEI/OVA nanoparticles, and fluoropolymer markedly boosted antigen cross-presentation, DC maturation and $CD8^+$ T cell immune responses *via* TLR4 signaling pathway for triggering post-surgical

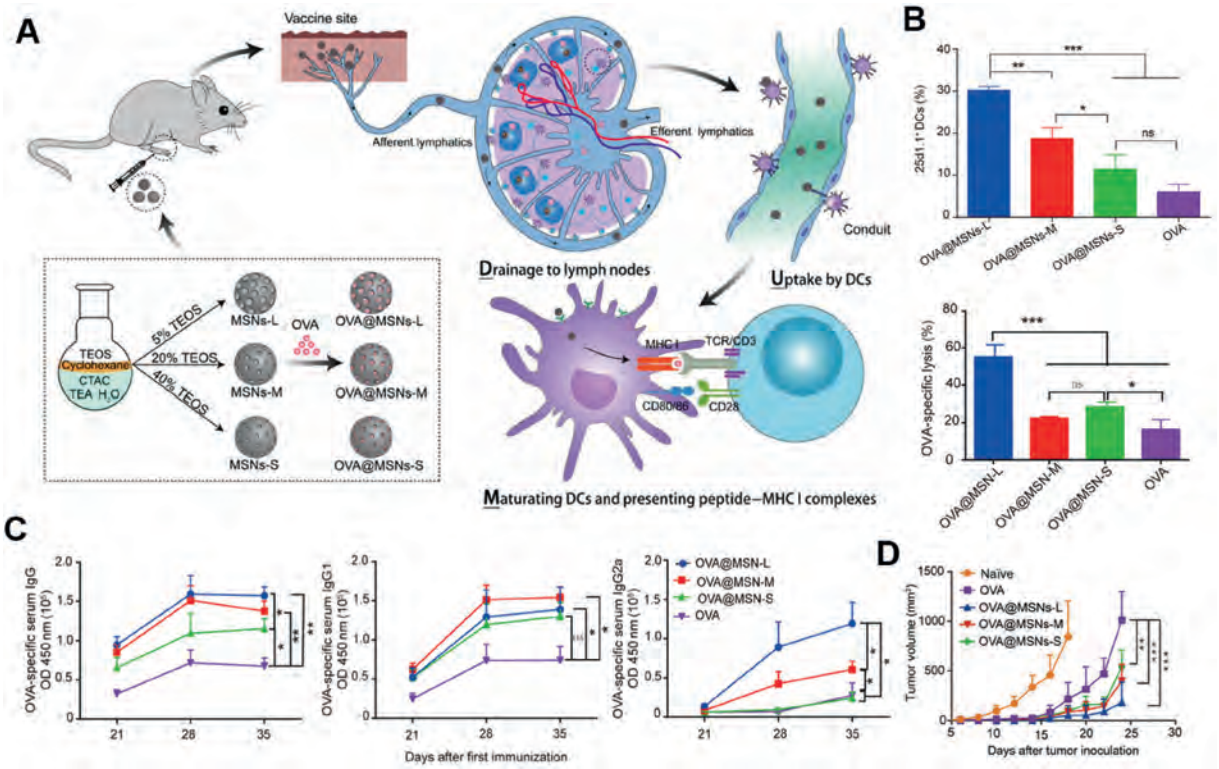

Figure 4 Mesoporous silica nanoparticles enhanced immune responses depending on pore size. (A) Schematic illustration of the DUMP cascade of antigen-loaded lymph node-targeting MSNs to induce adaptive immune response. (B) Cross-presentation of OVA in DC2.4 cells, and percentage of OVA-specific $CD8^+$ T cell lysis. (C) Levels of anti-OVA IgG, IgG1, and IgG2a antibodies in serum were assayed (10^5 dilution). (D) Tumor growth curves of mice immunized with free OVA and OVA@MSNs. Data are presented as mean ± SD (n = 3 to 10). *P < 0.05, **P < 0.01, ***P < 0.001. Reprinted with the permission from Ref. 63. Copyright © 2020 The American Association for the Advancement of Science.

cancer immunotherapy[71]. Moreover, the shape of particles also determined the adjuvant effect of polymeric nanoparticles[72]. While the physical mixture of PMA polymeric nanoparticles and antigen triggered more potent antibody production than chemical conjugation, rod-shaped PMA nanoparticles exhibited stronger immunostimulatory effects in producing antibody titers and cytokine secretion than worm-, sphere-, and tadpole-shaped nanoparticles[72]. Poly-γ-glutamic acid (γ-PGA)-based nanoparticles also acted as immune adjuvants to promote antigen uptake, DC activation, cytokine release and T cell responses, consequently stimulating anti-tumour immunity[73]. Additionally, the triblock copolymer TPCAH could encapsulate OVA antigen and efficiently yield powerful antibody production, $CD4^+$ T and $CD8^+$ T cell activation, and cytokines secretions as well as enhanced the complement system for adaptive immunity, indicating the adjuvant effect of this triblock copolymer[74]. Another polymeric nanoparticle pLHMGA could deliver peptide antigen and poly IC, and subsequently enhanced HPV-specific $CD8^+$ T cells in cancer vaccines[75], while TLR7/8 agonist-encapsulated PLGA nanoparticles were also effective in migrating to draining lymph nodes, DC activation and CTL responses for cancer immunotherapy[76].

4. Polysaccharide nanoparticles as potential adjuvants

Since nanoparticles possess dual functions of antigen delivery and immunostimulatory properties, nanosized polysaccharides may elicit more remarkable adjuvant functions than polysaccharides. Notably, lipid/polymer/inorganic materials-based nanoparticles have been widely investigated and put into clinical trials, and even some of them have been approved for use in vaccines, but they still have some problems like excessive immune activation and toxicity. Meanwhile, although there are rare clinical trials on polysaccharide nanoparticles, they with good immunogenicity and biodegradability can activate antigen-presenting cells, lymphocytes or NK cells, and promote productions of cytokines, antibodies and complement molecules for triggering excellent immune adjuvant effects, suggesting polysaccharide nanoparticles as potential adjuvant candidates. The advantages and disadvantages of polysaccharide nanoparticle adjuvants compared with other particulate adjuvants are summarized in Table 1. Unsurprisingly, there are numerous explorations on the construction, function and underlying adjuvant mechanism of polysaccharide nanoparticles, such as chitosan-based nanoparticles, glucan-based nanoparticles and mannan-based nanoparticles, although their immunomodulatory function and mechanism remain still unclear.

4.1. The adjuvant functions of polysaccharide nanoparticles

4.1.1. Chitosan-based nanoparticles

Chitosan nanoparticles have been widely investigated as potential immune adjuvants for delivering antigens and enhancing vaccine efficacy[9,26,27,77-81]. Our previous study has constructed chitosan nanoparticles (CS NPs) with three different MWs, and uncovered the MW-dependent functions and signaling mechanisms of chitosan nanoparticles[9]. Interestingly, CS NPs as cationic nanoparticle forms could efficiently deliver mRNA antigen and assist endosomal escape, and exhibited more excellent adjuvant effects in humoral and cellular immunity than chitosan. CS NP with high MW preferably elicited potent STING signaling and moderate NLRP3 inflammasome, while the robust induction of autophagy efficiently balanced NLRP3 signaling, ultimately initiating remarkable mRNA antigen-specific immune responses. In contrast, CS NPs with low or medium MW could not elicit strong immune responses since they failed to stimulate STING and autophagy activation and only induced strong NLRP3 signaling (Fig. 5)[9]. Noticeably, the problem of water insolubility also restricts the application of chitosan, and nanosized chitosans (chitosan nanoparticles) could promote the solubility of chitosan. Chitosan nanoparticles are usually fabricated under acid environment, whereas chitosan at physiological conditions cannot be dispersed completely, and therefore decreased concentration of chitosan and prolonged dissolution time are involved in the construction of chitosan nanoparticles for improving the solubility[9]. Alternatively, chitosan derivatives with excellent water solubility have been also constructed into nanoparticles and explored as potential adjuvants. It has been reported that chitosan and its derivatives HACC nanoparticles markedly elevated cytokine secretions, lymphocyte proliferation and $CD4^+$/$CD8^+$ T cellular immunity, but not humoral immunity, and triggered

Table 1 The advantages and disadvantages of polysaccharide nanoparticle adjuvants compared with other particulate adjuvants.

Nanoparticle	Basic composition	Advantage	Disadvantage
Lipid-based nanoparticles	Ionizable lipids, phospholipids, cholesterol, and polyethylene glycol	Plasticity and versatility, protecting mRNA antigen and increasing antigen stability, assisting mRNA to enter and transfect immune cells	Poor biological distribution, allergic or severe anaphylactic reactions, toxicity
Inorganic nanoparticles	Gold nanoparticles, mesoporous silica nanoparticles, sliver nanoparticles or iron oxide nanoparticles	Precise control of size, shape and charge, etc., easy to modification of nanoparticle surface, low price	Low biodegradability, toxicity, limited targeted delivery
Polymeric nanoparticles	Synthetic polymer nanoparticles, such as PLGA/pLHMGA/ TPCAH/PMA	Water-soluble, stable and capable of targeted delivery, precise control of loading efficiency, release kinetics and tissue-specific accumulation	Particle aggregation, toxicity
Polysaccharide nanoparticles	Chitosan, glucan, mannan, inulin or Chinese herbal medicine-derived polysaccharides	Intrinsic immune regulation, biocompatibility and biodegradability, inducing cellular immunity, humoral immunity and mucosal immunity	The purity and structure of polysaccharides affect immune activity

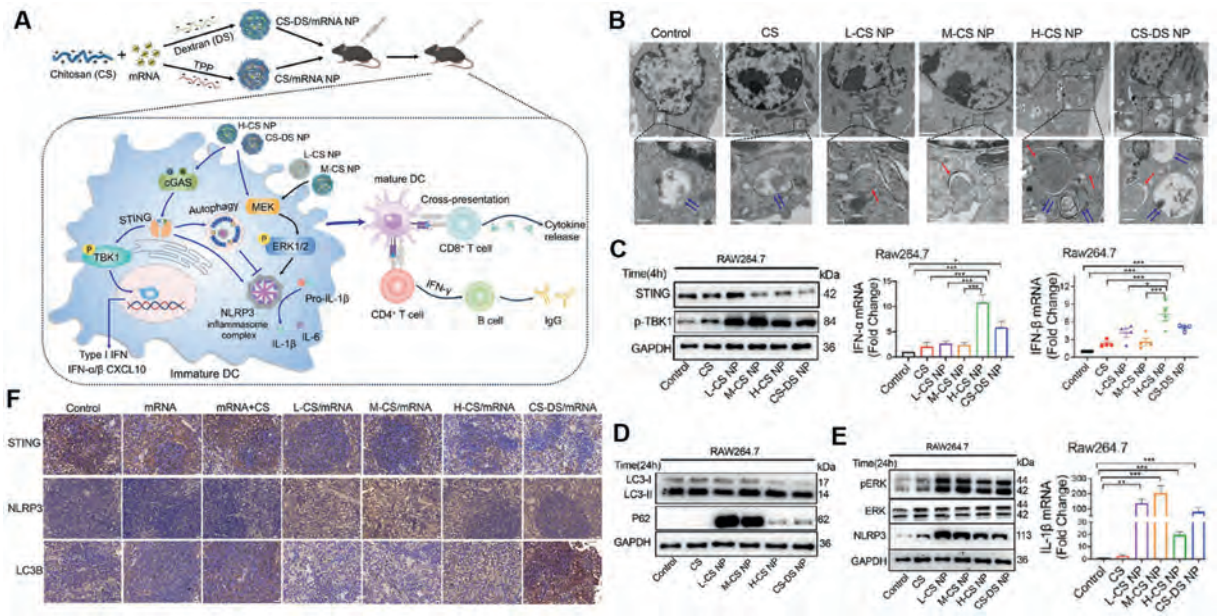

Figure 5 Polysaccharide nanoparticle tuned enhanced mRNA antigen-specific immune responses through MW-dependent STING, NLPR3 and autophagy signaling. (A) Schematical diagram of polysaccharide nanoparticle-enhanced mRNA antigen-specific immune responses and underlying mechanism. (B) TEM images of cells treated with different polysaccharide nanoparticles. Double-membrane autophagosomes (red arrows) and single-membrane autophagolysosomes (blue arrows). Scale bar = 1 μm. (C) The protein expressions of STING, and gene expression of *Ifnα* and *Ifnβ*. (D) The protein expressions of autophagy marker LC3I, LC3II and autophagy substrate. (E) The expressions of ERK, phosphorylated ERK, and NLRP3, and gene expression of *Il1β*. Data are presented as mean ± SEM ($n \geq 3$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Reprinted with the permission from Ref. 9. Copyright © 2023 American Chemical Society.

prevention against inactivated Newcastle disease[27]. Recent studies also showed that chitosan derivative nanoparticles, *N*-2-hydroxypropyl trimethyl ammonium chloride chitosan/*N*,*O*-carboxymethyl chitosan nanoparticles (*N*-2-HACC/CMCS NPs) *via* intranasal administration, exhibited the excellent immunomodulatory functions in enhancing both mucosal and systemic immune responses, which mainly depended on cGAS−STING/TBK1/IRF3 signaling[79,80].

Additionally, chitosan nanoparticles could be used for stabilizing Pickering emulsion, and chitosan nanoparticle-stabilized Pickering emulsion (CSPE) showed excellent adjuvant properties, which notably facilitated antigen uptake, antigen cross-presentation and T-cell activation to amplify both humoral and cellular immunity, triggering the potency in anti-tumour immunity[77]. Chitosan/γ-PGA nanoparticles also acted as immune adjuvants and possessed great potential in anti-tumor immunity *via* alleviating immunosuppressive myeloid cells and increasing $CD4^+$ T cell activation when cooperated with radiotherapy[78]. Chitosan/calcium phosphate nanosheet was notably effective in antigen delivery, antigen cross-presentation and Th1-type cytokine activation[82], suggesting their excellent capacity as antigen carriers and potential immunomodulators. Furthermore, chitosan was combined with another polysaccharide (dextran sulfate or hyaluronic acid) and Poly(I:C) to fabricate powerful polysaccharide adjuvant nanoparticles, which generate robust activation of antigen-presenting cells and immune responses against HIV peptide antigen[81]. Obviously, nanosized chitosan possessed enhanced adjuvant properties in both humoral and cellular immunity after chitosan was constructed into nanoparticles.

4.1.2. *Glucan-based nanoparticles*

Unlike chitosan nanoparticles, dextran (α-glucan) usually in combination with other materials or adjuvants in nanoparticle form elicited enhanced immunomodulatory functions in the research field of immune adjuvants. It has been reported that spermine-modified acetalated dextran-based nanoparticle delivered Nut3a and GM-CSF to trigger remarkable anti-tumor immunity, and this nanoparticle generated excellent adjuvant effects *via* modulating DC maturation, $CD3^+$ and cytotoxic $CD8^+$ T cell responses[83]. Acetalated dextran nanoparticles could be also combined with injectable alginate cryogels, which recruited and activated antigen-presenting cells, triggering potent chemo-immunotherapy[84]. Acetalated dextran microparticles with different degradation profiles also augmented humoral and cellular immune responses through controlled vaccine adjuvant and antigen delivery[85]. While fast-degrading antigen-loaded acetalated dextran microparticles exhibited superior antibody and cytokine productions, fast-degrading adjuvant-loaded microparticles triggered stronger potency at earlier time points and slow-degrading microparticles showed stronger potency at later time points. Additionally, dextran-modified hyaluronidase constructed as adjuvant nanomedicine could facilitate photodynamic-immunotherapy through ameliorating the immunosuppressive tumor microenvironment[86]. Dextran and β-cyclodextrin-based GSH-responsive nanoparticle platform largely promoted ROS-based immunogenic cell death and DC maturation, stimulating powerful anti-tumor therapeutics[87]. Dextran was also conjugated with mycophenolic acid to fabricate adjuvanted nanoparticles for markedly mitigating overactivated DCs and imiquimod-induced

psoriasis-like skin inflammation *via* modulating IL-23/Th17 axis (Fig. 6A)[88]. Furthermore, dextran-conjugated adjuvant CpG notably enhanced antigen uptake, lymph node-targeting as well as $CD8^+$ T cell activation to generate remarkable immunostimulatory effects for preventing tumour progression[89].

Alternatively, β-glucan-based nanoparticles which crosslinked with CpG-OND exhibited potent adjuvant activity in improving antigen uptake, DC maturation as well as Th1 and Th2-based immune responses[90], while β-glucan combined with chitosan and PLGA in a nanoparticle platform triggered promising immunostimulatory effects for tuberculosis treatment[91]. Moreover, β-glucan-modified inorganic nanoparticles showed excellent adjuvant properties. It has been reported that β-glucan-mesoporous silica nanoparticles with a particle size of ~100 nm exhibited a great potential as antigen delivery systems and adjuvants *via* elevating lymphatic-targeting capacity, antigen retention in lymph nodes and DC activation[92]. β-glucan-based superparamagnetic iron oxide nanoparticles also initiated trained immunity *via* mTOR signaling to provide robust prevention against sepsis (Fig. 6B)[93]. Additionally, yeast β-glucan-based nanoparticles could deliver methotrexate to initiate macrophage transformation from M1 to M2 and alleviate various proinflammatory cytokine expressions, largely inhibiting progression of rheumatoid arthritis[94].

4.1.3. *Mannan-based nanoparticles*

Since mannan can be recognized by pattern recognition receptors (PRRs) on various immune cells and especially can bind to DC-SIGN and mannose receptors highly expressed on DCs[95], mannan was usually decorated on the surface of nanoparticles for mediating the targeting and endocytosis of vaccines, and also exhibiting adjuvant effects. For instance, mannan coated polylactic acid-polyethyleneimine (PLA-PEI) nanoparticles were applied to deliver antigens and CpG. The mannan assisted nanoparticles with lymph node draining property and promote the capturing by DCs, while it as a TLR4 agonist also enhanced DC activation with CpG, consequently facilitating remarkable anti-tumor immunotherapy[96]. Furthermore, the decoration of mannan endowed allergen nanoparticles with DC-targeting capacity, and subsequently induced tolerogenic DCs and Treg cells to trigger preventative effects against allergen diseases (Fig. 6C and D)[97]. Mannan-coated STING-activating nanoparticles also showed great

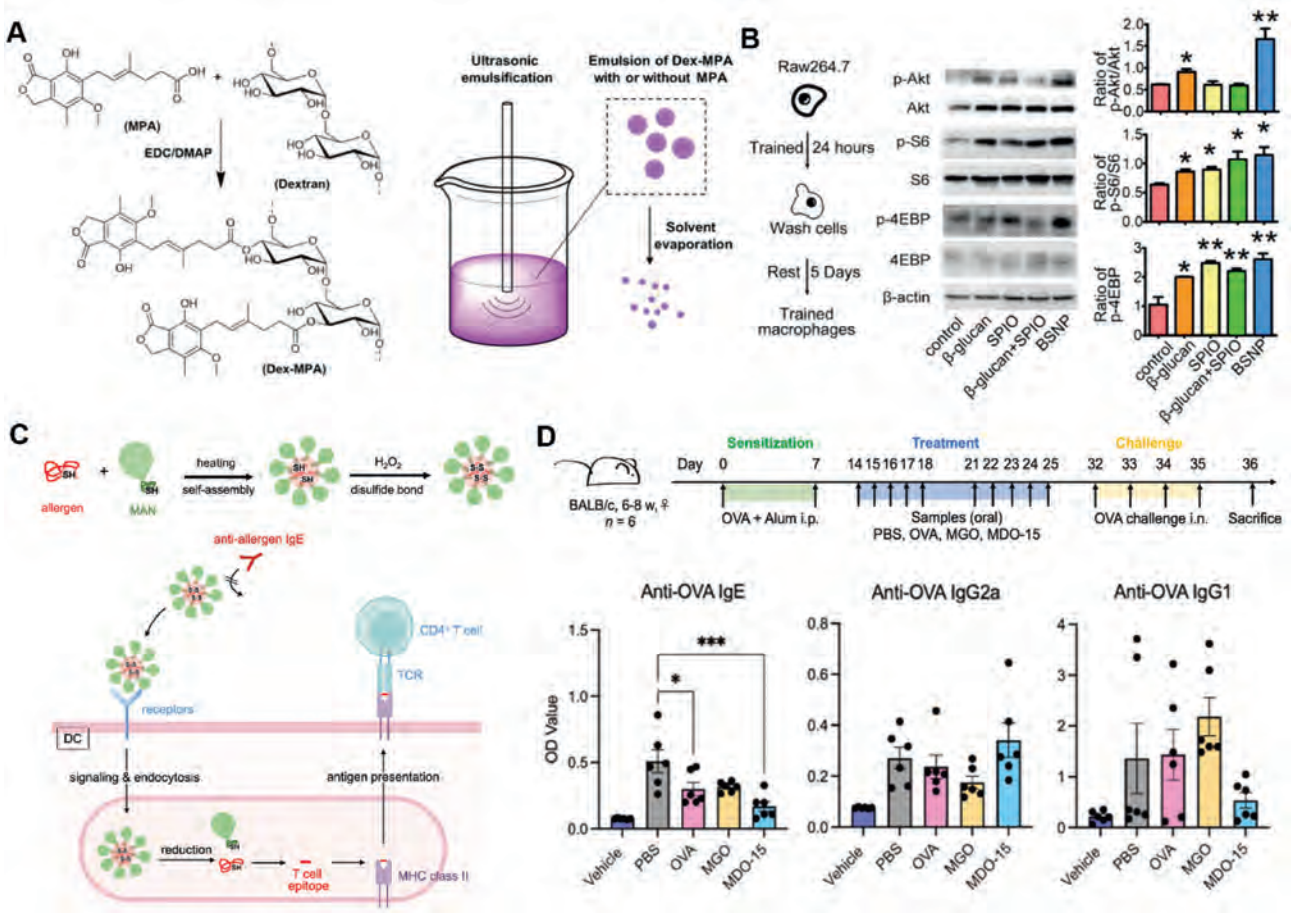

Figure 6 Glucan and Mannan-based nanoparticle facilitated immune responses. (A) Synthesis of Dex-MPA, and the preparation of dextran-based nanoparticles, Dex-MPA NPs or MPA@Dex-MPA NPs. (B) Schematic of *in vitro* trained immunity experimental setup, and the expression of proteins downstream mTOR pathway determined by Western blot. Data are presented as mean ± SEM ($n = 3$). $^*P < 0.05$, $^{**}P < 0.01$. (C) Preparation of mannan-based nanoparticles and functional mechanism of nanoparticles. (D) The treatment of allergic asthma by the oral administration of mannan-based nanoparticles, MDO-15, and serum anti-OVA antibodies measured by ELISA. Data are presented as mean ± SEM ($n = 6$). $^*P < 0.05$, $^{***}P < 0.001$. Reprinted with the permission from Refs. 88, 93 and 97 (Copyright © 2023 Elsevier Ltd., Copyright ©2021 Ivyspring International Publisher, Copyright ©2022 Ivyspring International Publisher).

potential in elevating DC-targeting ability and DC activation to suppress tumour progression, supporting the superior immunomodulatory effects of mannan-decoration[98]. Meanwhile, mannan-decorated mucoadhesive HPMCP microspheres elicited adjuvant functions in enhancing mannose receptor-mediated recognition and endocytosis as well as mucosal and systemic immune responses against *Actinobacillus pleuropneumoiae* infection[99]. Interestingly, the effect of chain length of mannan adjuvant potency of mannan was also investigated and uncovered that mannan-decorated lipid nanoparticles facilitated enhanced RNA antigen-specific immune responses with increasing the chain length of mannan from mono-to tetrasaccharide, but the booster dose responses plateaued above the length of disaccharide, suggesting the further enhancement of the length of disaccharide did not improve its immunostimulatory effects[100].

4.1.4. Other polysaccharide nanoparticles

In recent years, there are more and more investigations on other polysaccharide nanoparticles, such as Chinese medicinal herb-derived polysaccharide-based nanoparticles in the research field of adjuvant development. It has been reported that a natural polysaccharide from the rhizomes of *Bletilla striata* assembled with SARS-CoV-2 RBD protein to form nanovaccines, and this polysaccharide-based nanoparticle possessed a great potential in eliciting macrophages, B cells and dendritic cell activation, consequently amplifying humoral and cellular immune responses[101]. *Cistanche deserticola* polysaccharide-based nanoparticles also activate DCs, T cells and B cells to generate strong mixed Th1/Th2 response and mucosal immune responses[102]. Alternatively, polysaccharides can be loaded in a variety of particles, such as PLGA nanoparticles, $CaCO_3$ microparticles, chitosan-gold nanoparticles, alum particles and zinc oxide nanoparticles, to trigger excellent immunomodulatory functions[12,103-108]. For instance, *Viola philippica* polysaccharide loaded in chitosan-gold nanoparticles exhibited potent adjuvant functions to enhance robust protection against porcine circovirus type 2 virus mainly through antibody and cytokine production as well as T cell responses[106]. While *Alhagi honey* polysaccharide- or Chinese yam polysaccharides-encapsulated PLGA-based Pickering emulsion efficiently targeted DC to augment antigen uptake and DC activation for initiating strong and long-term cellular and humoral immune responses[103,105], *Polygonatum sibiricum* polysaccharide was loaded in $CaCO_3$ microparticles as promising adjuvants elicited enhanced secretion of IL-4, IL-6, IFN-γ, TNF-α and IgG, and ultimately increased the ratio of $CD4^+/CD8^+$ T cells and the frequency of $CD3^+$ $CD69^+$ T cells in spleen lymphocytes[104].

4.2. The immunoenhancing mechanism of polysaccharide nanoparticles

The modulation of immune cell signal transduction and functions by polysaccharide nanoparticles can remarkably assist the better understanding of their adjuvant effects. Over few decades, there are some explorations about the adjuvant mechanism of polysaccharide nanoparticles, such as the involvement of STING signaling, NLRP3 inflammasome, and autophagy[3,9]. However, the underlying immunomodulatory mechanism have not been fully elucidated and discussed.

4.2.1. cGAS−STING signaling

cGAS−STING signaling pathway is crucial for the detection of foreign DNA in innate immunity[109]. When cGAS detects double-stranded DNA (dsDNA) originated from pathogens like viruses or bacteria, cyclic GMP−AMP (cGAMP) is synthesized and binds to STING protein which causes STING to translocate from the endoplasmic reticulum to the Golgi apparatus. Once activated, STING triggers a signaling cascade involving phosphorylation of TBK1 and IRF3 protein, subsequently initiating type I interferons, such as IFN-α, and IFN-β[109]. While chitosan adjuvant enhanced cellular immune responses *via* cGAS−STING signaling[8], chitosan nanoparticles was also effective in eliciting STING activation[80,110,111]. The mannose-modified stearic acid-grafted chitosan micelles stimulated DC maturation and $CD3^+$ $CD8^+$ T infiltration depending on cGAS−STING signaling for enhanced anti-tumor immunity[110]. Chitosan could be assembled with PD-L1 antibody to form nanoparticles which also initiated the activation of cGAS−STING signaling to augment $CD8^+$ T cell responses for suppressing lung metastasis (Fig. 7A and B)[111]. Moreover, chitosan oligosaccharide-delivered DNA vaccine microneedle facilitates DC maturation as well as systemic and mucosal T cell immune responses against SARS-CoV-2 through activating cGAS−STING-mediated IFN signaling (Fig. 7C and D)[112]. Unsurprisingly, as described before, we have previously revealed that chitosan and chitosan/dextran nanoparticles with high MW elicited potent STING signaling and subsequently triggered excellent immunoenhancing capacity *via* maintaining the balance of NLRP3 inflammasome and autophagy signaling, whereas chitosan nanoparticles with low MWs could not induce STING and autophagy to evoke potent immunostimulatory effects[9].

Apart from chitosan nanoparticles, other polysaccharide nanoparticles possessed a great potential in eliciting cGAS−STING signaling for enhancing immune responses. For instance, dextran-based nanoadjuvants initiated phosphorylation of STING, TBK1 and IRF3 protein to promote macrophage M1 polarization, DC maturation as well as TNF-α and IL-6 production, suggesting the involvement of STING signaling in the immunoenhancing activity of dextran nanoparticles[113]. Another polysaccharide, fucoidan, derived from brown algae also constructed nanoparticles to efficiently deliver ferulic acid to protect against cisplatin-induced acute kidney injury *via* modulating cGAS−STING pathways[114]. Additionally, polysaccharide could assemble with metal elements to form nanoparticles as promising adjuvants by triggering the activation of STING signaling. The dendrobium polysaccharide hydrogel embedding manganese microsphere could promote cGAS−STING signaling to initiate immunogenic cell death of tumor cells and activation of immune cells for mitigating tumor growth and metastasis[115], while nanoparticles with Cu^{2+}-chitosan shell provoked superior STING activation and cuproptosis to promote DC maturation and innate and adaptive immunity, and subsequently induced anti-tumor immunity to alleviate lung metastasis[116]. Therefore, based on current studies, cGAS−STING is one of the most significant signaling pathways involving in the adjuvant mechanism of polysaccharide nanoparticles (Fig. 8A).

4.2.2. NLRP3 inflammasome signaling

NLRP3 (NOD-, LRR- and pyrin domain-containing 3) as an essential sensor in the innate immune system participates in recognizing microbial pathogens and cellular damage, and initiating inflammatory responses[117]. Moreover, NLRP3 is a key functional component which can assembled with an adaptor ASC and an effector caspase-1 to form NLPR3 inflammasome complex. The activation of NLRP3 inflammasome involves proteolytic cleavage of dormant procaspase-1 to active form of caspase-1,

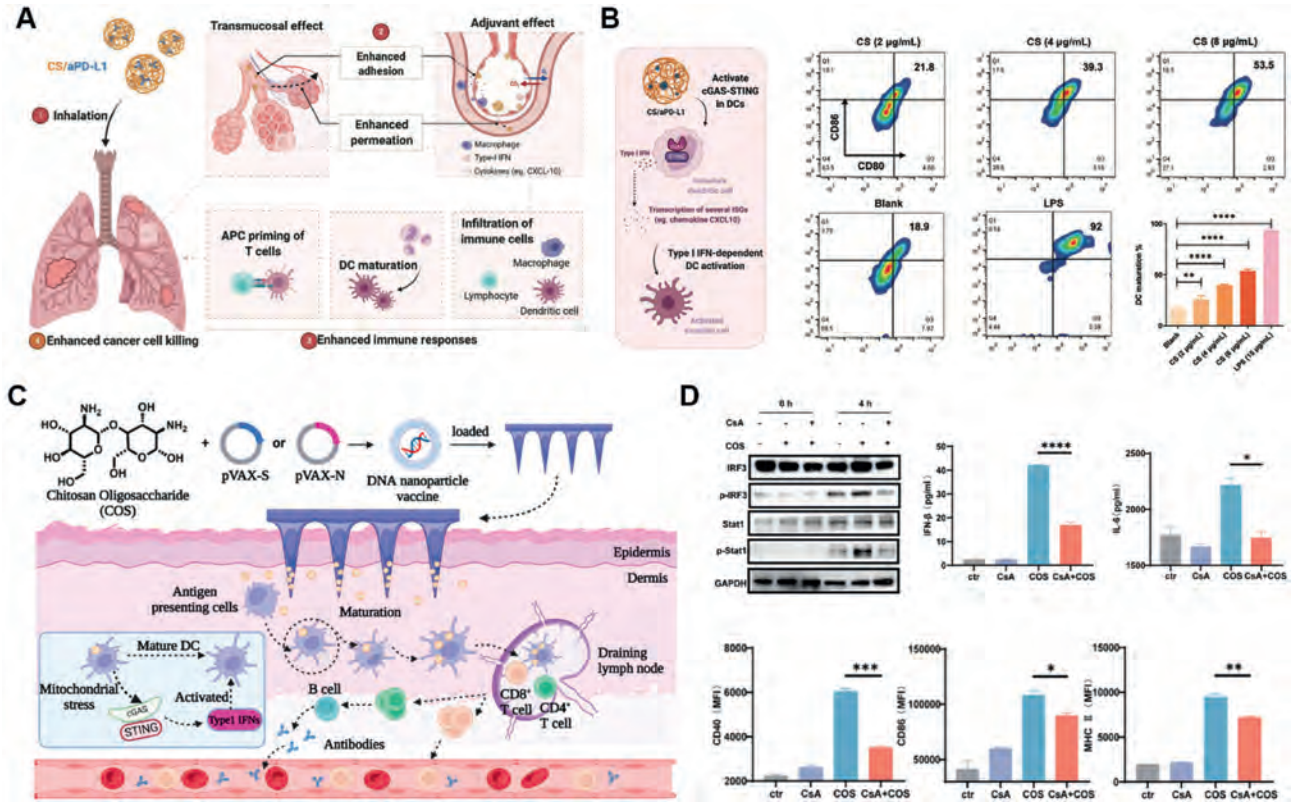

Figure 7 Polysaccharide-based nanoparticles enhanced immune responses through cGAS−STING signaling. (A) Schematic illustration of chitosan (CS)/aPD-L1 nanocomplex inhalation for suppressing lung metastases. (B) Illustration of adjuvant effects induced by CS *via* activating the cGAS−STING pathway, and the surface expressions of CD80 and CD86 on BMDCs incubated with various formulations analyzed by flow cytometry. Data are presented as mean ± SD ($n = 3$). $^{**}P < 0.01$, $^{****}P < 0.0001$. (C) Diagrammatic sketch to illustrate that the chitosan oligosaccharide (COS)-engineered DNA nanoparticle vaccine induced a robust innate immunity and adaptive immunity. (D) COS activated the major transcription factors involved downstream of the cGAS−STING-dependent pathway including IFR3, Stat1, IFN-β and IL-6, and CD40, CD86, and MHC II expression. Data are presented as mean ± SEM ($n = 3$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. Reprinted with the permission from Refs. 111 and 112 (Copyright © 2021 Wiley-VCH GmbH, Copyright © 2023 American Chemical Society).

subsequently eliciting IL-1β and IL-18 activation[118]. Interestingly, it has been reported that NLRP3 inflammasome was one of the most active signaling pathways participating in commercial adjuvants, such as aluminum[2]. Unsurprisingly, polysaccharide nanoparticles also modulated NLRP3 inflammasome activation to exhibit immunomodulatory functions (Fig. 8B). *Pleurotus ferulae* polysaccharides-gold nanoparticles promoted ERK, NF-κB and NLRP3 expressions to initiate Th1 cell responses and anti-tumor immunity, suggesting that the adjuvant effects of nanoparticles mainly depended on NLRP3 and TLR4 signaling[119]. Chitosan and carboxymethyl chitosan nanoparticles also assisted doxycycline to prevent periodontal disease *via* modulating NLRP3 inflammasome and IL-1β production[120]. Furthermore, the DDA of chitosan was crucial for NLRP3 inflammasome signaling, since chitosan with a DDA below 80% could not induce NLRP3 activation and fully deacetylated chitosan was the most potent immune adjuvant among chitosans with different DDA[121]. Alternatively, β-glucan-based nanoparticles possessed superior Dectin-1^{+} monocytes/macrophages-targeting capability to suppress cardiac ischemic/reperfusion injury with the active involvement of NLRP3 inflammasome[122]. Importantly, NLRP3 inflammasome was the downstream signaling pathway of STING and our previous study uncovered that chitosan nanoparticles or chitosan/dextran nanoparticles triggered STING-mediated NLRP3 activation which was balanced by autophagy to maintain moderate NLRP3 signaling to amplify humoral and cellular immune responses, suggesting the importance of NLRP3 signaling in the adjuvant effects of polysaccharide nanoparticles[9].

4.2.3. *TLRs signaling*

Toll-like receptors (TLRs) belong to pattern recognition receptors (PRRs) that detect microbial infections and subsequently initiate immune responses, particularly innate immunity, for host defence[123]. They possess an extracellular domain that recognizes specific patterns associated with pathogens (PAMPs) or damaged cells (DAMPs), and an intracellular domain that recruits adaptor proteins, such as MyD88 or TRIF to initiate downstream signaling pathways, including NF-κB and MAPK pathway, leading to pro-inflammatory cytokines productions. Among TLRs, TLR4 has been one of the most active participants in polysaccharide nanoparticle-mediated immunomodulatory functions (Fig. 8C). The *Astragalus* polysaccharide nanoparticles triggered activation of TLR4/NF-κB pathway to reduce the inflammatory response and consequently alleviated septic cardiac dysfunction[124], while *Pholiota adiposa* polysaccharide-based nanoparticles initiated macrophage polarization from M2 to M1 phenotype and enhanced T cell responses to suppress tumor progression *via* TLR4/MyD88/NF-κB signaling aixs[125]. Moreover, chitosan conjugated with

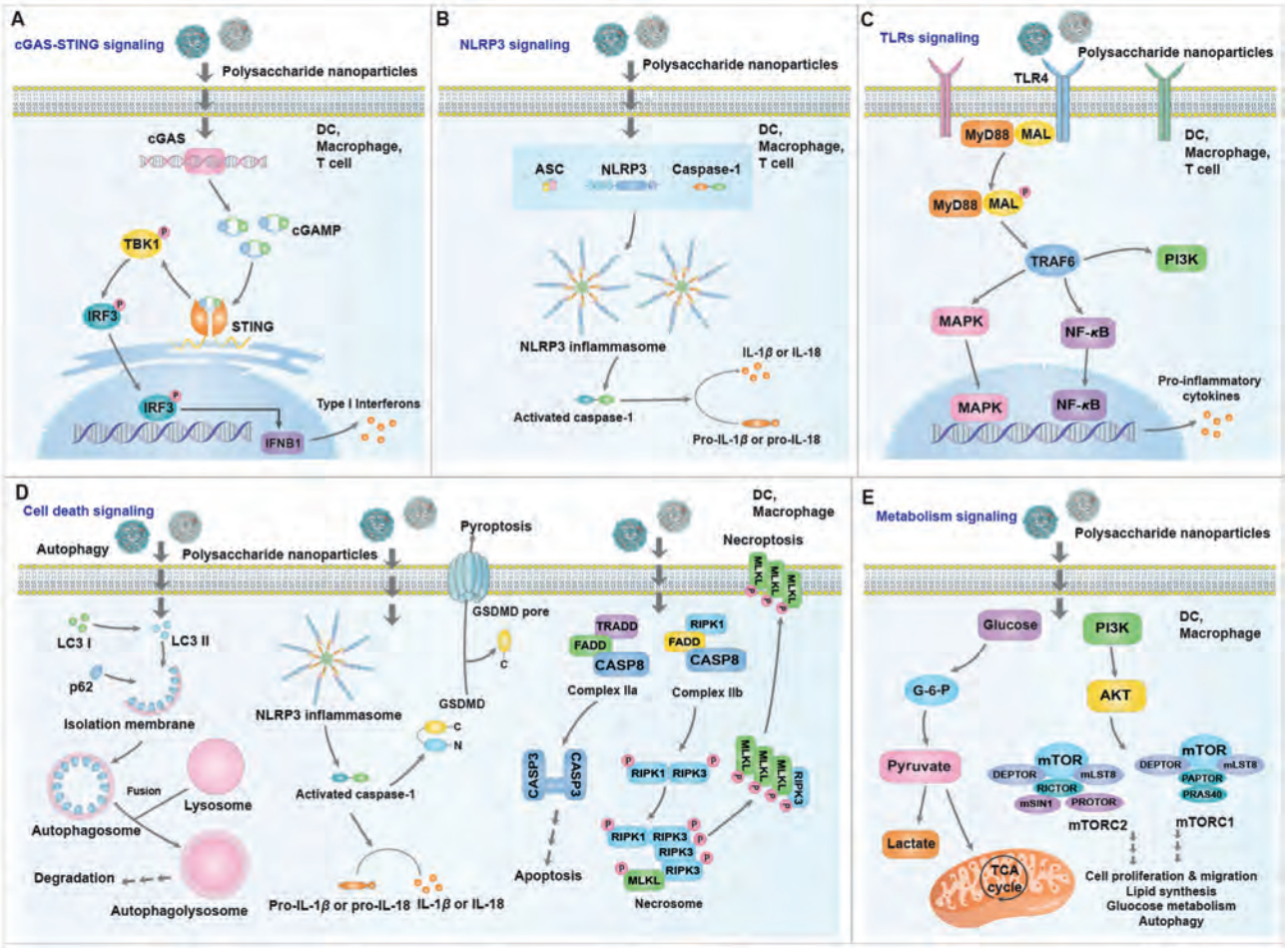

Figure 8 The potential signal mechanism of polysaccharide adjuvant-based nanoparticles. (A) cGAS−STING siganling. (B) NLRP3 signaling. (C) TLRs signaling. (D) Cell death signaling. (E) Metabolism signaling.

gentamicin could mitigate heat stress-induced mucosal damage mainly through attenuating TLR4/STAT6/MYLK signaling[126]. It has been also reported that mannan acted as a TLR4 agonist to exhibit synergistic immunostimulatory effects with CpG in a nanovaccine system, which markedly elicited DC activation and robust anti-tumor immune responses for suppressing tumor growth[96]. Additionally, TLR2 also played a significant role in the adjuvant effects of polysaccharide nanoparticles. β-Glucan could specifically interact with dectin-1 and TLR2 on the surface of APCs, which was beneficial for antigen uptake and processing as well as APC maturation, and consequently, β-glucan/CpG-OND-based nanoparticles showed great potential in amplifying antigen-specific immune responses against infectious diseases and cancers[90]. *Lactobacillus* exopolysaccharide nanoparticles as promising adjuvants also assisted the SARS-CoV-2 vaccine to trigger humoral and cellular immune responses through the positive involvement of TLR2 and TLR4[127].

4.2.4. Cell death

While the elimination of host immune cells is initially considered to benefit an infecting pathogen, emerging evidence suggests that cell death, such as apoptosis, autophagy, pyroptosis, and necroptosis, contributes to activating immune responses for combating pathogen[128,129]. Programmed cell death can not only destroy the niche of certain pathogens and coordinate subsequent immune responses, but also release intracellular pathogens, antigens, and danger signals detected by nearby immune cells, consequently triggering signal transduction, cytokine release and enhanced immune cell functions. Unsurprisingly, some studies have reported the possible participation of immune cell death in the immunomodulatory activity of adjuvants including polysaccharide nanoparticles (Fig. 8D). Autophagy as a crucial "self-eating" cellular process involves the degradation and recycling of cellular components, which was induced by polysaccharide adjuvants in immune cells, such as dendritic cells, macrophages, to facilitate immune responses. For instance, *Apios americana* Medikus tuber polysaccharide promoted HMGB1−Beclin1, Sirt1−FoxO1 and Akt−mTOR signaling to initiate the activation of autophagy in macrophages *via* increasing expressions of related proteins, LC3, Beclin1, Atg4, Atg5, and Atg7, and consequently exerted potent anti-inflammatory responses[130], indicating the effect of autophagy in macrophage functions. Moreover, chitosan hydrogel-loading melanin composite nanoparticles elicited the transformation of M1 to M2 macrophages and initiated the autophagy activation of M2 macrophages to modulate immune activation for wound healing[131]. β-glucan nanoparticles also stimulated NOX-2-mediated autophagy activation and phagosomal maturation to trigger excellent anti-microbial activity[132],

supporting the potential importance of autophagy activation for the adjuvant effect of polysaccharide nanoparticles. Importantly, the MW of polysaccharides largely affected the polysaccharide nanoparticle-modulated autophagy activation of immune cells. We have previously uncovered that chitosan-based nanoparticles with high MW, but not low MW, exerted more powerful effects in autophagy signaling of dendritic cells and macrophages, which could suppress the excessive NLRP3 inflammasome for triggering superior adjuvant potency[9].

Alternatively, pyroptosis as an inflammatory type of cell death is mediated by inflammasome, including NLRP3 inflammasome, in which NLRP3 inflammasome promoted caspase-1 activation as well as IL-1β and IL-18 secretion, and subsequently elicited the cleavage of gasdermin family proteins, particularly gasdermin D (GSDMD), to form pyroptotic pores in cell membrane[133]. While NLRP3 inflammasome is one of the significant immune signaling pathways, the downstream pyroptosis of immune cells actively participated in regulating immune responses[134]. Moreover, the essential role of NLPR3 signaling in polysaccharide nanoparticle-mediated immunoenhancing functions suggests the possible involvement of pyroptosis. However, although polysaccharides such as β-glucan modulated NLRP3-dependent pyroptosis and IL-1β secretion *via* dectin-1 in immune cells, most studies focus on polysaccharide-induced pyroptosis in tumor cells, but not immune cells[135]. Similarly, other cell death forms, like RIPK3/MLKL-mediated necroptosis and apoptosis, in immune cells play important roles in immune responses[128], however, there is rare investigation on their participation in the adjuvant effects of polysaccharide nanoparticles. Obviously, it needs further explorations about programmed cell death of immune cells, such as pyroptosis, necroptosis and apoptosis, in polysaccharide nanoparticle-mediated adjuvant effects assisting a better understanding of the immunoenhancing mechanism of polysaccharide nanoparticles.

4.2.5. Metabolism

The metabolism of myeloid cells can programme the innate immune responses and stimulation of T cell activation, and the mammalian target of rapamycin (mTOR) as a central metabolic regulator actively participates in shaping immune cell functionality[136]. It has been reported that extracellular or intracellular stimulus could bind to their cognate receptors, such as growth factor receptors, TLRs or cytokine receptors to activate mTOR complex 1 (mTORC1) and mTORC2, and then elicit PI3K and AKT phosphorylation, ultimately modulating innate immune cells[137]. For instance, the reprogramming of glycolytic monocytes and transient co-activation of mitochondrial pathways promoted TLR4-dependent DC maturation, while the immune profiles of DCs were highly dependent on mTOR/AMPK phosphorylation balance as well as glycolytic and fatty acid oxidation metabolism[138]. Therefore, it suggested that the metabolic modulations of immune cells were probably involved in the mechanism of adjuvants (Fig. 8E). Polysaccharides, such as *Astragalus* polysaccharide and *Dendrobium officinale* polysaccharide, regulated PI3K/AKT/mTOR or ROS−AMPK signaling to suppress Parkinson diseases or colon cancer[139,140], indicating the participation of metabolism in the immunomodulation of polysaccharides. Furthermore, betanin-encapsulated chitosan-based nanoparticles exhibited potent blockade in breast tumor progression *via* PI3K/AKT/mTOR signaling[141], while blackberry polysaccharide-based nanoparticles largely augmented glucose metabolism *via* PI3K/AKT singaling and lipid metabolism through AMPK singling for treating insulin resistance[142]. The self-assembled tea polysaccharide-based nanoparticles also exhibited strong inhibitory effects in type 2 diabetes *via* modulating glucogenesis and lipid metabolism[143]. Importantly, β-glucan-encapsulated nanoparticles mediated trained immunity through metabolic programming of immune cells, since β-glucan activated Dectin-1 to initiate phosphorylation of AKT/mTOR and inhibiting mTOR remarkably decreased trained effects[144]. However, current studies have not fully elucidated the involvement of metabolism signaling in the adjuvant mechanism of polysaccharide nanoparticles, and it still requires deep investigations.

5. Conclusions and perspectives

Polysaccharides, such as chitosan, glucan, mannan, Chinese medicinal herb-derived polysaccharides, with adjuvant effects have been widely investigated in a variety of vaccines[3]. Meanwhile, nanoparticles exhibited dual functions of antigen drug delivery system and intrinsic adjuvant property[17], suggesting that polysaccharides in nanoparticle construction were beneficial for improving immunoenhancing effects. Unsurprisingly, polysaccharide nanoparticles can function as potent vaccine adjuvants to markedly augmented humoral, cellular and mucosal immune responses, which was more potent than polysaccharide, probably through modulating signal transductions in immune cells, such as cGAS−STING signaling, NLRP3 signaling, TLRs signaling, cell death or metabolism (Fig. 8, Table 2[9,27,77-82,85,86,88-94,96-106,110−113,116,119,121,125,127,131,132,141,143,144]).

More importantly, the following research directions can be explored for the effective utilization of polysaccharide nanoparticles as promising immune adjuvants: (i) Optimized methods for fabrication of polysaccharide nanoparticles. As biomacromolecules, most polysaccharides, such as chitosan, exert some limitations on water solubility and degradation, which make it difficult to construct polysaccharide nanoparticles. Therefore, it needs more enhancements on the fabrication method of polysaccharide nanoparticles to address these problems; (ii) The effect of distinct physicochemical properties of polysaccharide nanoparticles. It has been reported that the adjuvant function of polysaccharides or nanoparticles was dependent on physicochemical properties including MW and DDA of polysaccharides as well as the shape and size of nanoparticles[9,72]. Moreover, the influence of physicochemical properties seems different in different types of polysaccharide nanoparticles, and further investigations on various polysaccharide nanoparticles are urgently required; (iii) Further explorations on the adjuvant mechanism of polysaccharide nanoparticles. Current studies about the immunoenhancing mechanism of polysaccharide nanoparticles mainly focus on STING and NLPR3 signaling pathways, and it lacks investigations on the involvement of other important immune cell signaling and functions, such as cell death (pyroptosis, necroptosis, apoptosis etc.), metabolism and epigenetics; (iv) The improvement of polysaccharide nanoparticles towards clinical application. Although polysaccharide nanoparticles notably enhanced immunomodulatory functions, their adjuvant potency and mechanism still need deep elucidations. The systemic understanding of adjuvanticity can provide a foundation for the modulation and improvement of polysaccharide nanoparticles for clinical applications. Additionally, a single adjuvant component may not trigger enough immunoenhancing effects, and polysaccharides combined with other adjuvants in nanoparticle forms can be another direction for developing efficient licensed adjuvants. Collectively, this review

Table 2 An updated summary on the adjuvant function and signal mechanism of polysaccharide nanoparticles.

Polysaccharide nanoparticle	*In vitro* (cell type)*/in vivo*	Function	Signal mechanism	Ref.
Chitosan nanoparticles, chitosan/dextran nanoparticles	DC, macrophage, *in vivo*	Enhanced immune responses in mRNA vaccines	Amplified STING, NLPR3 and autophagy signaling depending on the MW of chitosan	9
Chitosan nanoparticles, chitosan/HACC nanoparticles	*In vivo*	Increased cytokine secretions, lymphocyte proliferation and $CD4^+$/$CD8^+$ T cellular immunity against inactivated newcastle disease	−	27
Chitosan nanoparticle-stabilized pickering emulsion	DC, macrophage, *in vivo*	Augmented antigen uptake, antigen cross-presentation and T-cell activation to initiate both humoral and cellular immunity as well as anti-tumour immunity	−	77
Chitosan/γ-PGA nanoparticles	*In vivo*	Modulated $CD4^+$ T cell activation and immunosuppressive myeloid cells when cooperated with radiotherapy for anti-tumor immunity	−	78
N-2-HACC/CMCS nanoparticles	DC, *in vivo*	Promoted lymphocyte proliferation and pro-inflammatory factor secretion to enhance mucosal and systemic immune responses	Activated TLR4/NF-κB signaling pathway	79
N-2-HACC/CMCS nanoparticles	Macrophage, *in vivo*	Facilitated BMDC activation, cytokine release and Th1 responses	Stimulated cGAS−STING signaling pathway	80
Chitosan/dextran sulfate or hyaluronic acid/ Poly(I:C) Nanoparticles	*In vivo*	Generated activation of antigen-presenting cells and immune responses against a HIV peptide antigen	−	81
Chitosan/calcium phosphate nanosheet	DC, *in vivo*	Elevated antigen delivery, antigen cross-presentation and Th1-type cytokine activation	−	82
Acetalated dextran-based microparticles	DC, *in vivo*	Augmented humoral and cellular immune responses through controlled vaccine adjuvant and antigen delivery	−	85
Dextran-modified hyaluronidase nanoparticles	*In vivo*	Acted as nanoadjuvant to ameliorate immunosuppressive tumor microenvironment for enhancing photodynamic immunotherapy	−	86
Mycophenolic acid-conjugated dextran nanoparticles	DC, *in vivo*	Mitigated overactivated DCs and imiquimod-induced psoriasis-like skin inflammation	Modulated IL-23/Th17 axis	88
Dextran−CpG conjugates	DC, *in vivo*	Enhanced antigen uptake, lymph node-targeting and $CD8^+$ T cell activation to for preventing tumour progression	−	89
β-Glucan-CpG-OND nanopaticles	Macrophage, *in vivo*	Improved antigen uptake, DC maturation as well as Th1 and Th2-based immune responses	Interacted with TLR2 and dectin-1	90
β-Glucan-chitosan-PLGA nanoparticles	*In vivo*	Induced potent immunostimulatory effects for tuberculosis treatment	−	91
β-Glucan-mesoporous silica nanoparticles	DC, *in vivo*	Promoted lymphatic-targeting capacity, antigen retention in lymph nodes and DC activation	−	92
β-Glucan-based superparamagnetic iron oxide nanoparticles	Macrophage, *in vivo*	Elicited trained immunity to prevent against sepsis	Regulated mTOR signaling pathway	93
Yeast β-glucan-based nanoparticles	Macrophage, *in vivo*	Initiated macrophage transformation from M1 to M2 and decreased various proinflammatory cytokine expressions to suppress rheumatoid arthritis	−	94

(*continued on next page*)

Table 2 (*continued*)

Polysaccharide nanoparticle	*In vitro* (cell type)*/in vivo*	Function	Signal mechanism	Ref.
Mannan-coated PLA-PEI nanoparticles	DC, *in vivo*	Increased lymph node draining capacity and DC activation for anti-tumor immunotherapy	Regulated TLR4 signaling	96
Mannan-coated OVA nanoparticles	DC, *in vivo*	Augmented DC-targeting ability, and tolerogenic DCs and treg cells to prevent against allergen diseases	−	97
Mannan-coated STING-activating nanoparticles	DC, *in vivo*	Elevated DC-targeting ability and DC activation to suppress tumour progression	−	98
Mannan-decorated mucoadhesive HPMCP microspheres	Macrophage, *in vivo*	Facilitated mannose receptor-mediated recognition and endocytosis, and mucosal and systemic immune responses against infection	−	99
Mannan-conjugated liposome	*In vivo*	Potentiated RNA antigen-specific immune responses	−	100
Polysaccharide from the rhizomes of *Bletilla striata*-based nanovaccine	Macrophage, *in vivo*	Elicited macrophages, B cells and dendritic cell activation for enhancing humoral and cellular immune responses	−	101
Cistanche deserticola polysaccharide-based nanoparticles	DC, *in vivo*	Activated DCs, T cells and B cells to generate strong mixed Th1/Th2 response and mucosal immune responses	−	102
Alhagi honey polysaccharide- or *Chinese yam* polysaccharides-encapsulated PLGA-based pickering emulsion	*In vivo*	Targeted DC to increase antigen uptake and DC activation for initiating cellular and humoral immune responses	−	103,105
Polygonatum sibiricum polysaccharide-loaded $CaCO_3$ microparticles	*In vivo*	Enhanced secretion of IL-4, IL-6, IFN-γ, TNF-α and IgG, as well as $CD4^+$/$CD8^+$ T cells and $CD3^+CD69^+$ T cells in spleen lymphocytes	−	104
Viola philippica polysaccharide-loaded chitosan-gold nanoparticles	*In vivo*	Stimulated antibody, cytokine production and T cell responses for protection against porcine circovirus type 2 virus	−	106
Mannose-modified stearic acid-grafted chitosan micelles	DC, *in vivo*	Promoted DC maturation and $CD3^+CD8^+$ T infiltration for anti-tumor immunity	Triggered cGAS−STING signaling	110
Chitosan-antibody nanocomplex	*In vivo*	Amplified $CD8^+$ T cell responses for suppressing lung metastasis	Activated cGAS−STING signaling	111
Chitosan oligosaccharide-encapsulated DNA vaccine microneedles	DC, macrophage, *in vivo*	Facilitated DC maturation, systemic and mucosal T cell immune responses against SARS-cov-2	Elicited cGAS−STING-mediated IFN signaling	112
Dextran-based nanoadjuvants	Macrophage, *in vivo*	Improved macrophage M1 polarization, DC maturation, TNF-α and IL-6 production	Upregulated STING signaling	113
Cu^{2+}-chitosan shell-based nanoparticles	DC, *in vivo*	Induced DC maturation as wells as innate and adaptive immunity for alleviating lung metastasis	Provoked STING signaling and cuproptosis	116
Pleurotus ferulae polysaccharides-gold nanoparticles	DC, *in vivo*	Triggered Th1 cell responses and anti-tumor immunity	Stimulated NLRP3 and TLR4 signaling	119
Chitosan-based nanoparticles	DC, *in vivo*	Activated DCs, mitochondrial ROS and antigen-specific Th1 immune responses depending on DDA	Modulated NLRP3 and STING-type I IFN signaling	121
Pholiota adiposa polysaccharide-based nanoparticles	Macrophage, *in vivo*	Initiated macrophage polarization from M2 to M1 and T cell responses for anti-tumor immunity	Activated TLR4/MyD88/NF-κB signaling aixs	125

Table 2 (*continued*)

Polysaccharide nanoparticle	*In vitro* (cell type)/*in vivo*	Function	Signal mechanism	Ref.
Lactobacillus exopolysaccharide nanoparticles	Macrophage, *in vivo*	Assisted SARS-CoV-2 vaccine to induce humoral and cellular immune responses	Regulated TLR2 and TLR4 signaling pathway	127
Chitosan hydrogel loading melanin composite nanoparticles	DC, macrophage, *in vivo*	Stimulated transformation of M1 to M2 macrophage and immune activation for wound healing	Induced autophagy activation	131
β-Glucan nanoparticles	Macrophage	Triggered phagosomal maturation to trigger excellent anti-microbial activity	Initiated NOX-2-mediated autophagy activation	132
Betanin-encapsulated chitosan-based nanoparticles	−	Alleviated tumor cell migration and proliferation	Modulated PI3K/AKT/mTOR signaling	141
Self-assembled tea polysaccharide-based nanoparticles	*In vivo*	Exhibited strong inhibitory effects in type 2 diabetes	Regulated glucogenesis and lipid metabolism	143
β-Glucan-encapsulated nanoparticles	Macrophage, *in vivo*	Mediated trained immunity through metabolic programming of immune cells	Initiated AKT/mTOR signaling	144

gives an updated summary and perspective about the adjuvant function and mechanism of polysaccharide nanoparticles, providing a platform for wide applications of polysaccharide nanoparticles as potent immune adjuvants in various vaccine strategies.

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Author contributions

Yuhong Jiang conceived, drafted and refined the manuscript. Shanshan Qi and Canquan Mao assisted in revising the manuscript. All of the authors have read and approved the final manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

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