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Review

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Applications of polymeric adjuvants in studying autoimmune responses and vaccination against infectious diseases

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Polymers as an adjuvant are capable of enhancing the vaccine potential against various infectious diseases and also are being used to study the actual autoimmune responses using self-antigen(s) without involving any major immune deviation. Several natural polysaccharides and their derivatives originating from microbes and plants have been tested for their adjuvant potential. Similarly, numerous synthetic polymers including poly-electrolytes, polyesters, polyanhydrides, non-ionic block copolymers and external stimuli responsive polymers have demonstrated adjuvant capacity using different antigens. Adjuvant potential of these polymers mainly depends on their solubility, molecular weight, degree of branching and the conformation of polymeric backbone. These polymers have the ability not only to activate humoral but also cellular immune responses in the host. The depot effect, which involves slow release of antigen over a long duration of time, using different forms (particulate, solution and gel) of polymers, and enhances the co-stimulatory signals for optimal immune activation, is the underlying principle of their adjuvant properties. Possibly, polymers may also interact and activate various toll-like receptors and inflammasomes, thus involving several innate immune system players in the ensuing immune response. Biocompatibility, biodegradability, easy production and purification, and non-toxic properties of most of the polymers make them attractive candidates for substituting conventional adjuvants that have undesirable effects in the host.

1. Introduction

Over recent decades, material science research has been expanding exponentially to design and develop novel biomaterials for biomedical applications, and polymers have proved to be promising candidates. The easy and controllable synthesis of polymers in various formats with good mechanical properties, biocompatibility and biodegradability makes them more valuable in the biomedical field and in tissue engineering as scaffolds to grow mammalian cells for regeneration of damaged organs. Use of polymers in immunology as an adjuvant could be a useful substitute for conventional bacterial adjuvants. In immunology, an adjuvant is defined as the substance normally used with a weak antigen to enhance its immunogenic properties via activation of innate and adaptive immune responses. Adjuvants, such as polymers that are immunologically inert but capable of inducing an immune response when given with an antigen, have several advantages. Generally, they act as depot carriers through slow release of the antigen, thereby modulating the ensuing immune responses. Polymers mixed with an antigen can follow different signalling pathways. For example, the polymer–antigen can be phagocytosed and processed through proteasomes, activation of inflammasome pathway via secretion of IL-1 β cytokine, ligand for toll-like receptor(s) or directly interact with B cells. Alternatively, processed antigens can be presented by antigen-presenting cells via major histocompatibility complex (MHC) molecules to naive T cells, which in turn can become activated and release various cytokines leading to

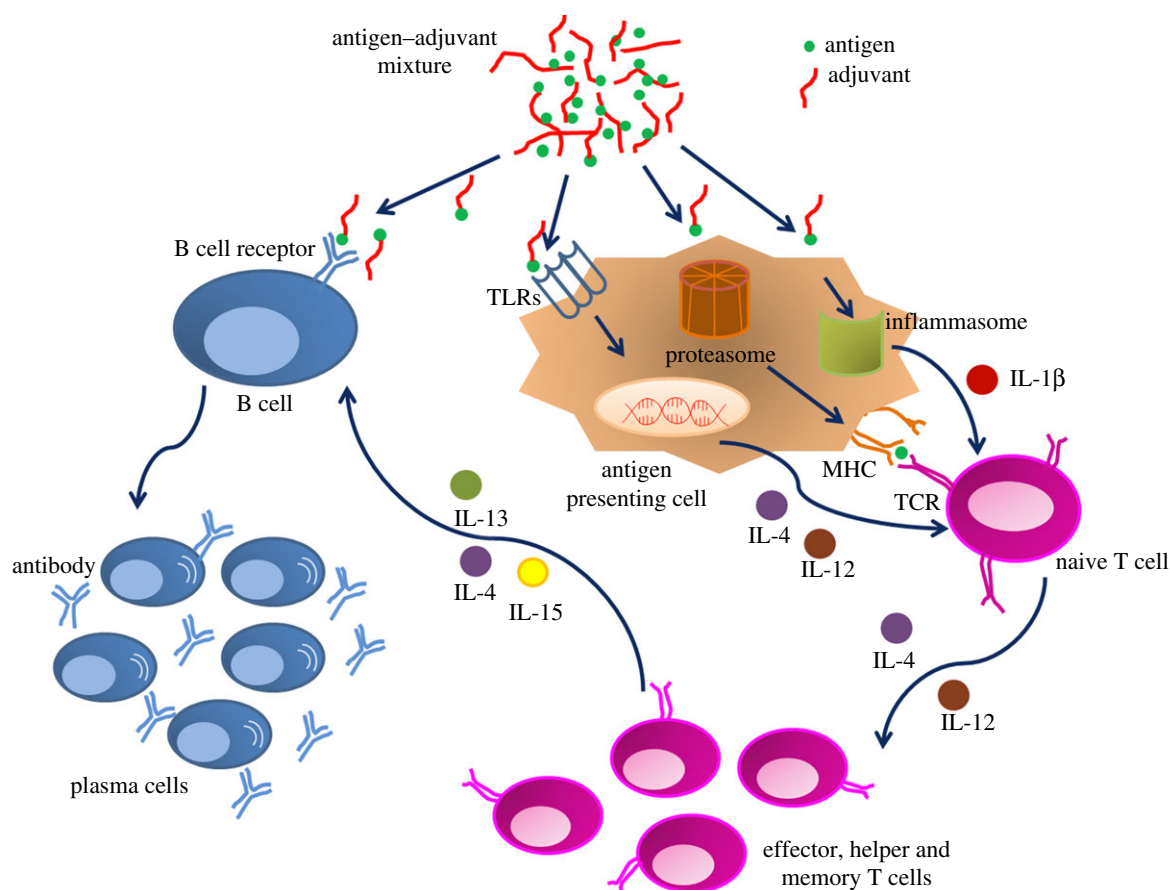


Figure 1. Schematic diagram of possible mode of action of polymeric adjuvants. TLR, toll-like receptor; TCR, T cell receptor; IL, interleukin.

enhanced T and B cell interactions. The activated B cells in turn can undergo differentiation into antibody-secreting plasma cells [1,2] (figure 1), and the antibodies thus produced can activate the downstream events of the effector phase of an immune response involving various chemokines, cytokines, proteases and effector cell populations such as neutrophils, macrophages, osteoclasts, mast cells and eosinophils [3].

Conventionally used adjuvants are alum compounds [4], Freund's adjuvants (complete Freund's adjuvant, CFA and incomplete Freund adjuvant, IFA) [5], diphosphoryl lipid A [6], muramyl dipeptide, monocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor [7] and oligodeoxynucleotides containing CpG motifs [8]. Among them, Freund's adjuvants are frequently used and well characterized, involving slow release of injected antigen and the pathogen-associated molecular patterns of the mycobacteria present in this adjuvant interacts with the pathogen recognition receptors such as toll-like receptors (TLRs), leading to the activation and proliferation of T cells [9]. The mycobacterium component of CFA specifically activates the Th1 pathway, resulting in the initiation of the delayed-type hypersensitivity reaction against the injected antigen [10]. Moreover, some systemic effects were also observed in the blood system characterized by proliferation of Mac-11⁺ immature myeloid cells. While, IFA does not have this mycobacterium content, it activates the Th2 pathway with a strong bias towards the humoral immune response [11]. However, the paraffin oil present in Freund's adjuvant has been shown to be non-degradable and cause toxicity problems [12]. In recent years, polymers have proved to be promising candidates to replace such conventional adjuvants with their biocompatible and biodegradable properties. These

polymers, together with an antigen, can activate the immune response effectively and have thus been used as an adjuvant in vaccination strategies and in the induction of autoimmune responses.

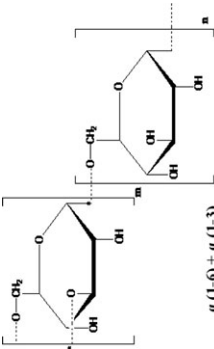
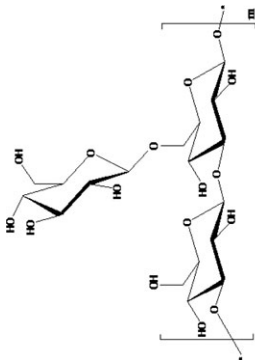
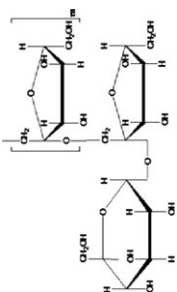
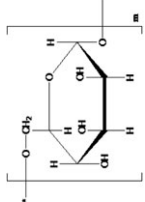
2. Polymers as vaccine adjuvants

Vaccination is the most effective way to control and prevent diseases. But multiple doses of conventional vaccines for the activation of the desired immune responses are very difficult and challenging, especially in developing countries. Interestingly, polymers in vaccine formulations could improve the delivery of antigens and thus reduce the booster doses of vaccines required for the activation of an appropriate immune response. For many decades, various biodegradable natural and synthetic polymers have been used for antigen delivery for the controlled release of vaccines over longer periods of time and as an adjuvant for enhancing immunogenicity of weak vaccines (table 1). In this review, we will discuss the application of polymers as adjuvants in vaccination.

2.1. Natural polymers

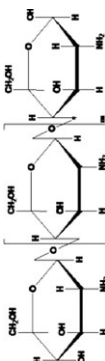
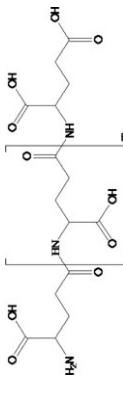
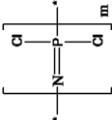
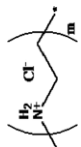
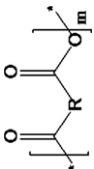
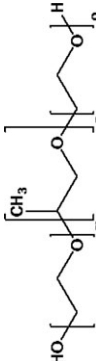
Numerous polysaccharides originated from plant and microbes have been tested for their adjuvant potential in vaccination. In this scenario, various derivatives of dextran have shown to have immunological properties. The sulphate-derivatized form of dextran sulphate was shown to have anti-inflammatory properties *in vivo* and it was used in the induction of inflammatory colitis in mice [13]. Another derivative, diethylaminoethyl dextran (DEAED), is polycationic in nature and has been used as a vaccine adjuvant. DEAED,

Table 1. Polymeric adjuvants used in vaccination.

polymer preparations	constituent/monomer	sources	variants	antigen/vaccine immunization
dextran	α-1,6-glucan with α-1,3-branches  $\alpha(1-6) + \alpha(1-3)$	<i>Leuconostoc mesenteroides</i>	sulphated dextran, diethylaminoethyl dextran, acetylated dextran	<i>Streptococcus bovis</i> vaccine [13] Venezuelan equine encephalomyelitis virus [14] TLR-7 agonist, imiquimod [15]
lentinan	β1,3-glucosylhexose with β-1,6-branches  $\beta(1-3) + \beta(1-6)$	<i>Lentinus edodes</i>	lentinan, lentinan sulphate	<i>Badillus Calmette</i> – Guérin (BCG) [16] Newcastle disease vaccine [17]
inulin	β-D-(2-1) poly(fructo-furanosyl) β-D-glucose  $\beta(1-3) + \beta(1-6)$	<i>Streptococcus mutans</i> , <i>Aspergillus</i> species, plants	γ -inulin, δ -inulin	H2N2 influenza virus [18] Japanese encephalitis, HIV [19]
mannan	α-D-mannose  $\alpha(1-6) + \alpha(1-3)$	yeast, bacteria and plants	mannan, acetylated mannan (acemannan)	A β antigen, HBsAg [20] feline leukaemia virus, feline immunodeficiency virus, heartworm antigen [21]

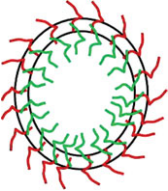
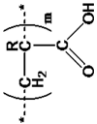
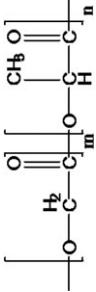
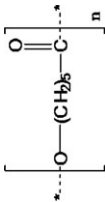
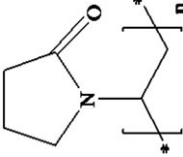
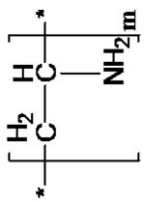
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Table 1. (Continued.)

polymer preparations	constituent/monomer	sources	variants	antigen/vaccine immunization
chitosan	β -1 – 4-linked polymer of D-glucosamine and N-acetyl-D-glucosamine 	β -(1 – 4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine	chitosan,	H5N1 vaccine, HBsAg antigen [22] influenza vaccine [23]
	glucosamine		N-trimethylchloride chitosan	meningococcal polysaccharide vaccine, HBsAg, plasmid DNA vaccine, <i>Helicobacter pylori</i> vaccine [24]
poly- γ -glutamic acid	γ -glutamic acid 	—	—	ovalbumin [25]
multiphasic emulsions	amphiphilic block polymer and mineral oil	—	PEG-based copolymers (PEG- <i>b</i> -PLA, PEG- <i>b</i> -PCL, PEG- <i>b</i> -PLACL)	oil ISA51-vaccine [26]
polyphosphazenes	inorganic polymer of phosphorus and nitrogen atom with organic side group 	—	poly(di(sodium carboxylatophenoxy)phosphazene)	influenza vaccine [27], retrovirus based vaccine [28]
polyelectrolytes	electrolyte 	—	polyelectrolytes multilayer capsules (PMLC)	B16 melanoma, influenza virus [29]
polyanhydrides	diacidic monomers 	—	liposome-polyethylene glycol-polyethyleneimine complex (LPPC)	bovine serum albumin [30]
		—	1,6-bis(<i>p</i> -carboxyphenoxy)hexane (CPH) and 1,8-bis(<i>p</i> -carboxyphenoxy)-3,6-dioxaoctane (CPTEG)	<i>Yersinia pestis</i> [31]
non-ionic	copolymer of ethylene oxide and propylene oxide	—	poly(methyl vinyl ether- <i>co</i> -maleic anhydride)	mucins [32]
copolymers		—	poloxamers/pluronic	bovine β -lactoglobulin (BLG) [33]

(Continued.)

Table 1. (Continued.)

polymer preparations		constituent/monomer	sources	variants	antigen/vaccine immunization
polymersomes		vesicle of amphiphilic synthetic block copolymers	—	polyethylene glycol- <i>b</i> -polybutadiene, poly(<i>g</i> -benzyl-L-glutamate)-K	Tat peptide of HIV [34] influenza haemagglutinin (HA) antigen [35]
polyacrylates		acrylic acid derivatives	—	polymethylmethacrylate poly(2-aminoethylmethacrylate)	inactivated influenza virus [36] DNA vaccine [37]
polyglycolic- ω -lactides		copolymer of glycolic and lactic acid	—	—	recombinant hepatitis B [38], <i>Plasmodium vivax</i> vaccine [39], hepatitis B vaccine [40], DNA vaccine [41]
poly- ϵ -caprolactone		ϵ -caprolactone	—	—	<i>Schistosoma mansoni</i> antigen [42], <i>Brucella ovis</i> antigen [43]
polyvinylpyrrolidone		<i>N</i> -vinylpyrrolidone	—	—	<i>Aspergillus fumigatus</i> antigen [44]
polyethylamines		vinylethylamines	—	—	DNA vaccine [45]

together with Venezuelan equine encephalomyelitis virus antigen, induced primary antibody responses after vaccination of rhesus monkeys [14]. In another study, adjuvant properties of DEAED were proved in mice immunized with whole-cell cholera vaccine [46]. Another derivative of dextran, acetylated dextran, in the form of microparticles was proven to be an activator of toll-like receptor pathways and to induce inflammatory cytokines [15].

As an adjuvant, lentinan, another polysaccharide composed of β -1,3-glucosamine monomer units with β -1,6 branching has been demonstrated in a vaccination study. Lentinan activated macrophages with an increasing respiratory burst activity and IL-6 production after lethal influenza virus infection [47]. It also enhanced antigen presentation through a dendritic cell vaccine procedure, leading to the activation of T and natural killer cell populations with a concomitant production of cytokines by splenocytes [48]. The sulphated derivative of lentinan was proven to be a powerful adjuvant in chickens immunized with Newcastle disease vaccine with an increased serum antibody titre and proliferation of lymphocytes [17]. Moreover, lentinan suppresses tumouricidal activity of macrophages non-specifically unlike other adjuvants such as lipopolysaccharide (LPS) [49].

Inulin, another polysaccharide, is a linear chain polymer linked through a β (2–1) glycosidic bond. Adjuvancy of inulin was linked to the activation of the complement cascades [50]. Structurally, inulin occurred in various isoforms α , β , γ and δ . Out of these, γ inulin was proven to be a powerful adjuvant in immune activation [18]. More recently, δ inulin has been characterized as a more potent adjuvant than the γ isoform, enhancing both humoral and cellular immune responses when administered together with an antigen [51]. The T cell memory immune response was also strong as evidenced by a higher cellular proliferation rate. In addition, enhanced production of antigen-specific antibodies was also noticed [52]. Furthermore, δ inulin has been effective with Japanese encephalitis [13] and HIV vaccine antigens [19]. Its mechanism of action is mainly through interactions with APCs, including monocytes, macrophages and dendritic cells. However, unlike other adjuvants, it is unable to activate the pro-inflammatory NF- κ B pathway. But a positive aspect of inulin adjuvants is that like many other carbohydrate compounds, they are well tolerated by the body and cause minimal reactogenicity [53].

In this direction, mannan polysaccharide is also known as a vaccine adjuvant that mainly binds to mannose receptors and leads to the activation of the complement pathway with enhanced phagocytosis of the antigen [54,55]. In addition, it also operates via the inflammasome pathway through secretion of IL1 β and as a ligand for TLR4 [56]. The reduced form of mannan (mannose) is known for the activation of the Th2 pathway when administered with an antigen, while oxidized mannan is known to activate the Th1 pathway [57]. Mannan coupled with a recombinant protein antigen was shown to enhance higher production of specific IgGs compared with cholera toxin [20]. The acetylated derivative of mannan (Acemannan) has also shown anti-viral and anti-tumour activities. It has the capacity to induce maturation of DCs through upregulation of MHC class II molecules and production of IL-12 [21]. Interestingly, mannan has been shown to induce not only transient arthritis in naive BALB/c mice but also Th17-mediated chronic arthritis in ZAP-70 mutated (SKG) mice via activation of innate

immunity through toll-like receptors and dectin-1 [58]. However, C5a receptor (C5aR) deficiency in SKG mice inhibited the differentiation/expansion of Th17 cells after mannan or β -glucan treatment, and consequently suppressed the development of arthritis [59]. Recently, we have also found in naive mice that mannan can induce inflammation that is dependent on MHC class II but not on complement factor 5 (Khmaladze *et al.* 2012, unpublished data).

Subsequently, another natural polymer, chitosan, was shown to exhibit a range of immunological properties, including macrophage activation, cytokine production and enhanced antibody synthesis when co-delivered with an antigen. Similar to mannan, chitosan interacts with dectin-1 and toll-like receptors. Mostly, chitosan has been used for antigen delivery in the form of particles [60–62]. Recently, inactivated influenza virus vaccine entrapped in chitosan particles was found to increase the antibody titre two- to 10-fold more after intramuscular immunization in mice than the controls [23]. In other study, chitosan polymer has been used as an adjuvant in the form of particles for delivery of HBsAg antigen with an enhancement of immunogenicity ninefold more compared with naive mice [22]. Owing to the mucoadhesive property of chitosan, the *N*-trimethylchloride derivative of chitosan was used to demonstrate enhanced immunogenicity and protective efficiency through increased absorption of proteins at the mucosal surfaces by opening tight junctions [24]. In few studies, chitosan particles have been used in combination with alginate for delivery of HBsAg with CpG adjuvant via the intranasal route, which induced significant antibody responses [63]. Furthermore, chitosan as an adjuvant enhanced protection against *Helicobacter pylori* through induction of Th2 responses [64]. Similarly, trimethyl and mono-*N*-carboxymethyl chitosan-based nanoparticles encapsulated with tetanus toxoid antigen enhanced both mucosal and systemic immune responses in response to vaccination via the nasal route [65]. To prove this concept, Verheul *et al.* [66] fabricated trimethylchitosan particles with hyaluronic acid and found improved adjuvant potential compared with trimethylchitosan particles alone for nasal and intradermal vaccinations.

Poly- γ -glutamic acid (PGA) is another polypeptide that is composed of gamma-linked glutamic acid units with an alpha carboxylate side group, produced by *Bacillus* bacteria. PGA with L-phenylalanine ethyl ester has shown immunologic activity in the form of particulates. Ovalbumin-entrapped γ -PGA nanoparticles have activated human monocyte-derived dendritic cells and enhanced the production of inflammatory cytokines and chemokines [25]. Moreover, it was involved in the upregulation of co-stimulatory molecules, leading to better T-cell priming. In another study, γ -PGA particles induced innate immune cells *in vitro* and produced antigen-specific immune responses through TLR4 and MyD88 signalling pathways [67]. Recently, γ -PGA and benzalkonium-chloride-based anionic complexes were successfully encapsulated with ovalbumin antigen that were shown to be efficiently taken by dendritic cell line, DC 2.4. Subcutaneous administration of these complexes induced antibodies with activation of both Th1 and Th2 pathways [68].

2.2. Synthetic polymers

Synthetic polymers as an adjuvant have several benefits over conventional metal-based adjuvants such as alum compounds. Polymers in the micro/nano-size range can be

directly internalized via the lymphatic system and retained for a longer period of time, which helps to maintain a significant level of immune response and thus reduce multiple doses of antigen required for activation of the immune system. They are biocompatible, easy to degrade and non-toxic to animals, unlike alum compounds.

2.2.1. Multiphase emulsions

Among the various adjuvants available for vaccination, adjuvants based on emulsion such as Freund's adjuvant, montanide ISA 51 have the advantage of being easy to manufacture at low cost. Generally, these adjuvants are defined as water-in-oil emulsion with dispersed antigen [26]. However, preparation time, injection difficulties and localized toxicities at the injection site are considerable problems with these emulsions [59,69]. To improve injectibility, a new method consisting of re-dispersion of these emulsions in an aqueous phase using the hydrophilic emulsifier Tween-80 (polyoxyethylene sorbitan monooleate) has been demonstrated [70]. The adjuvancy of Tween-80 proved more immunogenic than controls; however, Tween-80 has been restricted in use owing to its lipophilic toxicity and extra reactivity problems [71,72]. To reduce the toxicity problems, a synthetic block polymer with low molecular weight has been suggested as a good alternative to surfactants [73]. For example, TiterMax is squalene-based water-in-oil emulsion containing polyoxyethylene–polyoxypropylene–polyoxyethylene (POE–POP–POE) polymer [74]. However, mild toxicity has also been reported with the use of the TiterMax adjuvant owing to its poor biodegradation properties [75]. To further improve this adjuvant, recently Huang *et al.* have proposed multiphase emulsion based on the polymeric emulsifiers poly(ethylene glycol)-blockpolylactide (PEG-*b*-PLA), poly(ethylene glycol)-block-poly(ϵ -caprolactone) (PEG-*b*-PCL) and poly(ethylene glycol)-block-poly(lactide-*co*- ϵ -caprolactone) (PEG-*b*-PLACL) in the antigen phase of oily ISA51-adjuvant-based vaccines. Good biodegradability and biocompatibility of these polymers makes them promising candidates for vaccines [76].

2.2.2. Polyphosphazenes

Polyphosphazenes are one of most frequently used polymers as an adjuvant, which consists of alternate nitrogen and phosphorus elements in the backbone with organic side groups. Polyphosphazene-based polymers generally form stable complexes with an antigen and thus produce more potent adjuvant preparations [77,78]. Their synthetic flexibility and susceptibility towards hydrolysis produces a promising matrix for adjuvants in vaccine formulations. Among polyphosphazene, poly(di(sodium carboxylatophenoxy)phosphazene, PCPP) was demonstrated for its adjuvant activity with influenza and other retrovirus-based vaccines [27,28]. Recently, PCPP has been injected with influenza virus X31 antigen subcutaneously and strong B and T cell responses were observed [79]. In another study, the adjuvant effect of PCPP was studied in combination with CpG oligodeoxynucleotides via the subcutaneous route [80]. Recently, Shim and co-workers [81] demonstrated the adjuvant potential of PCPP via the mucosal route. Interestingly, PCPP has a film-forming nature and is thus able to encapsulate the antigen efficiently even under mild conditions. It is a water-soluble molecule, which can be easily formulated with proteins and has the capacity to dissolve in a highly hydrated

environment. Owing to this dual functionality of PCPP (as an adjuvant and film-forming nature), it is one of the most promising materials available for use in vaccine delivery [82].

2.2.3. Polyelectrolytes

Very recently, polyelectrolyte multilayer capsules (PMLC) have been demonstrated as a carrier for antigen delivery [83–85]. PMLC can easily be self-assembled under aqueous conditions without the aid of any other chemicals, which may denature the antigen. Usually, PMLC are fabricated in three steps: encapsulation of antigen in porous microtemplates [86] followed by layer-by-layer coating of polymers [87] and removal of microtemplates through decomposition into low-molecular-weight components. These porous multilayer capsules rapidly rupture during cellular uptake and permit rapid intracellular release of the encapsulated antigen. De Geest *et al.* [29] have fabricated PMLC composed of dextran sulphate and poly-L-arginine encapsulated antigen, which was rapidly phagocytosed by dendritic cells, resulting in better antigen presentation to CD4⁺ and CD8⁺ cells. Subsequently, these capsules were demonstrated as carriers for an antigen in pulmonary vaccination. PMLC are efficiently taken up by alveolar macrophages, followed by initiation of Th17 immune responses, which is considered a central part in mucosal immune responses. Furthermore, PMLC-based immunization was also shown to provide protective immunity against B16 melanoma and influenza infection. High doses of PMLC activate the NALP inflammasome pathway.

Similarly, a cationic lipopolymer, liposome–polyethylene glycol–polyethyleneimine complex (LPPC), was also evaluated for adjuvant and immunomodulation properties. LPPC strongly adsorbs the antigens over the surface and enhances the immune responses. It was found that this polymer–antigen complex activates immune responses through enhanced uptake and presentation of antigens, cell surface receptor expression and release of pro-inflammatory cytokines. When, LPPC was given with LPS or CpG oligodeoxynucleotides, a strong level of IgA antibody responses was observed [30].

Water-soluble, non-toxic and synthetic polyelectrolytes were found to be efficient immunomodulators [88]. A more prominent increase in antibody responses was achieved by complexing an outer membrane protein of *Salmonella*, porin, with the heteropolymer, polyoxydionium [89]. Similarly, *Salmonella* O and H antigens complexed with polyelectrolytes have been shown to induce several-fold higher antibody responses against respective antigens [90].

2.2.4. Polyanhydrides

Polyanhydrides are a novel class of biodegradable polymers that have been extensively used in protein/antigen delivery in vaccinations [91,92]. In recent years, this polymer has been approved by the FDA for human use owing to its tissue compatibility and controlled biodegradation properties [93]. Degradation of this polymer occurs through surface erosion mechanisms, resulting in controlled release of antigens. Normally, degradation can be extended up to one month depending on the polymer chemistry [74,94]. Furthermore, controlled release of antigen activates the immune system requiring only a single injection, which potentially improves patient compliance [95]. Intrinsic adjuvant activity of polyanhydrides has been reported that was independent of the type

of antigen delivered. Recently, Torres *et al.* [96] reported adjuvant activity of various polyanhydrides, including sebacic anhydride, 1,6-bis(*p*-carboxyphenoxy)hexane (CPH) and 1,8-bis(*p*-carboxyphenoxy)-3,6-dioxoctane (CPTEG). A specific combination of CPH and CPTEG copolymer microparticles has been used as vaccine adjuvants and carriers for antigen. These particles were efficiently phagocytosed by APCs and localized in phagolysosomes. Expression of surface markers CD86, CD40 and MHC class II on dendritic cells was significantly increased after phagocytosis of microparticles. Moreover, the presence of polyanhydride particles induced specific proliferation of CD4⁺ and CD8⁺ T-cells. Recently, molecular design of amphiphilic polyanhydride nanoparticles has been proposed for the activation of APCs mimicking pathogens [97]. The dose–response effect of poly(methyl vinyl ether-*co*-maleic anhydride) nanoparticles was studied in the stimulation of TLRs. These particles were also involved in the activation of the complement pathway [98].

2.2.5. Non-ionic block copolymers

Non-ionic block copolymers containing polyethylene (POE) and polypropylene (POP) blocks are known as poloxamers/pluronic [99]. Experiments have proven the capability of low molecular weight pluronic polymers (3 kDa) with 90 per cent POP and 10 per cent POE in the activation of macrophages. Intraperitoneal injection of these copolymers in mice induced high-level expression of MHC class II molecules, which is a direct indication of macrophage activation [100,101]. Although the exact mechanism of action of the block copolymers has not been delineated, these polymers are known to work through the activation of the alternative pathway of the complement system [102]. It is of interest to note that certain complement proteins are also involved in the activation of macrophages [74]. These copolymers are also known for the stabilization interactions of protein antigens at the interface of oil- and water-based emulsions [103]. One of the major limitations of these copolymers is their poor solubility in aqueous formulations owing to their low molecular weight (2000–5000 Da), making it necessary to use them in oil-based emulsions only. To improve the solubility, Todd *et al.* [33] synthesized a larger molecular weight block copolymer CRL1005 (9000 Da), which was compatible in aqueous formulations. The CRL1005 polymeric adjuvant has a higher POP content, around 95 per cent compared with 80–90% POP in small-molecular-weight copolymers. These large polymers are soluble in water at 18°C but form aggregates at body temperature.

Recently, an interesting form of block copolymers known as polymersomes have been demonstrated in vaccine delivery for the activation of immune responses [104,105]. Generally, polymersomes are composed of amphiphilic block copolymers, which after self-assembly form polymeric shells. Polymersomes composed of POE glycol-polybutadiene block copolymer nanoparticles functionalized with Tat peptide of HIV enhanced the cellular uptake by dendritic cells [34]. Similarly, Quer *et al.* synthesized poly(*g*-benzyl-L-glutamate)-K (PBLG50-K) polymersomes and tested them as an adjuvant for influenza haemagglutinin (HA) antigen [35]. For targeted delivery to antigen-presenting cells, specifically to dendritic cells, POP-sulphide-based nanoparticles have been designed. These nanoparticles can be easily taken up in to lymphatic system and retained in lymph nodes for a

longer period of time. A total of 40–50% nodal resident dendritic cells and other antigen-presenting cells internalized these nanoparticles [106]. The advantage of using polysulphide-based particles is that they are stimuli-responsive and can encapsulate hydrophobic drugs efficiently [107].

2.2.6. Polymethacrylates

Polymethacrylic-acid-based nanoparticles were shown to be effective as adjuvants for inactivated HIV-2 antigen compared with several other conventional adjuvants, including alum and Freund's adjuvant [108]. They have been reported to enhance immune responses against different antigens, including influenza virus and bovine serum albumin [36]. The optimum methylmethacrylic acid concentration for enhancing antibody response against influenza virus was 2 per cent w/v. Above this concentration, immune responses declined. This can be explained better by antigen protection at higher concentrations of polymer. Furthermore, antigen concentration also affects the antibody response. Higher antigenic doses induced better antibody responses than lower amounts of antigen [109]. Cytokine induction and the adjuvant effect of PMMA particles was also demonstrated by Lou *et al.* [37] in DNA vaccination. In this vaccination procedure, TNF- α production was enhanced when macrophages were exposed to PMMA particles. They have synthesized a number of PMMA particles for DNA vaccination, and different levels of tumour protection effect were observed, which was dependent on the size and charge of the particles. Kreuter & Liehl [110] also tested the adjuvant potential of polymethylmethacrylate and polyacrylamide copolymer-based nanocapsules using inactivated influenza virus vaccine with an increased vaccine potential against lethal influenza infection. Besides protection, polymers were also effective in the induction of antibody responses. Recently, cationic poly(2-aminoethylmethacrylate) polymers with controlled chain length and defined molecular weight were synthesized and characterized for delivery of DNA vaccine to dendritic cells [111]. In another study, pH-sensitive polydiethylaminoethyl methacrylate core polyaminoethyl methacrylate shell nanoparticles were designed for targeted delivery of membrane impermeable molecules to dendritic cells [112]. Cytosolic delivery of antigens by these particles decreased the dose required for the activation of immune responses, significantly [113].

2.2.7. Polyglycolic-*co*-lactides

The adjuvant potential of PLGA using the particulate system [38,39] has been well documented. This polymer enhanced the uptake of delivered antigens by APCs [114,115]. Besides antigen presentation, numerous antigens in the form of proteins, peptides, viruses or DNA can easily be encapsulated in the form of nanoparticles [116]. In such vaccines, controlled release of antigen(s) for a longer period of time can effectively activate the immune responses, thereby avoiding booster doses required for the induction of protective immunity [117]. PLGA particles can also act as the delivery system for more than one type of antigen or combinations of antigen–adjuvant formulations in the same particle [118]. Furthermore, PLGA microparticles can retain the antigens in local lymph nodes and protect them from proteolytic degradation, which ensures longer retention of antigen. It has been shown that low doses of antigen are effectively delivered using PLGA particles for strong induction of T

cell responses [119]. In addition, these particles can also be used for delivering exogenous antigens via MHC class I complexes to CD8⁺ cells [120]. An additional advantage of PLGA nanoparticles is their functionalization with ligands such as antibodies, proteins and polysaccharides binding to membrane receptors of the cells, which is relatively easier than other polymers [121]. Uptake of PLGA particles depend on the characteristic features of the delivered antigen and also on the size, shape, charge and nature of the particle surface [122]. Out of these properties, size is one of the critical factors that decides the uptake of antigen loaded nanoparticles. The optimum dimensions of nanoparticles are yet to be confirmed, but 20–100 nm is enough for internalization. Apart from the antigen uptake via APCs, peripheral lymph nodes can also directly internalize these particles [43,123]. Recently, PLGA was mixed with alginate to improve the encapsulation efficiency. The incorporated alginate also elicited higher humoral and cellular response after immunization with two malarial synthetic peptides, SPf66 and S3, in BALB/c mice. Furthermore, PLGA with alginate modified with RGD peptide have been shown to enhance immunogenicity by cell-specific targeting [124]. PLGA microparticles coated with protamine significantly enhanced the immunogenicity of weak antigen in comparison with uncoated particles. The protamine moiety facilitates better cell penetration of these particles [125].

The efficacy of PLGA-based microspheres also has been tested for immunotherapy against experimental tumours. The mice vaccinated with PLGA-MS loaded ovalbumin and CpG oligodeoxynucleotides elicited a strong cytotoxic T-lymphocyte response [126]. In another study, the efficacy of PLGA particles with poly-L-arginine was compared with poly-electrolyte microcapsules in the induction of Th1/Th2 responses [127].

2.2.8. Polycaprolactones

Poly-ε-caprolactone (PEC) is another biocompatible and biodegradable synthetic polymer, which is used as an adjuvant. Unlike PLGA polymer, PEC degrades very slowly and does not generate an acidic environment after its degradation. The good biodegradability and low acidic pH makes PEC a potential adjuvant candidate and carrier for different vaccines. The adjuvant property of PEC-based microparticles was demonstrated with an antigen of *Schistosoma mansoni* for the induction of immunity after a single administration [42]. Compared with PLGA, PEC particles encapsulated with a similar antigen induced immune responses for a longer duration after oral or nasal antigen administration [123]. Better adjuvant properties of PEC were again proven in the delivery of antigenic extract (HS) from *Brucella ovis* either subcutaneously or per orally in BALB/c mice. Moreover, PEC particles with HS extract were found to interact more strongly with mucosal and Peyer's patches than PLGA. PEC-induced immune responses are of the Th1-type, while PLGA-induced Th2 responses are characterized by significant production of IL-4 levels. Furthermore, PEC induced higher release of IFN-γ and IL-2 cytokines than PLGA [43]. PEC particles were also proven to be efficient carriers for *Diphtheria* toxoid in mucosal vaccines compared to PLGA, and the higher uptake of PEC particles is due to the more hydrophobic nature of the PEC polymer [128].

2.2.9. Polyvinylpyrrolidone

Another biocompatible polymer polyvinylpyrrolidone (PVP) was used as an adjuvant in a few studies. PVP was used successfully in entrapping an antigen extracted from *Aspergillus fumigatus* and was capable of significantly inducing IgG levels compared to free antigen. Interestingly, the IgE response to PVP particles loaded with antigen was lower than the free antigen [44].

2.2.10. Cationic polymers

In general, cationic polymers, polyethylamines, were used in DNA delivery and immunization of various antigens via different routes. The approaches for DNA delivery usually involve injection of DNA either in solution or with cationic polymers/lipids. Efficient delivery of DNA to APCs in such a way could enhance the efficacy of nucleic acid and produce a strong immune response [45]. Such cationic polymer-based microparticles can also be synthesized via the incorporation of DNA non-covalently [129].

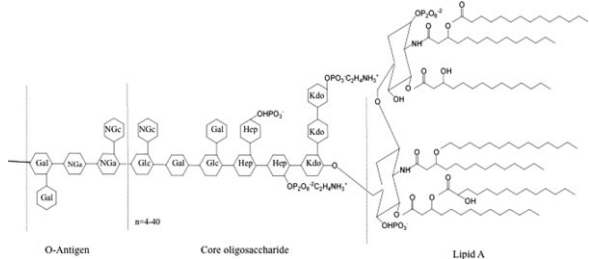
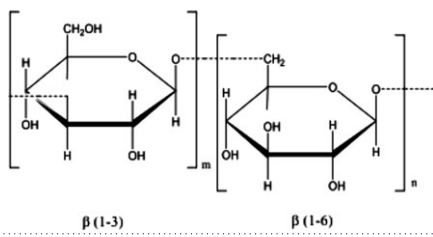
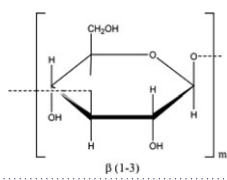
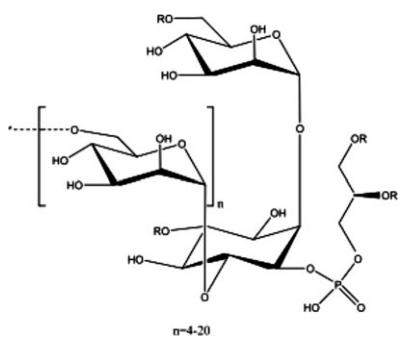
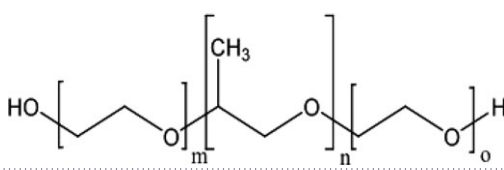
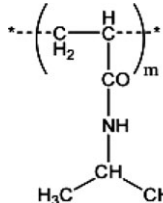
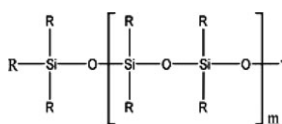
3. Polymers in the induction of autoimmune responses

Adjuvant potential of polymers in the induction of autoimmunity, mainly for the development of diseases models, has recently been explored (table 2). Animal models of autoimmune diseases have similarities to human disease phenotypes and are thus essential to delineate the disease pathways, genetics and, for developing and testing drug targets. Autoimmune disease models are developed mainly by disrupting the immune homeostasis either via immunization with autoantigens, spontaneous or induced genetic mutations or by using surgical procedures. Autoimmune diseases can be induced by immunization with polymers and autoantigens successfully. LPS is the first polymer that has been used in the activation of immune responses, which depend on time and dose of administration along with an antigen [141]. β-Glucans and zymosan are other natural polymers isolated from microbes that have been proven to be potential adjuvants for the development of autoimmune diseases [142,143]. Parallel to natural polymers, a few synthetic block copolymers of POP and POE has also been demonstrated for their adjuvant capacity in the development of myasthenia gravis, when injected along with torpedo acetylcholine receptor [136]. Recently, we have developed a synthetic polymer-based poly-N-isopropylacrylamide (PNiPAAm) adjuvant for the development of collagen-induced arthritis. When collagen type II (CII) purified from rat chondrosarcoma was injected with PNiPAAm, it efficiently activated the immune response in several different mouse strains and subsequently induced arthritis [137,138]. Most importantly, PNiPAAm was found to induce the immune responses independent of toll-like receptors.

3.1. Natural polymers

In adjuvant research, few natural polymers proved to be efficient adjuvants for the induction of autoimmune responses. LPS is the first natural polymer that has been used as an adjuvant and its immunomodulation activity

Table 2. Polymeric adjuvants used in autoimmunity.

polymer	monomer/components	source	autoimmune diseases
lipopolysaccharide	lipid A, O polysaccharide, trisaccharide 	Gram-negative bacteria	rheumatoid arthritis [130]
β -glucan	D-glucose 	<i>Candida albicans</i>	rheumatoid arthritis [131,132]
zymosan	β -glucan 	<i>Saccharomyces cerevisiae</i>	rheumatoid arthritis [133]
lipomannan	lipid and mannan 	<i>Mycobacterium tuberculosis</i>	rheumatoid arthritis [134,135]
TiterMax (TM)	squalene, non-ionic block polymer of polyoxypropylene and polyoxyethylene 	—	myasthenia gravis [136]
poly-N-isopropyl-acrylamide	N-isopropylacrylamide 	—	rheumatoid arthritis [137,138]
silicones	polymer of silica, carbon, hydrogen, oxygen 	—	scleroderma, rheumatoid arthritis, Sjogren's syndrome, systemic lupus erythematosus [139,140]

was first reported by Claman [144], who used LPS in conjunction with a T-dependent antigen. Recently, the adjuvant properties of LPS have been comprehensively characterized. LPS is a component of the cell wall of Gram-negative bacteria and used widely as the B cell mitogen [145]. It is composed of O antigen (polysaccharides) and lipid A moiety linked by a trisaccharide unit [146]. The mode of action of LPS is not fully understood yet, but lipid A is responsible for mitogenic activity and toxicity. In earlier experiments, it was demonstrated that LPS in conjunction with sheep red blood cells was capable of inducing significant antibody responses [147]. The mode of action of LPS is mainly via the humoral immune response to various T-independent antigens. Enhancement of antibody responses was observed when LPS and antigen were injected at the same time, and it was mainly dependent on the amount of antigen injected. Two- or threefold antibody increase was observed at the optimal amount of antigen, while the effect was strongest up to 40-fold when a low dose of antigen was given [130]. The effect was almost suppressed or absent when LPS was given 1–2 days after antigen injection [148]. Co-oral [131,149] administration of LPS along with CII enhanced both CII-specific antibody production and T-cell responses leading to a more chronic arthritis.

In this respect, β -glucans, another natural polysaccharide extracted from *Candida albicans*, has been tested as an adjuvant in the induction of experimental arthritis. β -Glucan is the polymer of D-glucose monomeric units linked via β -type glycosidic linkage and mainly occurs in variety of microbes. β -Glucan as an adjuvant has immunologic properties, which depend on solubility, molecular weight, degree of branching and conformation of the polymer [150–152]. Additionally, β -glucan has the ability to activate leukocytes, production of reactive oxygen species (ROS) and TNF- α cytokine [132,153]. Mice injected with β -glucan polymer are able to produce autoantibodies [149]. Hida and co-workers studied autoimmunity of the particulate form of oxidized β -glucans isolated from *C. albicans* in DBA/1 mice [131]. *In vitro*, these glucans activated the alternative complement pathway, enhanced vascular permeability and induced a higher level of IL-6 production by macrophages [152]. Furthermore, they have tested adjuvant activity of a water-soluble β -glucan fraction released from *C. albicans* and this fraction accelerated cytokine synthesis and activated immune cells leading to autoimmune arthritis [150,151]. Similarly, the adjuvant potential of β -glucan polymer isolated from a pathogenic fungus *Laminaria digitata* was tested in the spontaneous SKG model of autoimmune arthritis. These mice were unable to develop spontaneous arthritis in a specific pathogen-free environment but were able to develop arthritis after an injection of β -glucans intraperitoneally [132].

Furthermore, zymosan has also been demonstrated to activate autoimmune responses. Zymosan is a polysaccharide extracted from the cell wall of the yeast, *Saccharomyces cerevisiae* and it mainly contains β -1,3-glucan residues. As an adjuvant, zymosan binds to the TLR-2 receptor and activates the NF- κ B signalling pathway, thereby inducing inflammatory cytokine production [153,154]. β -Glucan derived from zymosan and their brief oxidation product act as a powerful adjuvant (carbohydrate adjuvant) for collagen-induced arthritis and at first, Keystone *et al.* [133] studied the arthritogenicity of zymosan independently. Induction of arthritis was mediated via activation of the

alternative complement pathway and macrophages, through binding to the membrane receptor dectin-1.

In this direction, the potential effect of lipomannan as an adjuvant has been analysed in collagen-antibody-induced arthritis, a model widely used to study the effector phase of arthritis without involving the priming phase. Lipomannan enhanced arthritis in the presence of functional phagocytes, which produced more ROS when injected with a cocktail of anti-collagen antibodies binding to well-defined CII epitopes by mainly interacting with TLR2, and the disease phenotype was mainly from the activity of granulocytes [134,135].

3.2. Synthetic polymers

Similarly, the adjuvant potential of synthetic polymers was studied using animal models of autoimmune diseases. Shenoy and Christadoss used TiterMax non-ionic block copolymer as an adjuvant with torpedo acetylcholine receptor antigen in C57BL/6 mice to induce autoimmune myasthenia gravis disease, characterized by weakness in muscles with electrophysiological defects with a significant increase in serum IgG levels [136]. Non-ionic block polymers are a copolymer of hydrophobic POP and hydrophilic POE blocks [74]. Block copolymers with high hydrophobicity were showing greater adjuvant potential, which could be changed by altering hydrophobicity and hydrophilicity content of the copolymer. These copolymers act as surface-active agents and form a stable water-in-oil emulsion, which act as a depot for slow release of antigen through the polymer.

Very recently, the adjuvant potential of temperature responsive PNiPAAm has been studied in the development of murine rheumatoid arthritis. PNiPAAm was synthesized through free radical polymerization (having a molecular weight of 120 kDa) and purified through repeated cycles of cooling and heating. This polymer, when injected with CII showed gelation properties, which led to clear precipitation and subsequent slow release of antigen (figure 2). More importantly, the polymer alone was unable to induce an immune response and only mice immunized with the polymer mixed with CII induced antigen-specific autoimmunity leading to polyarthritis. All the mice immunized with PNiPAAm-CII developed an antibody response significantly comprising of all the major IgG subclasses and an antigen-specific recall immune response was also observed using lymphocyte proliferation assay. All polymer immunized arthritic mice had massive infiltration of effector cells, including macrophages, neutrophils, eosinophils and osteoclasts, with extensive damage to the joint architecture. CII mixed with the polymer retained its native confirmation, which is a requirement for arthritis induction. CII mixed with a high-molecular-weight form of moderately hydrophobic PNiPAAm induced a significantly higher anti-CII response compared with covalently linked CII and polymer. Interestingly, all the polymer-CII immunized TLR deficient mice developed anti-CII antibodies, demonstrating adjuvancity of PNiPAAm could be independent of TLR pathways. This polymer PNiPAAm grafted with gelatine has been also used as a scaffold to support growth of primary chondrocytes for regeneration of cartilage [155].

Despite the use of polymers in animal models, autoimmunity has also been reported owing to polymer implants. Lately, a new autoimmune syndrome induced by adjuvants

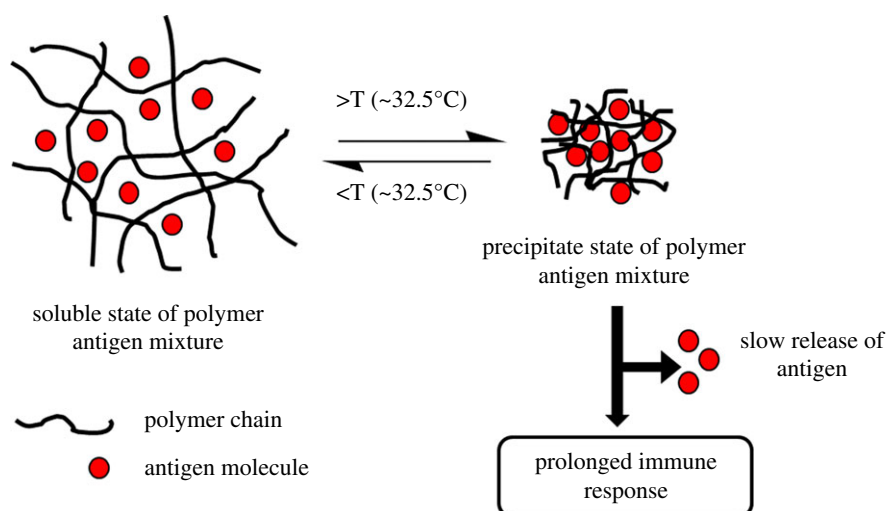


Figure 2. Schematic of reversible behaviour of temperature responsive poly-*N*-isopropylacrylamide (PNiPAAm). Below the lower critical solution temperature, PNiPAAm-antigen mixture is in the soluble state and it becomes a precipitate above this temperature.

has been reported. A well-known example is silicone polymer implantation [156]. Patients with silicone implants were developing a high level of anti-silicone antibodies in the surrounding tissues [139], and the presence of autoantibodies was demonstrated via immuno-fluorescence staining in the capsular tissue [140]. In another study, the presence of autoantibodies, including rheumatoid factor and anti-dsDNA antibodies, was observed in experimental mice after silicone implantation, leading to the development of autoimmune diseases [157]. Lidar and co-workers extensively studied autoimmunity in symptomatic women, who had silicone breast implants compared with asymptomatic women [158] and a 20 per cent increase in IgG titre was observed, and most of the antibodies were against dsDNA, ss-DNA, silicone and collagen type II. Also, chronic fatigue syndrome was triggered in patients with hepatitis B vaccination after silicone implantation [159]. Similarly, various autoimmune diseases including RA, systemic lupus erythematosus and systemic sclerosis were observed in several patients who had undergone silicone implantation [160]. At the cellular level, various phenotypic and functional alterations of T-cell subsets, B-cell activation, autoantibodies directed against endothelial and nuclear antigens were observed as a result of the implanted silicone polymer [161].

4. Conclusions

In conclusion, polymers as an adjuvant have the ability to enhance the immunogenicity of antigens in vaccination as well as in the induction of autoimmune responses. They can induce immune responses efficiently through different signalling mechanisms (toll-like receptors, inflammasome pathways) and offer the possibility of substituting bacterial-based adjuvants, which may cause toxicity and undesired activation of the immune system. Polymers have the ability to induce both humoral and cellular immune responses, when given along with an antigen, although they are not capable of eliciting any immune response on their own. Adjuvant potential of polymers depends on the solubility, molecular weight, degree of branching and conformation of the polymer used. Usually, they work on the principle of depot generation for slow release of the antigen for a longer period of time and act as an immuno-modulator via strong antigen presentation. Thus, polymeric adjuvants are highly valuable for better vaccination strategies and in studying basic pathogenic mechanisms involved in autoimmune diseases.

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References

1. Bomford R. 1998 Will adjuvants be needed for vaccines of the future. In *Modulation of the immune response to vaccine antigens Dev Biol Stand*, vol. 92 (eds F Brown, LR Haaheim), pp. 13–18. Basel, Switzerland: Karger.
2. Vogel FR. 1998 Adjuvants in perspective. In *Modulation of the immune response to vaccine antigens Dev Biol Stand*, vol. 92 (eds F Brown, LR Haaheim), pp. 241–248. Basel, Switzerland: Karger.
3. Nandakumar KS, Holmdahl R. 2006 Antibody-induced arthritis: disease mechanisms and genes involved at the effector phase of arthritis. *Arthritis Res. Ther.* **8**, 223. (doi:10.1186/ar2089)
4. Lindblad EB. 1995 Aluminium adjuvants. In *The theory and practical application of adjuvants* (ed. DES Stewart-Tull), pp. 21–35. Chichester, UK: John Wiley & Sons Ltd.
5. Johnson HE, DeAvila DM, Chang F, Reeves JJ. 1988 Active immunization of heifers against luteinizing hormone-releasing hormone, human chorionic gonadotropin and bovine luteinizing hormone. *J. Anim. Sci.* **66**, 719–726.
6. Jacobs DM. 1979 Synergy between T cell-replacing factor and bacterial lipopolysaccharide (LPS) in the primary antibody response *in vitro*: a model for lipopolysaccharide adjuvant action. *J. Immunol.* **122**, 1421–1426.
7. Heufler C, Koch F, Schuler G. 1988 Granulocyte/macrophage colony-stimulating factor and interleukin 1 mediate the maturation of murine epidermal Langerhans cells into potent immunostimulatory dendritic cells. *J. Exp. Med.* **167**, 700–705. (doi:10.1084/jem.167.2.700)
8. Segal BM, Chang JT, Shevach EM. 2000 CpG oligonucleotides are potent adjuvants for the activation of autoreactive encephalitogenic T cells *in vivo*. *J. Immunol.* **164**, 5683–5688.
9. Freund J. 1956 The mode of action of immunologic adjuvants. *Adv. Tuberc. Res.* **7**, 130–148.
10. Matthys P, Vermeire K, Billiau A. 2001 Mac-1⁺ myelopoiesis induced by complete Freund's

- adjuvant (CFA): a due to the paradoxical effects of IFN- γ in autoimmune disease models. *Trends Immunol.* **22**, 367–371. (doi:10.1016/S1471-4906(01)01937-8)
11. Heeger PS, Forsthuber T, Shive C, Biekert E, Genain C, Hofstetter HH, Karulin A, Lehmann PV. 2000 Revisiting tolerance induced by autoantigen in incomplete Freund's adjuvant. *J. Immunol.* **164**, 5771–5781.
 12. Stills HF, Bailey MQ. 1991 The use of Freund's complete adjuvant. *Lab. Anim.* **20**, 25–30.
 13. Bauer C *et al.* 2010 Colitis induced in mice with dextran sulfate sodium (DSS) is mediated by the NLRP3 inflammasome. *Gut* **59**, 1192–1199. (doi:10.1136/gut.2009.197822)
 14. Houston WE, Crabbs CL, Kremer RJ, Springer JW. 1976 Adjuvant effects of diethylaminoethyl-dextran. *Infect. Immun.* **13**, 1559–1562.
 15. Bachelder EM, Beaudette TT, Broaders KE, Frechet JMJ, Albrecht MT, Mateczun AJ, Ainslie KM, Pesce JT, Keane-Myers AM. 2010 *In vitro* analysis of acetalated dextran microparticles as a potent delivery platform for vaccine adjuvants. *Mol. Pharm.* **7**, 826–835. (doi:10.1021/mp900311x)
 16. Drandarska I, Kussovski V, Nikolaeva S, Markova N. 2005 Combined immunomodulating effects of BCG and Lentinan after intranasal application in guinea pigs. *Int. Immunopharmacol.* **5**, 795–803. (doi:10.1016/j.intimp.2004.12.008)
 17. Guo Z, Hu Y, Wang D, Ma X, Zhao X, Zhao B, Wang J, Liu P. 2009 Sulfated modification can enhance the adjuvant activity of lentinan and improve the immune effect of ND vaccine. *Vaccine* **27**, 660–665. (doi:10.1016/j.vaccine.2008.11.038)
 18. Cooper PD, Steele EJ. 1988 The adjuvant activity of γ inulin. *Immunol. Cell Biol.* **66**, 345–352. (doi:10.1038/icb.1988.45)
 19. Cristillo AD *et al.* 2011 Induction of mucosal and systemic antibody and T-cell responses following prime-boost immunization with novel adjuvanted human immunodeficiency virus-1-vaccine formulations. *J. Gen. Virol.* **92**, 128–140. (doi:10.1099/vir.0.023242-0)
 20. Stambas J, Pietersz G, McKenzie IF, Cheers C. 2002 Oxidised mannan as a novel adjuvant inducing mucosal IgA production. *Vaccine* **20**, 1068–1078. (doi:10.1016/S0264-410X(01)00456-X)
 21. Lee JK, Lee MK, Yun YP, Kim Y, Kim JS, Kim YS, Kim K, Han SS, Lee CK. 2001 Acemannan purified from *Aloe vera* induces phenotypic and functional maturation of immature dendritic cells. *Int. Immunopharmacol.* **1**, 1275–1284. (doi:10.1016/S1567-5769(01)00052-2)
 22. Prego C, Paolicelli P, Díaz B, Vicente S, Sánchez A, González-Fernández A, Alonso MJ. 2010 Chitosan-based nanoparticles for improving immunization against hepatitis B infection. *Vaccine* **28**, 2607–2614. (doi:10.1016/j.vaccine.2010.01.011)
 23. Ghendon Y *et al.* 2009 Evaluation of properties of chitosan as an adjuvant for inactivated influenza vaccines administered parenterally. *J. Med. Virol.* **81**, 494–506. (doi:10.1002/jmv.21415)
 24. Kotze AF, Luessen HL, de Leeuw BJ, de Boer BG, Verhoef JC, Junginger HE. 1997 N-trimethyl chitosan chloride as a potential absorption enhancer across mucosal surfaces: *in vitro* evaluation in intestinal epithelial cells (Caco-2). *Pharm. Res.* **14**, 1197–1202. (doi:10.1023/A:1012106907708)
 25. Akagi T, Kaneko T, Kida T, Akashi M. 2005 Preparation and characterization of biodegradable nanoparticles based on poly(γ -glutamic acid) with L-phenylalanine as a protein carrier. *J. Control. Release* **108**, 226–236. (doi:10.1016/j.jconrel.2005.08.003)
 26. Aucouturier J, Dupuis L, Deville S, Ascarateil S, Ganne V. 2002 Montanide ISA 720 and 51: a new generation of water in oil emulsions as adjuvants for human vaccines. *Expert Rev. Vaccines* **1**, 111–118. (doi:10.1586/14760584.1.1.111)
 27. Andrianov AK, Marin A, Chen J. 2006 Synthesis, properties, and biological activity of poly[di(sodium carboxylatoethylphenoxy)phosphazene]. *Biomacromolecules* **7**, 394–399. (doi:10.1021/bm050790a)
 28. McNeal MM, Rae MN, Ward RL. 1999 Effects of different adjuvants on rotavirus antibody responses and protection in mice following intramuscular immunization with inactivated rotavirus. *Vaccine* **17**, 1573–1580. (doi:10.1016/S0264-410X(98)00359-4)
 29. De-Geest BG *et al.* 2012 Polymeric multilayer capsule-mediated vaccination induces protective immunity against cancer and viral infection. *ACS Nano* **6**, 2136–2149. (doi:10.1021/nn205099c)
 30. Chen CH *et al.* 2012 Liposome-based polymer complex as a novel adjuvant: enhancement of specific antibody production and isotype switch. *Int. J. Nanomed.* **7**, 607–621.
 31. Conde BC, Schiltz E, Yu J, Minion FC, Phillips GJ, Wannemuehler MJ, Narasimhan B. 2010 Encapsulation into amphiphilic polyanhydride microparticles stabilizes *Yersinia pestis* antigens. *Acta Biomater.* **6**, 3110–3119. (doi:10.1016/j.actbio.2010.01.040)
 32. Yoncheva K, Lizarraga E, Irache JM. 2005 Pegylated nanoparticles based on poly(methyl vinyl ether-co-maleic anhydride): preparation and evaluation of their bioadhesive properties. *Eur. J. Pharm. Sci.* **24**, 411–419. (doi:10.1016/j.ejps.2004.12.002)
 33. Todd CW, Pozzi LAM, Guarnaccia JR, Balasubramanian M, Henkf WJ, Youngert LE, Newman MJ. 1997 Development of an adjuvant-active nonionic block copolymer for use in oil-free subunit vaccines formulations. *Vaccine* **15**, 564–570. (doi:10.1016/S0264-410X(97)00209-0)
 34. Christian NA *et al.* 2007 Tat-functionalized near-infrared emissive polymersomes for dendritic cell labeling. *Bioconjug. Chem.* **18**, 31–40. (doi:10.1021/bc0601267)
 35. Quer CB, Marsden HR, Romeijn S, Zope H, Kros A, Jiskoot W. 2011 Polymersomes enhance the immunogenicity of influenza subunit vaccine. *Polym. Chem.* **2**, 1482–1485. (doi:10.1039/c1py00010a)
 36. Kreuter J. 1992 Physicochemical characterization of nanoparticles and their potential for vaccine preparation. *Vac. Res.* **1**, 93–98.
 37. Lou PJ, Cheng WF, Chung YC, Cheng CY, Chiu LH, Young TH. 2009 PMMA particle-mediated DNA vaccine for cervical cancer. *J. Biomed. Mater. Res. A* **88**, 849–857. (doi:10.1002/jbm.a.31919)
 38. Saini V, Jain V, Sudheesh MS, Jaganathan KS, Murthy PK, Kohli DV. 2011 Comparison of humoral and cell mediated immune responses to cationic PLGA microspheres containing recombinant hepatitis B antigen. *Int. J. Pharm.* **408**, 50–57. (doi:10.1016/j.ijpharm.2011.01.045)
 39. Moon JJ, Suh H, Polhemus ME, Ockenhouse CF, Yadava A, Irvine DJ. 2012 Antigen-displaying lipid-enveloped PLGA nanoparticles as delivery agents for a *Plasmodium vivax* malaria vaccine. *PLoS ONE* **7**, e31472. (doi:10.1371/journal.pone.0031472)
 40. Shi L, Caulfield MJ, Chern RT, Wilson RA, Sanyal G, Volkin DB. 2002 Pharmaceutical and immunological evaluation of a single-shot hepatitis B vaccine formulated with PLGA microspheres. *J. Pharm. Sci.* **91**, 1019–1035. (doi:10.1002/jps.10042)
 41. He XW *et al.* 2005 Induction of mucosal and systemic immune response by single-dose oral immunization with biodegradable microparticles containing DNA encoding HBsAg. *J. Gen. Virol.* **86**, 601–610. (doi:10.1099/vir.0.80575-0)
 42. Benoit MA, Baras B, Poulain-Godefroy O, Schacht AM, Capron A, Gillard J, Riveau G. 1998 Evaluation of the antibody response after oral immunization by microparticles containing as antigen from *Schistosoma mansoni*. In *Biomedical science and technology recent developments in the pharmaceutical and medical sciences* (eds A Hincal, H Kas), pp. 137–144. New York, NY: Plenum.
 43. Murillo M, Goni MM, Irache JM, Arango MA, Blasco JM, Gamazo C. 2002 Modulation of the cellular immune response after oral or subcutaneous immunization with microparticles containing *Brucella ovis* antigens. *J. Control. Release* **85**, 237–246. (doi:10.1016/S0168-3659(02)00276-6)
 44. Madan T, Munshi N, De TK, Maitra A, Sarma PU, Aggarwal SS. 1997 Biodegradable nanoparticles as a sustained release system for the antigens/allergens of *Aspergillus fumigatus*: preparation and characterisation. *Int. J. Pharm.* **159**, 135–147. (doi:10.1016/S0378-5173(97)00278-0)
 45. Mastrobattista E, Hennink WE. 2012 Polymers for gene delivery charged for success. *Nat. Mater.* **11**, 10–12. (doi:10.1038/nmat3209)
 46. Kaistha J, Sockey J, Singh S, Kumar S, John PC, Sharma NC. 1996 Adjuvant effect of DEAE-dextran and tetanus toxoid on whole cell heat inactivated phenol preserved typhoid vaccine. *Indian J. Pathol. Immunol.* **39**, 287–292.
 47. Irinoda K, Masihi KN, Chihara G, Kaneko Y, Katori T. 1992 Stimulation of microbicidal host defence mechanisms against aerosol influenza virus infection by lentinan. *Int. J. Immunopharmacol.* **14**, 971–977. (doi:10.1016/0192-0561(92)90140-G)
 48. Wang J, Zhou ZD, Xia DJ. 2007 Study on effect of lentinan in enhancing anti-tumor action of dendritic cytoma vaccine and its mechanism. *Zhongguo Zhong Xi Yi Jie He Za Zhi* **27**, 60–64.
 49. Bruley-Rosset M, Florentin I, Mathe G. 1976 *In vivo* and *in vitro* macrophage activation by systemic adjuvants. *Agents Actions* **6**, 251–255. (doi:10.1007/BF01972217)

50. Cooper PD, Carter M. 1986 Anti-complementary action of polymorphic 'solubility forms' of particulate inulin. *Mol. Immunol.* **23**, 895–901. (doi:10.1016/0161-5890(86)90075-1)
51. Cooper PD, Petrovsky N. 2011 δ inulin: a novel, immunologically-active, stable packing structure comprising β -D-[2 $\rightarrow$ 1] poly(fructo-furanosyl) α -D-glucose polymers. *Glycobiology* **21**, 595–606. (doi:10.1093/glycob/cwq201)
52. Lobigs M, Pavy M, Hall RA, Lobigs P, Cooper P, Komiya T, Toriwa H, Petrovsky N. 2010 An inactivated Vero cell-grown Japanese encephalitis vaccine formulated with Advax, a novel inulin-based adjuvant, induces protective neutralizing antibody against homologous and heterologous flaviviruses. *J. Gen. Virol.* **91**, 1407–1417. (doi:10.1099/vir.0.019190-0)
53. Petrovsky N, Cooper PD. 2011 Carbohydrate-based immune adjuvants. *Expert Rev. Vaccines* **10**, 523–537. (doi:10.1586/erv.11.30)
54. Thiel S, Gadjeva M. 2009 Humoral pattern recognition molecules: mannan-binding lectin and ficolins. *Adv. Exp. Med. Biol.* **653**, 58–73. (doi:10.1007/978-1-4419-0901-5_5)
55. Takahara K, Yashima Y, Omatsu Y, Yoshida H, Kimura Y, Kang YS, Steiman RM, Park CG, Inaba K. 2004 Functional comparison of the mouse DC-SIGN, SIGNR1, SIGNR3 and Langerin, C-type lectins. *Int. Immunol.* **16**, 819–829. (doi:10.1093/intimm/dxh084)
56. Sheng KC, Pouniotis DS, Wright MD, Tang CK, Lazoura E, Pietersz GA, Apostolopoulos V. 2006 Mannan derivatives induce phenotypic and functional maturation of mouse dendritic cells. *Immunology* **118**, 372–383. (doi:10.1111/j.1365-2567.2006.02384.x)
57. McKenzie IF, Apostolopoulos V, Lees C, Xing PX, Lofthouse S, Osinski C, Popovski V, Acres B, Pietersz G. 1998 Oxidised mannan antigen conjugates preferentially stimulate T1 type immune responses. *Vet. Immunol. Immunopathol.* **63**, 185–190. (doi:10.1016/S0165-2427(98)00094-4)
58. Yoshitomi H *et al.* 2005 A role for fungal β -glucans and their receptor Dectin-1 in the induction of autoimmune arthritis in genetically susceptible mice. *J. Exp. Med.* **201**, 949–960. (doi:10.1084/jem.20041758)
59. Hashimoto M *et al.* 2010 Complement drives Th17 cell differentiation and triggers autoimmune arthritis. *J. Exp. Med.* **207**, 1135–1143. (doi:10.1084/jem.20092301)
60. Arca HC, Gunbeyaz M, Senel S. 2009 Chitosan-based systems for the delivery of vaccine antigens. *Expert Rev. Vaccines* **8**, 937–953. (doi:10.1586/erv.09.47)
61. Wen ZS, Xu YL, Zou XT, Xu ZR. 2011 Chitosan nanoparticles act as an adjuvant to promote both Th1 and Th2 immune responses induced by ovalbumin in mice. *Mar. Drugs* **9**, 1038–1055. (doi:10.3390/md9061038)
62. Zhao K, Shi X, Zhao Y, Wei H, Sun Q, Huang T, Zhang X, Wang Y. 2011 Preparation and immunological effectiveness of a swine influenza DNA vaccine encapsulated in chitosan nanoparticles. *Vaccine* **29**, 8549–8556. (doi:10.1016/j.vaccine.2011.09.029)
63. Borges O, Cordeiro-da-Silva A, Tavares J, Santarém N, de Sousa A, Borchard G, Junginger HE. 2008 Immune response by nasal delivery of hepatitis B surface antigen and codelivery of a CpG ODN in alginate coated chitosan nanoparticles. *Eur. J. Pharm.* **69**, 405–416. (doi:10.1016/j.ejpb.2008.01.019)
64. Gong YF, Xie Y, Zhou NJ, Chen J, Zhou XJ, Lu NH, Wang CW. 2007 Cellular immunity induced by H. pylori vaccine with chitosan as adjuvant. *Chin. J. Cell Mol. Immunol.* **23**, 595–599.
65. Sayin B, Somavarapu S, Li XW, Sesardic D, Senel S, Alpar OH. 2009 TMC–MCC (N-trimethyl chitosan–mono-N-carboxymethyl chitosan) nanocomplexes for mucosal delivery of vaccine. *Eur. J. Pharm. Sci.* **38**, 362–369. (doi:10.1016/j.ejps.2009.08.010)
66. Verheul RJ, Slütter B, Bal SM, Bouwstra JA, Jiskoot W, Hennink WE. 2011 Covalently stabilized trimethyl chitosan-hyaluronic acid nanoparticles for nasal and intradermal vaccination. *J. Control. Release* **156**, 46–52. (doi:10.1016/j.jconrel.2011.07.014)
67. Uto T, Akagi T, Yoshinaga K, Toyama M, Akashi M, Baba M. 2011 The induction of innate and adaptive immunity by biodegradable poly(γ -glutamic acid) nanoparticles via a TLR4 and MyD88 signaling pathway. *Biomaterials* **32**, 5206–5212. (doi:10.1016/j.biomaterials.2011.03.052)
68. Kurosaki T, Kitahara T, Nakamura T, Nishida K, Fumoto S, Kodama Y, Nakagawa H, Higuchi N, Sasaki H. 2012 Development of effective cancer vaccine using targeting system of antigen protein to APCs. *Pharm. Res.* **29**, 483–489. (doi:10.1007/s11095-011-0571-x)
69. Chang JCC, Diveley JP, Savary JR, Jensen FC. 1998 Adjuvant activity of incomplete Freund's adjuvant. *Adv. Drug Deliv. Rev.* **32**, 173–186. (doi:10.1016/S0169-409X(98)00009-X)
70. Vogel FR, Powell MF. 1995 A compendium of vaccine adjuvants and excipients. In *Vaccine design: the subunit and adjuvant approach* (eds MF Powell, MJ Newman), pp. 141–228. New York, NY: Plenum.
71. Podda A. 2001 The adjuvanted influenza vaccines with novel adjuvants: experience with MF59-adjuvanted vaccine. *Vaccine* **19**, 2673–2680. (doi:10.1016/S0264-410X(00)00499-0)
72. Leroux-Roels I, Borkowski A, Vanwolleghem T, Dramé M, Clement F, Hons E, Devaster JM, Leroux-Roels G. 2007 Antigen sparing and cross-reactive immunity with an adjuvanted rH5N1 prototype pandemic influenza vaccine: a randomised controlled trial. *Lancet* **370**, 580–589. (doi:10.1016/S0140-6736(07)61297-5)
73. Gaucher G, Poreba M, Ravenelle F, Leroux JC. 2007 Poly(*N*-vinyl-pyrrolidone)-block-poly(D,L-lactide) as polymeric emulsifier for the preparation of biodegradable nanoparticles. *J. Pharm. Sci.* **96**, 1763–1775. (doi:10.1002/jps.20833)
74. Newman MJ, Balusubramanian M, Todd CW. 1998 Development of adjuvant-active nonionic block copolymers. *Adv. Drug Deliv. Rev.* **32**, 199–223. (doi:10.1016/S0169-409X(98)00011-8)
75. Jeong B, Bae YH, Lee DS, Kim SW. 1997 Biodegradable block copolymers as injectable drug-delivery systems. *Nature* **388**, 860–862. (doi:10.1038/42218)
76. Huang MH, Huang CY, Lien SP, Siao SY, Chou AH, Chen HW, Liu SJ, Leng CH, Chong P. 2009 Development of multi-phase emulsions based on bioresorbable polymers and oily adjuvant. *Pharm. Res.* **26**, 1856–1862. (doi:10.1007/s11095-009-9898-y)
77. Andrianov AK, Payne LG. 1998 Polymeric carriers for oral uptake of microparticulates. *Adv. Drug Deliv. Rev.* **34**, 155–170. (doi:10.1016/S0169-409X(98)00038-6)
78. Payne LG, Jenks SA, Woods AL, Grund EM, Geribo WE, Loebelez JR, Andrianov AK, Roberts BE. 1998 Poly[di(carboxylatophenoxy)phosphazene] (PCPP) is a potent immunoadjuvant for an influenza vaccine. *Vaccine* **17**, 92–98. (doi:10.1016/S0264-410X(97)00149-7)
79. Mutwiri G, Benjamin P, Soita H, Townsend H, Yost R, Roberts B, Andrianov AK, Babiuk LA. 2007 Poly[di(sodium carboxylatoethylphenoxy)phosphazene] (PCEP) is a potent enhancer of mixed Th1/Th2 immune responses in mice immunized with influenza virus antigens. *Vaccine* **25**, 1204–1213. (doi:10.1016/j.vaccine.2006.10.011)
80. Mutwiri G, Benjamin P, Soita H, Babiuk LA. 2008 Co-administration of polyphosphazenes β with CpG oligodeoxynucleotides strongly enhances immune responses in mice immunized with hepatitis B virus surface antigen. *Vaccine* **26**, 2680–2688. (doi:10.1016/j.vaccine.2008.03.031)
81. Shim DH, Ko HJ, Volker G, Potter AA, Mutwiri G, Babiuk LA, Kwon MN. 2010 Efficacy of poly[di(sodium carboxylatophenoxy)phosphazene] (PCPP) as mucosal adjuvant to induce protective immunity against respiratory pathogens. *Vaccine* **28**, 2311–2317. (doi:10.1016/j.vaccine.2009.12.069)
82. Andrianov AK, DeColibus DP, Gillis HA, Kha HH, Marin A, Prausnitz MR, Babiuk LA, Townsend H, Mutwiri G. 2009 Poly[di(carboxylatophenoxy)phosphazene] is a potent adjuvant for intradermal immunization. *Proc. Natl Acad. Sci. USA* **106**, 18 936–18 941. (doi:10.1073/pnas.0908842106)
83. De-Geest BG, Sanders NN, Sukhorukov GB, Demeester J, De-Smedt SC. 2007 Release mechanisms for polyelectrolyte capsules. *Chem. Soc. Rev.* **36**, 636–649. (doi:10.1039/b600460c)
84. Sexton A, Whitney PG, Chong SF, Zelikin AN, Johnston APR, De-Rose R, Brooks AG, Caruso F, Kent SJ. 2009 A protective vaccine delivery system for *in vivo* T cell stimulation using nanoengineered polymer hydrogel capsules. *ACS Nano* **3**, 3391–3400. (doi:10.1021/nn900715g)
85. De-Koker S, Naessens T, De-Geest BG, Bogaert P, Demeester J, De-Smedt S, Grooten J. 2010 Biodegradable polyelectrolyte microcapsules: antigen delivery tools with Th17 skewing activity after pulmonary delivery. *J. Immunol.* **184**, 203–211. (doi:10.4049/jimmunol.0803591)

86. Yu AM, Wang YJ, Barlow E, Caruso F. 2005 Mesoporous silica particles as templates for preparing enzyme-loaded biocompatible microcapsules. *Adv. Mater.* **17**, 1737–1741. (doi:10.1002/adma.200402045)
87. Decher G. 1997 Fuzzy nanoassemblies: toward layered polymeric multicomposites. *Science* **277**, 1232–1237. (doi:10.1126/science.277.5330.1232)
88. Khaitov RM. 1990 Molecular bases for the construction of artificial immunogens. *J. Biomed. Sci.* **1**, 122–126.
89. Nandakumar KS, Muthukkaruppan VR. 1999 Influence of immunopotentiators on the antiporin immunoglobulin G subclass: distribution and protective immunity against murine salmonellosis. *Scand. J. Immunol.* **50**, 188–194. (doi:10.1046/j.1365-3083.1999.00576.x)
90. Petrov RV, Khaitov RM, Ataulakhov RI. 1987 Artificial antigens and vaccines based on non-natural polyelectrolytes. *Sov. Med. Rev. D Immunol. Rev.* **1**, 241–443.
91. Mallapragada SK, Narasimhan B. 2008 Immunomodulatory biomaterials. *Int. J. Pharm.* **364**, 265–271. (doi:10.1016/j.ijpharm.2008.06.030)
92. Torres MP, Determan AS, Anderson GL, Mallapragada SK, Narasimhan B. 2007 Amphiphilic polyanhydrides for protein stabilization and release. *Biomaterials* **28**, 108–116. (doi:10.1016/j.biomaterials.2006.08.047)
93. Adler AF, Petersen LK, Wilson JH, Torres MP, Thorstenson JB, Gardner SW, Mallapragada SK, Wannemuehler MJ, Narasimhan B. 2009 High throughput cell-based screening of biodegradable polyanhydride libraries. *Comb. Chem. High Throughput Screen* **12**, 634–645. (doi:10.2174/138620709788923764)
94. Shen EE, Kipper MJ, Dziadul B, Lim MK, Narasimhan B. 2002 Mechanistic relationships between polymer microstructure and release kinetics in bioerodible polyanhydrides. *J. Control. Release* **82**, 115–125. (doi:10.1016/S0168-3659(02)00125-6)
95. Kipper MJ, Wilson JH, Wannemuehler MJ, Narasimhan B. 2006 Single dose vaccine based on biodegradable polyanhydride microspheres can modulate immune response mechanism. *J. Biomed. Mater. Res. A* **76**, 798–810. (doi:10.1002/jbm.a.30545)
96. Torres MP, Wilson-Welder JH, Lopac SK, Phanse Y, Carrillo-Conde B, Ramer-Tait AE, Bellaire BH, Wannemuehler MJ, Narasimhan B. 2011 Polyanhydride microparticles enhance dendritic cell antigen presentation and activation. *Acta Biomater.* **7**, 2857–2864. (doi:10.1016/j.actbio.2011.03.023)
97. Petersen LK, Ramer-Tait AE, Broderick SR, Kong CS, Ulerly BD, Rajan K, Wannemuehler MJ, Narasimhan B. 2011 Activation of innate immune responses in a pathogen-mimicking manner by amphiphilic polyanhydride nanoparticle adjuvants. *Biomaterials* **32**, 6815–6822. (doi:10.1016/j.biomaterials.2011.05.063)
98. Camacho AI, Costa Martins RD, Tamayo I, de-Souza J, Lasarte JJ, Mansilla C, Esparza I, Irache JM, Gamazo C. 2011 Poly(methyl vinyl ether-co-maleic anhydride) nanoparticles as innate immune system activators. *Vaccine* **29**, 7130–7135. (doi:10.1016/j.vaccine.2011.05.072)
99. Moghimi SM, Hunter AC. 2000 Poloxamers and poloxamines in nanoparticles engineering and experimental medicine. *Trends Biotechnol.* **18**, 412–420. (doi:10.1016/S0167-7799(00)01485-2)
100. Howerton DA, Hunter RL, Ziegler HK, Check IJ. 1990 Induction of macrophage Ia expression *in vivo* by synthetic copolymer L81. *J. Immunol.* **144**, 1578–1584.
101. Robinson SN, Chavez JM, Pisarev VM, Mosley RL, Rosenthal GJ, Blonder JM, Talmadge JE. 2003 Delivery of Flt3 ligand (Flt3L) using a poloxamer-based formulation increases biological activity in mice. *Bone Marrow Transplant.* **31**, 361–369. (doi:10.1038/sj.bmt.1703816)
102. Hunter RL, Bennett B. 1984 The adjuvant activity of non-ionic block polymer surfactants. II. Antibody formation and inflammation to the structure of triblocks and octablock copolymers. *J. Immunol.* **133**, 3167–3175.
103. Hunter RL, Bennett B. 1986 The adjuvant activity of non-ionic block polymer surfactants. III. Characterization of selected biologically active surfaces. *Scand. J. Immunol.* **23**, 287–300. (doi:10.1111/j.1365-3083.1986.tb01970.x)
104. Discher DE, Ahmed F. 2006 Polymerasomes. *Annu. Rev. Biomed. Eng.* **8**, 323–341. (doi:10.1146/annurev.bioeng.8.061505.095838)
105. Holowka EP, Sun VZ, Kamei DT, Deming TJ. 2007 Polyarginine segments in block copolypeptides drive both vesicular assembly and intracellular delivery. *Nat. Mater.* **6**, 52–57. (doi:10.1038/nmat1794)
106. Reddy ST, Rehor A, Schmoekel HG, Hubbell JA, Swartz MA. 2006 *In vivo* targeting of dendritic cells in lymph nodes with poly(propylene sulfide) nanoparticles. *J. Control. Release* **112**, 26–34. (doi:10.1016/j.jconrel.2006.01.006)
107. Rehor A, Hubbell JA, Tirelli N. 2005 Oxidation-sensitive polymeric nanoparticles. *Langmuir* **21**, 411–417. (doi:10.1021/la0478043)
108. Stieneker F, Kersten G, van Bloois L, Crommelin DJ, Hem SL, Löwer J, Kreuter J. 1995 Comparison of 24 different adjuvants for inactivated HIV-2 split whole virus as antigen in mice. Induction of titres of binding antibodies and toxicity of the formulations. *Vaccine* **13**, 45–53. (doi:10.1016/0264-410X(95)80010-B)
109. Kreuter J, Mauler R, Gruschkau H, Speiser PP. 1976 The use of new polymethylmethacrylate adjuvants for split influenza vaccines. *Exp. Cell Biol.* **44**, 12–19.
110. Kreuter J, Liehl E. 1978 Protection induced by inactivated influenza virus vaccines with polymethylmethacrylate adjuvants. *Med. Microbiol. Immunol.* **165**, 111–117. (doi:10.1007/BF02122746)
111. Ji W, Panus D, Palumbo RN, Tang R, Wang C. 2011 Poly(2-aminoethyl methacrylate) with well defined chain length for DNA vaccine delivery to dendritic cells. *Biomacromolecules* **12**, 4373–4385. (doi:10.1021/bm201360v)
112. Hu Y, Litwin T, Nagaraja AR, Kwong B, Katz J, Watson N, Irvine DJ. 2007 Cytosolic delivery of membrane-impermeable molecules in dendritic cells using pH-responsive core-shell nanoparticles. *Nano Lett.* **7**, 3056–3064. (doi:10.1021/nl071542i)
113. Hu Y, Atukorale PU, Lu JJ, Moon JJ, Um SH, Cho EC, Wang Y, Chen J, Irvine DJ. 2009 Cytosolic delivery mediated via electrostatic surface binding of protein, virus, or siRNA cargos to pH-responsive core-shell gel particles. *Biomacromolecules* **10**, 756–765. (doi:10.1021/bm801199z)
114. Beaudette TT *et al.* 2009 *In vivo* studies on the effect of co-encapsulation of CpG DNA and antigen in aciddegradable microparticle vaccines. *Mol. Pharmacol.* **6**, 1160–1169. (doi:10.1021/mp900038e)
115. Jong S *et al.* 2007 Encapsulation in liposomal nanoparticles enhances the immunostimulatory, adjuvant and anti-tumor activity of subcutaneously administered CpG ODN. *Cancer Immunol. Immunother.* **56**, 1251–1264. (doi:10.1007/s00262-006-0276-x)
116. Prasad S, Cody V, Sautier-Sawyer JK, Saltzman WM, Sasaki CT, Edelson RL, Birchall MA, Hanlon DJ. 2011 Polymer nanoparticles containing tumor lysates as antigen delivery vehicles for dendritic cell-based antitumor immunotherapy. *Nanomedicine* **7**, 1–10.
117. Slutter B *et al.* 2010 Nasal vaccination with N-trimethyl chitosan and PLGA based nanoparticles: nanoparticle characteristics determine quality and strength of the antibody response in mice. against the encapsulated antigen. *Vaccine* **28**, 6282–6291.
118. Brunner R, Jensen-Jarolim E, Pali-Schöll I. 2010 The ABC of clinical and experimental adjuvants: a brief overview. *Immunol. Lett.* **128**, 29–35. (doi:10.1016/j.imlet.2009.10.005)
119. Diwan M, Elamanchili P, Cao M, Samuel J. 2004 Dose sparing of CpG oligodeoxynucleotide vaccine adjuvants by nanoparticle delivery. *Curr. Drug Delivery* **1**, 405–412. (doi:10.2174/1567201043334597)
120. Shen H, Ackerman AL, Cody V, Giodini A, Hinson ER, Cresswell P, Edelson RL, Saltzman WM, Hanlon DJ. 2006 Enhanced and prolonged crosspresentation following endosomal escape of exogenous antigens encapsulated in biodegradable nanoparticles. *Immunology* **117**, 78–88. (doi:10.1111/j.1365-2567.2005.02268.x)
121. Kelly C, Jefferies C, Cryan SA. 2011 Targeted liposomal drug delivery to monocytes and macrophages. *J. Drug Delivery* **2011**, 1–11. (doi:10.1155/2011/727241)
122. Jain RA. 2000 The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. *Biomaterials* **21**, 2475–2490. (doi:10.1016/S0142-9612(00)00115-0)
123. Baras B, Benoit MA, Dupre L, Poulain-Godefroy O, Schacht AM, Capron A, Gillard J, Riveau G. 1999 Single-dose mucosal immunization with biodegradable microparticles containing a *Schistosoma mansoni* antigen. *Infect. Immun.* **67**, 2643–2648.
124. Mata E, Igartua M, Patarroyo ME, Pedraz JL, Hernández RM. 2011 Enhancing immunogenicity to

- PLGA microparticulate systems by incorporation of alginate and RGD-modified alginate. *Eur. J. Pharm. Sci.* **44**, 32–40. (doi:10.1016/j.ejps.2011.05.015)
125. Martínez Gómez JM, Csaba N, Fischer S, Sichelstiel A, Kündig TM, Gander B, Johansen P. 2008 Surface coating of PLGA microparticles with protamine enhances their immunological performance through facilitated phagocytosis. *J. Control. Release* **130**, 161–167. (doi:10.1016/j.jconrel.2008.06.003)
 126. Mueller M, Schlosser E, Gander B, Groettrup M. 2011 Tumor eradication by immunotherapy with biodegradable PLGA microspheres—an alternative to incomplete Freund's adjuvant. *Int. J. Cancer* **129**, 407–416. (doi:10.1002/ijc.25914)
 127. Temmerman MLD, Reijman J, Vandenbroucke RE, Koker SD, Libert C, Grooten J, Demeester J, Gander B, Smedt SCD. 2012 Polyelectrolyte LbL microcapsules versus PLGA microparticles for immunization with a protein antigen. *J. Control. Release* **158**, 233–239. (doi:10.1016/j.jconrel.2011.10.029)
 128. Singh J, Pandit S, Bramwell VW, Alpar HO. 2006 Diphtheria toxoid loaded poly-(ϵ -caprolactone) nanoparticles as mucosal vaccine delivery systems. *Methods* **38**, 96–105. (doi:10.1016/j.ymeth.2005.11.003)
 129. Palumbo RN, Zhong X, Panus D, Han W, Ji W, Wang C. 2012 Transgene expression and local tissue distribution of naked and polymer-condensed plasmid DNA after intradermal administration in mice. *J. Control. Release* **159**, 232–239. (doi:10.1016/j.jconrel.2012.01.012)
 130. Makela O, Seppala IJ, Vaara M. 1983 LPS greatly enhances the antibody response to hapten-polysaccharide conjugates, but not to hapten-protein conjugates. *Annu. Rev. Immunol.* **13**, 25–36.
 131. Ohno N *et al.* 1999 Solubilization of yeast cell-wall β -(1/3)-D-glucan by sodium hypochlorite oxidation and dimethyl sulfoxide extraction. *Carbohydrate Res.* **316**, 161–172. (doi:10.1016/S0008-6215(99)00049-X)
 132. Hida S, Miura NN, Adachi Y, Ohno N. 2007 Cell wall β -glucan derived from *Candida albicans* acts as a trigger for autoimmune arthritis in SKG mice. *Biol. Pharm. Bull.* **30**, 1589–1592. (doi:10.1248/bpb.30.1589)
 133. Keystone EC, Schorlemmer HU, Pope C, Allison AC. 1977 Zymosan-induced arthritis. *Arthritis Rheum.* **20**, 1396–1401. (doi:10.1002/art.1780200714)
 134. Kelkka T, Hultqvist M, Nandakumar KS, Holmdahl R. 2012 Enhancement of antibody-induced arthritis via toll-like receptor 2 stimulation is regulated by granulocyte reactive oxygen species. *Am. J. Pathol.* **181**, 141–150. (doi:10.1016/j.ajpath.2012.03.031)
 135. Hultqvist M, Nandakumar KS, Björklund U, Holmdahl R. 2010 Rabeximod reduces arthritis severity in mice by decreasing activation of inflammatory cells. *Annal. Rheum. Dis.* **69**, 1527–1532. (doi:10.1136/ard.2009.121178)
 136. Shenoy M, Christadoss P. 1983 Induction of experimental autoimmune myasthenia gravis with acetylcholine receptors using a non-ionic copolymer as adjuvant. *Immunol. Invest.* **22**, 267–282. (doi:10.3109/08820139309063408)
 137. Shakya AK, Kumar A, Nandakumar KS. 2011 Adjuvant properties of a biocompatible thermo-responsive polymer of N-isopropylacrylamide in autoimmunity and arthritis. *J. R. Soc. Interface* **8**, 1748–1759. (doi:10.1098/rsif.2011.0114)
 138. Shakya AK, Kumar A, Klaczewska D, Hultqvist M, Hagenow K, Holmdahl R, Nandakumar KS. 2011 Collagen type II and a thermo-responsive polymer of N-isopropylacrylamide induce arthritis independent of Toll-like receptors: a strong influence by major histocompatibility complex class II and Ncf1 genes. *Am. J. Pathol.* **179**, 2490–2500. (doi:10.1016/j.ajpath.2011.07.034)
 139. Goldblum RM, Pelley RP, O'Donnell AA, Pyron D, Heggers JP. 1992 Antibodies to silicone elastomers and reactions to ventriculoperitoneal shunts. *Lancet* **340**, 510–513. (doi:10.1016/0140-6736(92)91710-P)
 140. Bekercioglu M, Onat AM, Tercan M, Buyukhatipoglu H, Karakok M, Isik D, Bulut O. 2008 The association between silicone implants and both antibodies and autoimmune diseases. *Clin. Rheumatol.* **27**, 147–150. (doi:10.1007/s10067-007-0659-1)
 141. Louis JA, Lambert PH. 1979 Lipopolysaccharides: from immunostimulation to autoimmunity. *Immunopathology* **2**, 215–228.
 142. Hida S, Miura NN, Adachi Y, Ohno N. 2005 Effect of *Candida albicans* cell wall glucan as adjuvant for induction of autoimmune arthritis in mice. *J. Autoimmunity* **25**, 93–101. (doi:10.1016/j.jaut.2005.06.002)
 143. Hida S, Nagi-Miura N, Adachi Y, Ohno N. 2006 β -Glucan derived from zymosan acts as an adjuvant for collagen-induced arthritis. *Microbiol. Immunol.* **50**, 453–461.
 144. Claman HN. 1963 Tolerance to a protein antigen in adult mice and the effect of non-specific factors. *J. Immunol.* **91**, 833–839.
 145. Seppala IJ, Mäkelä O. 1984 Adjuvant effect of bacterial LPS and/or alum precipitation in responses to polysaccharide and protein antigens. *Immunology* **53**, 827–836.
 146. Liideritz O, Westphal O, Staub AM, Nikaido H. 1971 Isolation and chemical and immunological characterization of bacterial lipopolysaccharide. In vol. 4 (eds G Weinbaum, S Kadis, SJ Aji), pp. 145. New York, NY: Academic Press.
 147. Louis JA, Chiller JM, Weigle WO. 1973 The ability of bacterial lipopolysaccharide to modulate the induction of unresponsiveness to a state of immunity. Cellular parameters. *J. Exp. Med.* **138**, 1481–1495. (doi:10.1084/jem.138.6.1481)
 148. Franzl RE, McMaster PD. 1968 The primary immune response in mice. I. The enhancement and suppression of hemolysin production by a bacterial endotoxin. *J. Exp. Med.* **127**, 1087–1107. (doi:10.1084/jem.127.6.1087)
 149. Adachi Y, Suzuki Y, Ohno N, Yadamae T. 1998 Adjuvant effect of grifolan on antibody production in mice. *Biol. Pharm. Bull.* **21**, 974–977. (doi:10.1248/bpb.21.974)
 150. Kurihara K, Shingo Y, Miura NN, Horie S, Usui Y, Adachi Y, Yadamae T, Ohno N. 2003 Effect of CAWS, a mannoprotein-beta-glucan complex of *Candida albicans*, on leukocyte, endothelial cell, and platelet functions *in vitro*. *Biol. Pharm. Bull.* **26**, 233–240. (doi:10.1248/bpb.26.233)
 151. Ohno N. 2003 Chemistry and biology of angitis inducer, *Candida albicans* water-soluble mannoprotein-beta-glucan complex (CAWS). *Microbiol. Immunol.* **47**, 479–490.
 152. Tokunaka K, Ohno N, Adachi Y, Tanaka S, Tamura H, Yadamae T. 2000 Immunopharmacological and immunotoxicological activities of a watersoluble (1/3)-beta-D-glucan, CSBG from *Candida* spp. *Int. J. Immunopharmacol.* **22**, 383–394. (doi:10.1016/S0192-0561(99)00093-4)
 153. Sato M *et al.* 2003 Direct binding of Toll-like receptor 2 to zymosan, and zymosan induced NF- κ B activation and TNF- α secretion are down-regulated by lung collectin surfactant protein A. *J. Immunol.* **171**, 417–425.
 154. Dillon S *et al.* 2006 Yeast zymosan, a stimulus for TLR2 and dectin-1, induces regulatory antigen-presenting cells and immunological tolerance. *J. Clin. Invest.* **116**, 916–928. (doi:10.1172/JCI27203)
 155. Ibusuki S, Matsuda T, Iwamoto Y. 2003 Tissue engineered cartilage using thermo-responsive gelatin as an *in situ* forming and moldable scaffold with chondrocytes: *in vitro* and *in vivo* performances. *Arthritis Res. Ther.* **5**, 61. (doi:10.1186/ar862)
 156. Agmon-Levin N, Hughes GRV, Shoenfeld Y. 2012 The spectrum of ASIA: 'autoimmune (auto-inflammatory) syndrome induced by adjuvants. *Lupus* **21**, 118–120. (doi:10.1177/0961203311429316)
 157. Schaefer CJ, Wooley PH. 1999 The influence of silicone implantation on murine lupus in MRL lpr/lpr mice. *J. Rheumatol.* **26**, 2215–2221.
 158. Zandman-Goddard G, Blank M, Ehrenfeld M, Gilburd B, Peter J, Shoenfeld Y. 1999 A comparison of auto-antibody production in asymptomatic and symptomatic women with silicone breast implants. *J. Rheumatol.* **26**, 73–77.
 159. Nancy AL, Shoenfeld Y. 2008 Chronic fatigue syndrome with autoantibodies—the result of an augmented adjuvant effect of hepatitis-B vaccine and silicone implant. *Autoimmun. Rev.* **8**, 52–55. (doi:10.1016/j.autrev.2008.07.026)
 160. Kumagai Y, Shiokawa Y, Medsger TAJ, Rodnan GP. 1984 Clinical spectrum of connective tissue disease after cosmetic surgery. Observations on eighteen patients and a review of the Japanese literature. *Arthritis Rheum.* **27**, 1–12. (doi:10.1002/art.1780270101)
 161. Chizzolini C. 2007 Update on pathophysiology of scleroderma with special reference to immunoinflammatory events. *Annal. Med.* **39**, 42–53. (doi:10.1080/07853890601098152)