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

Akhilesh Kumar Shakya and Kutty Selva Nandakumar

Medical Inflammation Research, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden

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, polysters, 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-1β 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
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], muramylidipeptide, 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.

<table>
<thead>
<tr>
<th>polymer preparations</th>
<th>constituent/monomer</th>
<th>sources</th>
<th>variants</th>
<th>antigen/vaccine immunization</th>
</tr>
</thead>
<tbody>
<tr>
<td>dextran</td>
<td>α-1,6-glucan with α-1,3-branches</td>
<td><em>Leuconostoc mesenteroides</em></td>
<td>sulphated dextran, diethylaminoethyl dextran, acetylated dextran</td>
<td><em>Streptococcus bovis</em> vaccine [13], Venezuelan equine encephalomyelitis virus [14], TLR-7 agonist, imiquimod [15]</td>
</tr>
<tr>
<td>lentinan</td>
<td>β1,3-glucohexaose with β-1,6-branches</td>
<td><em>Lentinus edodes</em></td>
<td>lentinan, lentinan sulphate</td>
<td><em>Bacillus Calmette–Guérin</em> (BCG) [16], Newcastle disease vaccine [17]</td>
</tr>
<tr>
<td>inulin</td>
<td>β-α-(2-1) poly(fructofuranosyl) β-α-glucose</td>
<td><em>Streptococcus mutate, Aspergillus</em> species, plants</td>
<td>γ-inulin, δ-inulin</td>
<td><em>H2N2 influenza</em> virus [18], Japanese encephalitis, HIV [19]</td>
</tr>
<tr>
<td>mannan</td>
<td>α-α-mannose</td>
<td>yeast, bacteria and plants</td>
<td>mannan, acetylated mannan (acemannan)</td>
<td>Aβ antigen, HBsAg [20], feline leukaemia virus, feline immunodeficiency virus, heartworm antigen [21]</td>
</tr>
</tbody>
</table>

(Continued.)
<table>
<thead>
<tr>
<th>polymer preparations</th>
<th>constituent/monomer</th>
<th>sources</th>
<th>variants</th>
<th>antigen/vaccine immunization</th>
</tr>
</thead>
<tbody>
<tr>
<td>chitosan</td>
<td>β-(1→4)-linked polymer of D-glucosamine and N-acetyl-D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine</td>
<td></td>
<td>chitosan,</td>
<td>H5N1 vaccine, HBsAg antigen [22] influenza vaccine [23]</td>
</tr>
<tr>
<td>chitosan</td>
<td>N-trimethylchloride chitosan</td>
<td></td>
<td>N-trimethylchloride chitosan</td>
<td>meningococcal polysaccharide vaccine, HBsAg, plasmid DNA vaccine, <em>Helicobacter pylori</em> vaccine [24]</td>
</tr>
<tr>
<td>poly-γ-glutamic acid</td>
<td>γ-glutamic acid</td>
<td></td>
<td></td>
<td>ovalbumin [25]</td>
</tr>
<tr>
<td>multiphasic emulsions</td>
<td>amphiphilic block polymer and mineral oil</td>
<td></td>
<td></td>
<td>PEG-based copolymers (PEG-b-PLA, PEG-b-PCL, PEG-b-PLACL) oil ISAS1-vaccine [26]</td>
</tr>
<tr>
<td>polyphosphazenes</td>
<td>inorganic polymer of phosphorus and nitrogen atom with organic side group</td>
<td></td>
<td>poly(di(sodium carboxylatophenoxy phosphazene)</td>
<td>influenza vaccine [27], retrovirus based vaccine [28]</td>
</tr>
<tr>
<td>polyelectrolytes</td>
<td>electrolyte</td>
<td></td>
<td></td>
<td>polyelectrolytes multilayer capsules (PMLC) B16 melanoma, influenza virus [29]</td>
</tr>
<tr>
<td>polyanhydrides</td>
<td>diacidic monomers</td>
<td></td>
<td>1,6-bis(p-carboxyphenoxo)hexane (CPH) and 1,8-bis(p-carboxyphenoxo)-3,6-dioxoanote (CPTEG)</td>
<td><em>Yersinia pestis</em> [31]</td>
</tr>
<tr>
<td>non-ionic copolymers</td>
<td>copolymer of ethylene oxide and propylene oxide</td>
<td></td>
<td>poloxamers/pluronics</td>
<td>bovine β-lactoglobulin (BLG) [33]</td>
</tr>
<tr>
<td>polymer preparations</td>
<td>constituent/monomer</td>
<td>sources</td>
<td>variants</td>
<td>antigen/vaccine immunization</td>
</tr>
<tr>
<td>-----------------------</td>
<td>---------------------</td>
<td>---------</td>
<td>----------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>polymersomes</td>
<td>vesicle of amphiphilic synthetic block copolymers</td>
<td>—</td>
<td>polyethylene glycol-b-polybutadiene, poly(g-benzyl-l-glutamate)-K</td>
<td>Tat peptide of HIV [34], influenza haemagglutinin (HA) antigen [35]</td>
</tr>
<tr>
<td>acrylates</td>
<td>acrylic acid derivatives</td>
<td>—</td>
<td>polymethylmethacrylate, poly(2-aminoethylmethacrylate)</td>
<td>inactivated influenza virus [36], DNA vaccine [37]</td>
</tr>
<tr>
<td>polyglycolic-co-lactides</td>
<td>copolymer of glycolic and lactic acid</td>
<td>—</td>
<td>recombiant hepatitis B [38], Plasmodium vivax vaccine [39], hepatitis B vaccine [40], DNA vaccine [41]</td>
<td></td>
</tr>
<tr>
<td>poly-ε-caprolactone</td>
<td>ε-caprolactone</td>
<td>—</td>
<td>Schistosoma mansoni antigen [42], Brucella ovis antigen [43]</td>
<td></td>
</tr>
<tr>
<td>polyvinylpyrrolidone</td>
<td>N-vinylpyrrolidone</td>
<td>—</td>
<td>Aspergillus fumigates antigen [44]</td>
<td></td>
</tr>
<tr>
<td>polyethylamines</td>
<td>vinylethylamines</td>
<td>—</td>
<td>DNA vaccine [45]</td>
<td></td>
</tr>
</tbody>
</table>
together with Venezuelan equine encephalomyelitis virus antigen, induced primary antibody responses after vaccination of rhesus monkeys [14]. In another study, adjuvant properties of DEAE-D 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-glucan hexaose 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 cells 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 administrated 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-κB 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 IL-1β and as a ligand for TLR4 [56]. The reduced form of mannan (mannose) is known for the activation of the Th2 pathway when administrated with an antigen, while oxidized mannan in 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 mann (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 (Khaladze 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 mucoadhesiveness property of chitosan, the N-trimethylcholoride 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 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 showed 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 α-phenylalanine ethyl ester has shown immunological 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 bio compatible, 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 monoleate) 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)-block-poly(lactide-c-caprolactone) (PEG-b-PLA), poly(ethylene glycol)-block-poly(e-caprolactone) (PEG-b-PCL) and poly(ethylene glycol)-block-poly(lactide-co-e-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. Polypolypelectrolytes
Polyelectrolytes are one of the most frequently used polymers as an adjuvant, which consists of alternate nitrogen and phosphorus elements in the backbone with organic side groups. Polypolypelectrolyte-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 the polypelectrolytes, poly(di(sodium carboxylatophenoxyl)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 oligonucleotides 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. Polyphosphazenes
Polyphosphazenes are one of the 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 the various adjuvants available for vaccination, Freund's adjuvant, and 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 monoleate) 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)-block-poly(lactide-c-caprolactone) (PEG-b-PLA), poly(ethylene glycol)-block-poly(e-caprolactone) (PEG-b-PCL) and poly(ethylene glycol)-block-poly(lactide-co-e-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. Polypolypelectrolytes
Polyelectrolytes are one of the most frequently used polymers as an adjuvant, which consists of alternate nitrogen and phosphorus elements in the backbone with organic side groups. Polypolypelectrolyte-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 the polypelectrolytes, poly(di(sodium carboxylatophenoxyl)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 oligonucleotides 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. Polyphosphazenes
Polyphosphazenes are one of the 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 the polyphosphazenes, poly(di(sodium carboxylatophenoxyl)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 oligonucleotides 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].
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-dioxoacane (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/pluronics [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. Intraportal 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-polylbutadiene 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 polylaminoethyl 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 withprotamine 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 polyelectrolyte microcapsules in the induction of Th1/Th2 responses [127].

2.2.8. Polycaprolactones
Poly-e-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 fumigates 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, polyethyleneimines, 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 autoimmune, 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.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Monomer/Components</th>
<th>Source</th>
<th>Autoimmune Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipopolysaccharide</td>
<td>Lipid A, O polysaccharide, trisaccharide</td>
<td>Gram-negative bacteria</td>
<td>Rheumatoid arthritis [130]</td>
</tr>
<tr>
<td>β-glucan</td>
<td>D-glucose</td>
<td>Candida albicans</td>
<td>Rheumatoid arthritis [131,132]</td>
</tr>
<tr>
<td>Zymosan</td>
<td>β-glucan</td>
<td>Saccharomyces cerevisiae</td>
<td>Rheumatoid arthritis [133]</td>
</tr>
<tr>
<td>Lipomannan</td>
<td>Lipid and mannan</td>
<td>Mycobacterium tuberculosis</td>
<td>Rheumatoid arthritis [134,135]</td>
</tr>
<tr>
<td>TiterMax (TM)</td>
<td>Squalene, non-ionic block polymer of polyoxypropylene and polyoxyethylene</td>
<td>—</td>
<td>Myasthenia gravis [136]</td>
</tr>
<tr>
<td>Poly-N-isopropylacrylamide</td>
<td>N-isopropylacrylamide</td>
<td>—</td>
<td>Rheumatoid arthritis [137,138]</td>
</tr>
<tr>
<td>Silicones</td>
<td>Polymer of silica, carbon, hydrogen, oxygen</td>
<td>—</td>
<td>Scleroderma, rheumatoid arthritis, Sjogren’s syndrome, systemic lupus erythematosus [139,140]</td>
</tr>
</tbody>
</table>
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 β-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 [132,133]. 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-glucan polymer 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-kB 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 antigenspecific 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 adjuvancy 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
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.

Supported by Swedish Rheumatism Association, King Gustaf V:s 80-years foundation, Swedish Research Council and Karolinska Institute funding agencies.

**References**


10. Matthys P, Vermette K, Billiau A. 2001 Mac-1+ myelopoiesis induced by complete Freund's


28 Biomaterials 138620709788923764)


