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Polymers as immunological adjuvants: An update on recent developments

ABSTRACT

Polymers are used in several immunological applications, for example in designing new generation vaccines, implantation studies, therapeutics and for the development of animal models that are mimicking human pathological conditions. Polymers can function as attenuators to modulate host immune responses in tissue transplantation, as suppressors to inhibit immune responses against therapeutics or as an adjuvant in the activation of immune responses. Among them, polymers as adjuvants are highly promising and are being developed in the vaccination and autoimmunity fields. As an adjuvant, polymers can efficiently deliver antigens and have the ability to modulate immune responses toward an antigen. Adjuvant properties of the polymers are mainly dependent on their extrinsic and intrinsic properties such as polymer chemistry, format, charge and a fine balance between hydrophobicity and hydrophilicity. Polymers can also be easily coupled with an antigen/immuno-modulator/ligand either physically or chemically. Efficient delivery and protection against degradation of antigens *in vivo* are some of the advantages of these polymeric adjuvants. Present review focuses on various recent developments in polymeric adjuvants and responsible factors that affect their adjuvant properties.

Key words: polymeric adjuvant, vaccine, animal model

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Article info:
Received: 10 December 2012
Accepted: 21 December 2012

INTRODUCTION

Over the past few decades, focus of materials science is shifted more towards soft materials research, especially polymers are gaining importance due to their easy and controllable synthesis with a number of desirable modifications for various biological applications. Polymers are highly useful in different biotechnological as well as biomedical applications such as in the delivery of drugs/antigens/modified genetic elements, tissue engineering, regenerative medicine and implantation of medical devices (Figure 1). Polymer based drug delivery systems enable release of drugs for a longer period of time in a controlled way. Specific ligands can easily be coupled with polymers to target specific tissues or cells. The appropriate drugs can be conjugated with biocompatible polymers to make "polymeric pro-drugs" to release such active drugs inside the body (Qian et al., 2012; Xiao et al., 2012). Recently, researchers are designing responsive/smart polymer based drug delivery systems to release drugs in the presence or absence of a specific environmental stimuli such as pH, temperature, ionic strength etc. (Colson et al., 2012). Uses of these polymers have also been reported in the fabrication of biocompatible scaffolds for growing several different mammalian cells to regenerate damaged organs/tissues. These polymeric scaffolds can be designed in the form of three dimensional porous networks, which will allow proper circulation of nutrients for the optimal growth of the cells. These scaffolds are mechanically stable but can easily be degradable (Tripathi et al., 2009; Bhat et al., 2011). Polymeric materials have also been used in the fabrication of biosensors, and in the assessment of medical devices *in vivo* (Jenke et al., 2012; McGraw et al., 2012). Polymeric systems used for the implantation of medical devices should be biocompatible at least on their surface. Though many medical devices are designed as biocompatible, after implantation, they become isolated from devices in the form of fibrous encapsulates that are completely rejected by the body (Grodzinski, 1999). However, these devices did not show or induce any undesirable effects.

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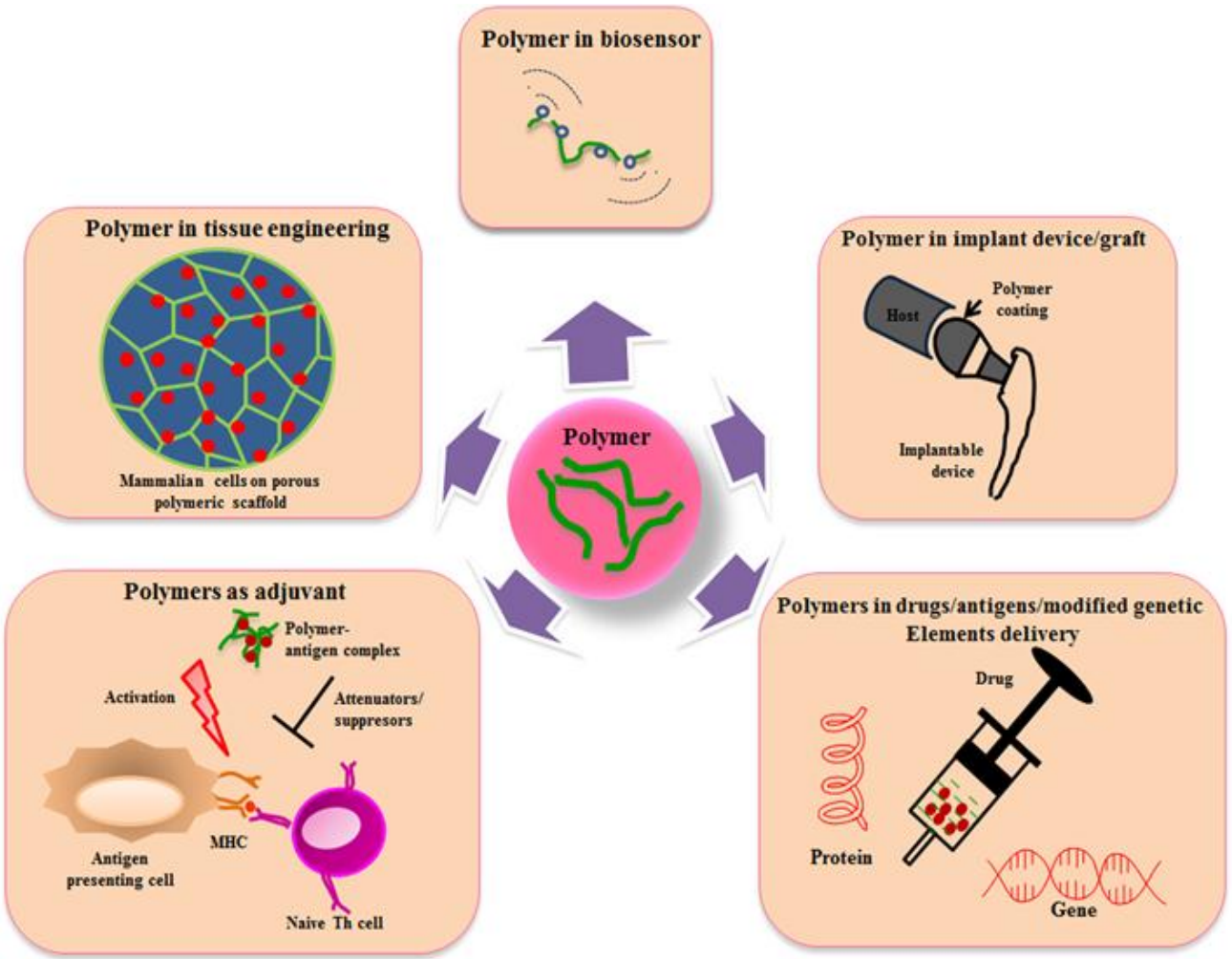

Figure 1. *Schematic representation of various polymer applications in the biomedical field. Polymers can be used in tissue engineering as scaffolds for growth of mammalian cells, in the activation or suppression of immune responses, carrier for delivery of drugs/antigens/modified genetic elements, in biosensors and in coating implantable devices/grafts to minimize rejection.*

Generally, polymers used for biomedical purposes should be biodegradable and are not generating any harmful (toxic) effects within the host as the result of their degradation. Biodegradable polymers can be natural or synthetic and also can be classified as bioerodible or bioresorbable polymers. In other biological applications, polymers can also prevent biological fouling of surfaces due to non-specific adhesion of cells or proteins, which can be toxic for the body (Diagne et al., 2012).

Polymers are extensively used in immunology as an adjuvant, attenuator, stimulator or suppressor of immune responses. As an adjuvant, polymers are promising candidates for enhancing immune responses of purified recombinant and/or weak antigens, in the development of animal disease models without any major bias in the ensuing immune responses, possibly by inducing all classes of cytokines and in vaccination studies as carriers. They can induce specific immune responses, when given along with an antigen (Shakya et al., 2013). As an attenuator, they can reduce binding or adhesion of host immune cells over implanted surfaces. Polymers can also be used as an activator or suppressor of immune responses (Jalilian, 2012; Kim et al., 2012). These points will be discussed in detail below.

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I. Polymers as immunological attenuators

Polymers can be used as an agent for reducing immune responses during transplantation of organs or tissues to prevent adverse immunological reactions. Thus, they can replace conventional immunosuppressive drugs such as cyclosporin, which is normally associated with risks of infections and induction of certain forms of cancer. Blood transfusion studies revealed that covalent conjugation of monomethoxypolyethylene glycol (mPEG), a linear chain amphiphilic molecule, to tissues or cells can significantly reduce chances of rejection and can possibly induce tolerance (Scott et al., 1997; Scott et al., 1998). Mainly loss of antigen recognition and inability of antibodies to bind with epitopes are major mechanisms involved in PEGylation. Dampening of T cell activity because of increased resistance to proteolytic degradation after PEGylation was reported with egg-white lysozyme antigen (So et al., 1996). In addition, PEGylation does not impair any cellular signalling pathways as confirmed by transplantation of PEGylated isogenic rat pancreatic islets (Chen et al., 2001). Therefore, unlike other immuno-suppressive drugs, PEGylation works physically on alterating immunogenicity of donor tissue without disturbing recipient's immune responses. Apart from PEGylation, coating of polyelectrolytes on the cell surface can also attenuate host immune responses. Recently a versatile approach has emerged based on layer-by-layer self-assembly of polyelectrolytes over red blood cells with an aim to make universally acceptable RBCs (Mansouri et al., 2011). Polyelectrolytes can attenuate or suppress binding of antibodies to the donor red blood cells. These polyelectrolyte coatings were composed of four bilayers of alginate and modified chitosan with phosphorylcholine, which was further surrounded by another two layers of alginate and modified polyethylene glycol. While encapsulating with polyelectrolytes, RBCs retained viability and functionality as confirmed by haemolysis assay. The immunologic attenuation was confirmed by reduction in the recognition of antigens on RBC surface by respective antibodies.

Recently, biological therapy (including monoclonal antibodies to cytokines to specifically inhibit their functions, receptor blocking antibodies and new fusion receptors) has emerged as a promising approach to treat allergic and autoinflammatory diseases (Broderick et al., 2011). One of the major disadvantages of using these bioactive proteins is their immunogenicity. One approach is to humanize these therapeutic proteins for better tolerance (Almagro et al., 2008). PEGylation is another well-known strategy for improvement of pharmacokinetic, pharmacodynamic and immunological properties of therapeutic proteins/peptides. Polyethylene glycol (PEG) is a typical polymer, which has been used for conjugation of therapeutic proteins. Generally, PEG conjugation improves circulating half-life, reduces toxicity and minimizes immunogenicity of therapeutic proteins. In 1977, for the first time, Auchowski et al., demonstrated that conjugation of PEG to protein reduced the immunogenicity and antigenicity of protein with minimum loss of functional activity (Abuchowski et al., 1977). Later, the molecular weight ratio of mPEG to protein was shown to determine immunotolerogenicity of mPEG proteins (So et al., 1999). In pharmaceutical research, various PEG coupled therapeutics like l-asparaginase, growth hormone, cytokines are widely approved for human use (Abuchowski et al., 1984; Jevsevar et al., 2010). However, few studies have also reported significant loss of activity of protein by PEGylation (Bowen et al., 1999). Moreover, because of the difficulties involved with the PEGylation reactions, separation and, characterization of the structure and activity of the products, new bio-processing conditions are being developed for wider application of PEGylated conjugates in medicine (Gonzalez-Valdez et al., 2012). Furthermore, site-selective PEGylation is used currently to obtain a single isomer with increasing degree of homogeneity and bioactivity by targeting protein N-terminus and free cysteines but further amino acid positions can also be PEGylated by using disulphide bridges, glutamines and C-terminus and, sites of O- and N-glycosylation or the glycans of a glycoprotein (Pasut et al., 2011).

Several labs are also designing stimuli-responsive polymers for conjugation of therapeutic proteins, which can be used for treating tumours and inflammation. The major advantage with these polymers is the ease with which their functional activities can be switched on/off in the presence or absence of a specific stimulus such as pH, temperature and ionic strength (Chilkoti et al., 2002). Similar to PEGylation, conjugation of proteins with polyvinyl alcohol (PVA) was also shown to cause loss of most of the antigenicity of antigens (Atassi et al., 1991).

II. Polymers as immuno-suppressors

Suppression of immune system can either be desirable or dangerous for the body during organ transplantation. Supression of the host immune system against infections is highly undesirable but increasing the chances of therapy for

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allergies and autoimmune diseases by dampening an ongoing immune response is favourable. Generally, state of immuno-suppression achieved by immunosuppressive drugs are associated with undesirable consequences of toxicities (Descotes, 2004). These consequences may be circumvented by the use of polymeric nanoparticles. Recently, few studies were reported the immuno-suppressive role of polymeric nanoparticles directly, such as inhalation of canban nanotubes (CNTs), which suppressed the B cell responses and TGF-α was suggested as a key element in the suppression process (Mitchell et al., 2009). Another example is the water soluble polyhydroxy C60 (fullerene) that has shown inhibitory effect on type 1 hypersensitive reaction (Ryan et al., 2007). Similarly, other polymers such as chitosan, poly(lactide-*co*-glycolide) (PLGA), polymethyvinylether-*co*-maleic anhydride based nanoparticles and dendrosomes have also been used in the suppression of type I and III hypersensitive reactions (Balenga et al., 2006; Gomez et al., 2008). Recently, a synthetic peptide based dendrimers have shown inhibitory effect on experimental allergic encephalomyelitis, model for multiple sclerosis (MS) (Wegmann et al., 2008). On the otherhand, natural polymers like water soluble polysaccharides from the alga, *Sargassum fusiforme*, composed of d-fucose, l-xylose, d-mannose and d-galactose was shown to protect against cyclophosphamide induced immunosuppression with a possibility of reducing the severity of chemotherapeutic immunosuppression (Chen et al., 2012).

Polymeric nanoparticles as carriers of immunosuppressors

On the otherhand, there are few studies reporting the effect of polymeric nanoparticles as carriers in treating autoimmune diseases. For example, PLGA particles loaded with an autoantigen collagen type II, which is present abundantly on the articular cartilage, site of inflammatory attack in arthritis, suppressed arthritis significantly (Kim et al., 2002). Similarly, suppression of diabetes in a mouse model was observed with the delivery of IL-10 cytokine expressing DNA using polymeric nanoparticles (Basarkar et al., 2009). It was also possible to treat experimental autoimmune uveoretinitis with betamethasone loaded polylactide particles (Sakai et al., 2006). Furthermore, silica nanoparticles loaded with cytotoxic T cell associated antigen was used for the treatment of thyroiditis in canine model (Choi et al., 2008). Mechanisms of action of these particles *in vivo* are yet to be deciphered. However, these nanaoparticles are not only biocompatible and biodegradable but also can deliver therapeutic proteins efficiently (Yun et al., 2012). Recently, polymeric miscelles made up of methoxy poly(ethylene oxide)-*b*-poly(ε-caprolactone) as vehicles for the solubilization and delivery of cyclosporine A (Hamdy et al., 2011) and rapamycin loaded pH-sensitive, biocompatible acetylated dextran (Ac-DEX) microparticles were used (Kauffman et al., 2011) for immunosuppression applications.

III. Polymers as immunological adjuvants

An adjuvant is not only an important component in animal model(s) development, which are essential tools to delineate pathogenesis of human diseases, but also in potential vaccine formulations to prevent/contain such diseases. Usually, adjuvants along with an antigen can be taken by antigen presenting cells (APCs) and the processed antigen can be presented on its cell surface to activate T cells. Apart from cell-cell contact and co-stimulatory signals, stimulation via secreted proteins like cytokines may also be required for the optimal activation of T cells, which in turn could potentially activate other T and B cells in the vicinity into memory as well as effector cell populations. Activated B cells can further differentiate into short and long-lived memory B cells and plasma B cells, which produce sustained antibodies against the antigen (Shlomchik et al., 2012; Winter et al., 2012).

There are only few adjuvants that are approved for human use by US Food and Drug Administration (FDA) including alum, MF59, virosomes and montanide ISA 51. Among them, alum is a well-studied material and is known for tremendous activation of antibody responses but with less cellular activation (HogenEsch, 2002). Alum can induce good immune responses, but safety concerns are still associated with these salts (Tomljenovic et al., 2012). Although emulsions have the capacity to encapsulate various antigens to induce antibody and cell mediated immune responses, they are unable to induce Th1-type immune responses. Therefore, there is a potential need for immunologically inert adjuvants. In this context, polymers have the potential to be used as alternatives because of their effectiveness and safety. Polymers are known to enhance the shelf-life of antigens and are capable of inducing long lasting antibody responses as well as cell-mediated immunity (Shakya et al., 2011a). The immunomodulators or ligands targeting specific cells can easily be coupled to polymers, which can easily be developed in the form of micro/nano based particulate adjuvants such as emulsions, microparticles, liposomes (Huang et al., 2009; Chen CH et al., 2012). Normally these particulate systems are

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directly taken up by APCs and present the associated antigens on their cell surface to T cells.

Beside these formulations, researchers focus is now shifting towards nanoparticles based on polymethacrylates, poly(lactide-*co*-glycolide) PLGA, polycaprolactones (PCL), polyvinylpyrrolidone (PVP) etc. (Madan et al., 1997; Benoit et al., 1998; Lou et al., 2009; Moon et al., 2012). Unlike conventional adjuvants, these polymeric particles are biocompatible, degradable and are capable of delivering antigens more efficiently. They can release antigen *in vivo* in a controlled manner apart from stabilizing these antigens, which is a major concern in vaccine formulations due to the liable nature of many protein antigens. For example, amphiphilic polymer composed of 1,6-bis(p-carboxyphenoxy) hexane and 1, 8-bis(p-carboxyphenoxy)-3,6-dioxaoctane has shown to stabilize *Bacillus anthracis* antigen in the form of nanoparticles. These nanoparticles can also be stored for a longer time, thus decreasing costs of vaccination considerably (Petersen et al., 2012).

For other applications, oil based adjuvants are more popular, for example in the induction of autoimmunity for the development of new disease models to study pathological mechanisms in human diseases. Apart from oil and alum based compounds, various carbohydrates (acylated dextran, mannan, lipomannan, inulin isoforms, lentinnan, chitosan) are also capable of activating the immune system (Petrovsky et al., 2011). Despite their good adjuvant potential, they still possess considerable level of toxicity and degradation problems. Recently our group discovered a novel application for the thermo-responsive polymer, poly-*N*-isopropylacrylamide (PNiPAAm), which acts as an adjuvant for enhancing the immunogenicity of the self-antigen, collagen type II (CII) to induce a variant of collagen induced arthritis (CIA), a classical animal model of Rheumatoid arthritis. CIA is used to study disease pathogenesis of RA and to discover appropriate targets for its therapy. Mice immunized with polymer-CII developed arthritis with a concomitant development of an anti-CII antibody response comprising of all the IgG subclasses. After mixing with polymer, CII still retained its native epitopes and native confirmation of CII is a pre-requsite for its arthritogenicity (Schulte et al., 1998). Usually, adjuvants enhance immunogenicity by acting as a depot for slow release of an antigen for sustained period of time, as an immunomodulator or help in the processing of an antigen in a better way by antigen presenting cells. In our experiments, gelated PNiPAAm acted as a reservoir for CII and released CII at physiological concentrations and also acted as a modulator of immune system at the cellular level. A physical interaction of CII with polymer was better compared to covalent linkage for the induction of an antibody response. Inside the host, polymer precipitates around the CII (gelation) and entrapped it. Moreover, high molecular weight of polymer was better for inducing robust immune responses due to its strong precipitation property compared to small chains of the polymer (Shakya et al., 2011a). Unlike the conventional adjuvants, immune response induced by PNiPAAm is independent of TLRs. In addition, polymer as an adjuvant is immunologically inert and at the same time did not alter the genetic susceptibility of CII. Activation of serum cytokines IFN-γ, IL-4 and IL-17 were observed few days after the immunization of mice with polymer-CII (Shakya et al., 2011b).

Polymers have also been reported as a promising candidate for their use as a scaffold for growing chondrocytes in the regeneration process of articular cartilage within arthritic joints. Natural biopolymers such as collagen based polymers are known as good polymers for supporting the growth of primary chondrocytes (Li et al., 2005). Apart from these natural polymers, various synthetic polymer(s) based scaffolds have also been tried for cartilage regeneration. For example, neocartilage formation was observed using poly-*N*-isopropylacrylamide-*co*-acrylic acid (PNiPAAm-*co*-AAc) polymer seeded with rabbit chondrocytes in the presence of transforming growth factor-β3 (TGF-β3) (Yun et al., 2008). In another study, acrylate based polymethacrylate-gelatin cryogel scaffold has shown promising scaffold property for primary chondrocytes during cartilage tissue engineering (Singh et al., 2010). Recently, a more improved microtubular oriented PLGA scaffold was developed for *in vitro* cartilage regeneration (Zhang et al., 2012).

IV. Factors affecting the immunogenicity of an antigen with polymeric adjuvants

Although polymers are considered as promising candidates for induction of immune responses, there are several factors which could affect adjuvant potential of them such as size, shape, format, polymer chemistry and a fine balance of hydrophobicity and hydrophilicity (Figure 2). These factors are playing an important role in determining their adjuvant property, which will be discussed in detail below.

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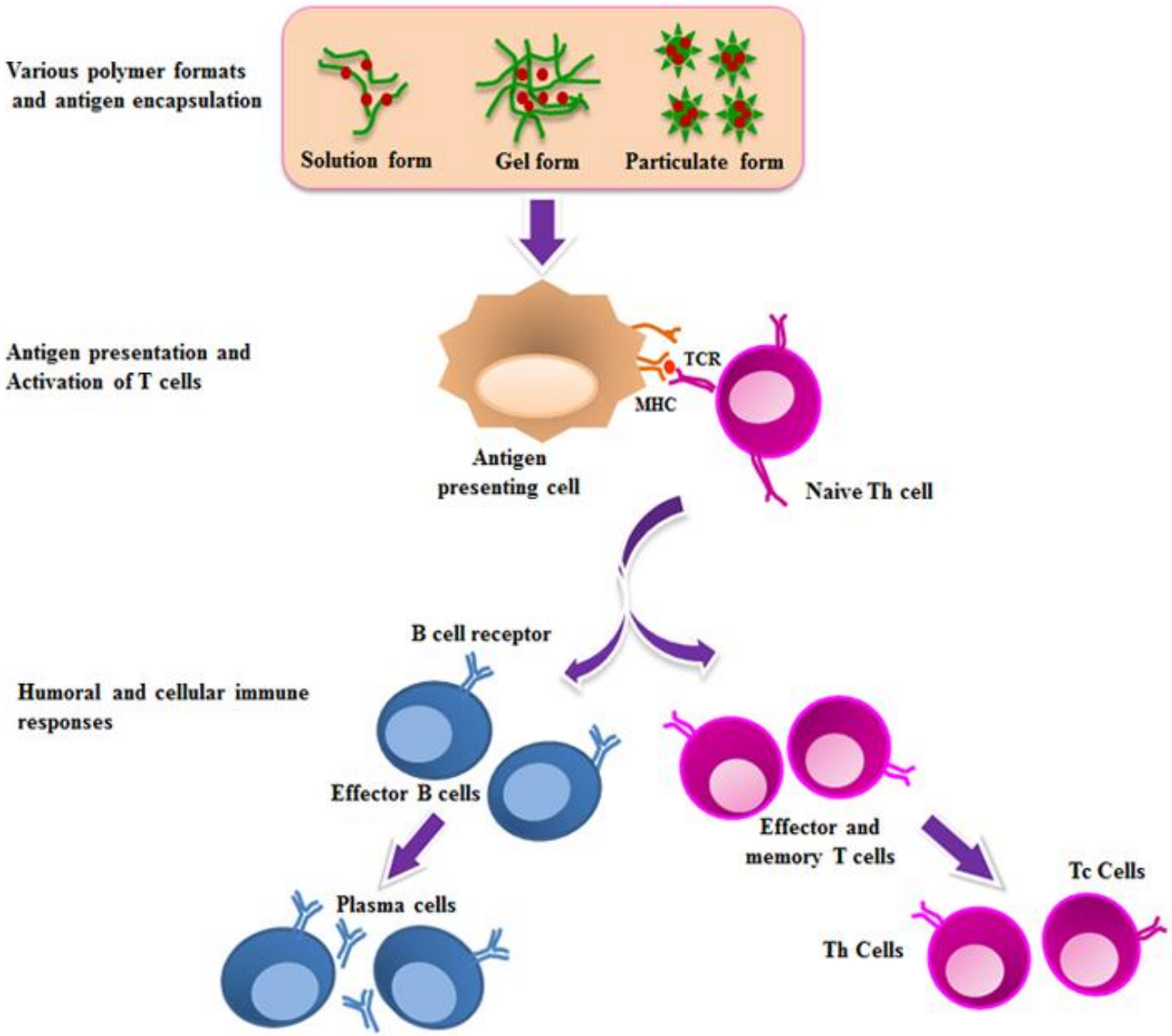

Figure 2. *Schematic representation of different polymer formats and the activation of the immune system. Polymers can exist in solution, gel and particulate formats and antigen can be easily encapsulated within them. Antigen released from these polymers is taken up by antigen presenting cells, processed and presented in the form of peptides to T cells leading to their activation, secretion of different cytokines, an effective T-B co-operation, antibody production and the effector functions.*

1. Polymer formats versus adjuvant potential

Particulate size of the polymers is an important factor for their internalization by APCs and the efficacy of an encapsulated antigen. These particulates can be taken into lymphoid organs either within interstitial space or by peripheral dendritic cells (DCs) (Swartz et al., 2008). The particles with more than 100 nm are directly internalized by peripheral DCs and taken into lymph nodes. While particles with less than 50 nm diameters are directly transported into lymphatic vessels within interstitial space and internalized by resident immature DCs (Reddy et al., 2006a; Reddy et al., 2006b). Moreover, particle size is also of importance during antigen delivery across the mucosal surfaces in mucous based vaccines. Generally, mucus layer restricts entry of particles of the size more than 100 nm, while particles with 50 nm size

can easily penetrate through this barrier (Cone, 2009). Because of this, it is a challenge to deliver vaccines through mucosal route. Particles in micro/nano range can also determine other immunological properties like antigen uptake by APCs and their maturation (Kim et al., 2010). Normally, higher cell uptake is observed with nano-sized particles compared to micro-sized particles (Yue et al., 2010). Nanoparticles in vaccine research are promising vehicles for better antigen delivery due to tunable chemical and physical properties, biocompatibility, stability, high loading capacity, specificity to target cells, and other immuno-modulator properties. They can also be made responsive for the target cell environment by using smart/intelligent or stimuli-responsive polymer based particulates (Cui et al., 2012). Nanoparticles could overcome the natural barriers such as reticuloendothelial system and can easily be cleared though kidney glomeruli. Non-specific accumulation can also be minimized by modulation of physio-chemical properties of the nanoparticles. Particles in micro/nano size can be synthesized by various approaches like physical assembly of interactive polymer chains, polymerization of monomers and cross-linking of pre-formed polymers. Mostly, chitosan, poly-L-lysine, dextran, alginic acid, hyalauronic acid, and heparin natural polymers can be fabricated in the form of nanoparticles. In the synthetic class, polymethacrylates, polyglycolides, poly(d, l-lactic-*co*-glycolic acid), poly(ε-caprolactanes) based particulate systems have been designed for different vaccines (Benoit et al., 1998; Lou et al., 2009; Moon et al., 2012).

The particulate system can be designed in a way to stimulate or suppress an immune response based on the need of the application. Kreuter and his group in 1986 analyzed the size effect of the polymethylmethacrylate and polystyrene particles encapsulated with influenza vaccines, which were synthesized through gamma radiation. Due to less aggregation of these polymers, they have synthesized different size-based particles in the range of 62-306 nm and found a better adjuvant effect with smaller particles compared to bigger particles (Kreuter et al., 1986). Moreover, an advantage with smaller sized particles is the availability of high surface area for adsorption of the antigens. This allows high encapsulation of antigens on the particles. Stimulation of macrophages and other immuno-competent cells are probable mode of action of these particles-based adjuvants (Gaspar et al., 1992). Other than the particulate format, polymers are also being used in solution form as adjuvants. Recently, our group demonstrated the adjuvant capacity of synthetic stimuli-sensitive polymer based poly-*N*-isopropylacrylamide as an adjuvant in solution form. Poly-*N*-isopropylacrylamide based polymers are temperature-sensitive and show reversible phase transition at their cloud point around ~32°C. These polymers in solution form can be mixed with any antigen, in our case with an autoantigen CII, and can be injected easily. This polymer precipitated over the antigen inside the host. The exact mechanism of action of these polymers is yet to be completely delineated. However, slow release of the antigen during long period of time could possibly be considered as the mechanism of action. With an antigen, these polymers can stay in the lymphatic system for more than one month. As an adjuvant, these polymers are biocompatible and do not show any type of toxicities both *in vitro* and *in vivo*. Polymer-CII mixture can induce both antibody and cell mediated immune responses significantly (Shakya et al., 2011a). Interestingly, unlike the conventional adjuvants like CFA and IFA, polymer based adjuvants induced all the types of effector T cells, thereby effectively precluding any major bias in the ensuing immune responses. Simialarly, we observed adjuvant property of poly-*N*-isopropylacrylamide is TLR independent unlike TLR2 dependent conventional complete Freund adjuvant (Shakya et al., 2011b).

2. Polymer chemistry

Polymer chemistry is another important factor for the adjuvant capacity of polymers. Earlier Bennett et al., (Hunter et al., 1984) studied adjuvant potential of non-ionic block copolymers based on the variation of copolymer composition. They have tested 17 surface active agents that can enhance humoral immune responses and inflammation. These agents were composed of hydrophobic polyoxypropylene (POP) and hydrophilic polyoxyethylene (POE) blocks, which are different from each other in terms of molecular weight and types of linking between the blocks. These agents were emulsified in oil-in-water and loaded with bovine serum albumin as an antigen. Large and insoluble copolymers with POE blocks flanking with POP chains were effective for antibody formation. Moreover, activation of complement system with the production of chemotactic factors were observed with these block copolymers. Adjuvant activity and inflammation decreased substantially with as increasing POE content in the block copolymers. In another preparation, block copolymers with POP blocks flanking with POE induced granuloma formation rather than antibody formation. While low molecular weight block copolymers with equal

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proportions of POE and POP content induced low immune response with an increased inflammation (Hunter et al., 1984). Moreover, in a comparison study, effect of polymer composition on immunological properties of uricase antigen was studied in conjugation with different polymers; polyvinylpyrrolidone (PVP), poly(*N*-acryloylmorpholine), branched monomethoxy polyethyleneglycol and linear monomethoxypolyethyleneglycol. Antigenicity and immunogenicity of uricase was altered and dependent on the type of polymer used. Out of these four conjugates, PVP has shown to have highest adjuvant property while monomethoxypolyethylene glycol reduced the antigenicity of uricase significantly (Caliceti et al., 1999). Recently, we have tested the temperature responsive copolymers poly-N-isopropylacrylamide-*co*-allylamine (PNiPAAm-*co*-AAm) as an adjuvant with CII autoantigen in mice. PNiPAAm based copolymers were synthesized through copolymerization of different proportions of allylamine (AAm). We observed reduced antibody formation with an increasing AAm content in the PNiPAAm-*co*-AAm terpolymer. In this case, copolymerization of AAm decreased the cloud point of PNiPAAm and as a result of this inherent precipitation property immune responses were affected significantly (Shakya et al., 2011a).

3. Molecular weight

Molecular weight significantly affects the adjuvant potential of a polymeric adjuvant. Higher molecular weight polymers enhanced the immune responses significantly compared to low molecular weight polymers. Besides molecular weight, the distribution of polymeric chains is also another important factor that can affect adjuvant potency of a polymer. For the first time Hunter et al., in 1991 has observed the effect of molecular weight of the copolymer using polyoxypropylene (POP) and polyoxyethylene (POE) adjuvant system. Mean antibody titre values were effectively increased as the molecular weight of POP blocks of copolymer increased in POP-*b*-POE (Hunter et al., 1991). Similarly, we have also observed the effect of increasing molecular weight of PNiPAAm on its adjuvant effect. 120 kDa average molecular weight of PNiPAAm induced a higher antibody response when injected along with CII compared to PNiPAAm with 70 kilodaltons (Shakya et al., 2011a).

4. Hydrophilic and hydrophobic balance

Hydrophilic and hydrophobic effect of polymers is playing an important role in determining their adjuvant effect. Hydrophobic domain of polymers is favourable for protein adsorption, which recruits more primary antigen presenting cells for phagocytosis of the antigen, thus affecting potential retention of antigens with the polymers (Nguyen et al., 2009). For the first time, the effect of particle(s) hydrophobicity on its adjuvant properties was demonstrated with oral immunization (Eldridge et al. 1990), where higher hydrophobicity favoured a significantly higher immune response (Eldridge et al., 1991). This observation was further supported by the study of Youan et al., who synthesized hydrophobic polycaprolactone (PCL) particles, which enhanced the phagocytosis of PCL particles by macrophages (Youan et al., 1999). Cellular mechanisms by which hydrophobic property affects adjuvant activity are not yet clear. However, how they affect the humoral immune responses through involvement of TLRs and induced maturation of dendritic cells are well documented (Ferreira et al., 2012). Apart from hydrophobicity, hydrophilic moieties of polymers are also important to determine their adjuvant potential. Generally, hydrophilic moiety of an adjuvant mixture prevents non-specific interactions of proteins with cells. Physically, hydrophilic part helps in the solubility of antigens with an adjuvant, which helps in homogenous distribution of antigen within the polymer-antigen mixture. For example, PLGA nanoparticles coated with PEG molecules help in non-specific interaction of particles with other cells. On other hand, other property like loading efficiency of antigens was substantially decreased after coating with PEG molecules. Hydrophilic domain of an adjuvant is also important in the aggregation of nanoparticles (NPs) on mucus and their transport across the mucus layer. Generally, short and dense grafting of PEG molecular chains stabilized the NPs compared to longer and well dispersed grafted PEG. Larger PEG chain might get entangled with each other and prevent NPs phagocytosis by APCs. Therefore, optimum hydrophilic effect is an important factor for a better adjuvant effect (Huang et al., 2000; Lai et al., 2009). Recently, contradictory findings were reported with the adjuvant properties of saponins obtained from *Chiococca alba*, a flowering plant in the coffee family, *Rubiaceae*. Usually saponins are tripene bidesmosides, which contain glycidic moieties. The adjuvant activity of saponins increased with the increasing length and hydrophilicity of the sugar chain attached to C-28 (Nico et al., 2012).

Recently, we found that a fine balance of hydrophilic and hydrophobic moieties determined the adjuvant effect of PNiPAAm and its copolymers in the induction of auto-

immune responses with the CII antigen. We screened four different synthetic polymers having hydrophilic and/or hydrophobic moiety, viz., poly(*N*-allylisopropylamine), poly-*N*-isopropylacrylamide, poly-*N*-isopropylacrylamide, polybut-3-en-2-one. In both mice and rats, poly-N-isopropylacrylamide showed highest adjuvant activity among all the polymers. Structurally this polymer have isopropyl group attached to an amide group, which is contributing more to its hydrophobic nature. Interestingly, adjuvant activity was decreased in polyacrylamide followed by polybut3-en-2-one, which has ketone group contributing to the hydrophobicity of this polymer. Furthermore, poly(*N*-allylisopropylamine) did not show any adjuvant effect because of its complete hydrophilic nature due to the presence of amine groups. Thus, a fine balance of hydrophilicity and hydrophobicity is important for determining the adjuvant activity of a polymeric adjuvant.

5. Charge

Charge of the polymers is another factor for deciding their adjuvant efficacy. Charge can affect the loading efficiency, stability, bio-adhesive properties of antigens, especially in DNA vaccines (Sharma et al., 2009). Generally, cationic nano-gels can easily form complexes with DNA by electrostatic interactions, thereby increasing the stability of an antigen. Positive charge can also facilitates higher transfection efficiency by favouring interactions of the polymer with the negatively charged proteins of cell membrane (Mao et al., 2001). Moreover, cationic polymer(s) mediated delivery systems enhanced both localized and systemic immunogenicity compared to other polymers (Kumar et al., 2002).

A series of polyethylamines (PEI) complexed with DNA and their *in vivo* expression was checked in a study to enhance adaptive immune responses. All PEI were able to increase 20 to 400 fold DNA expression *in vivo* and 10 to 25 fold more $CD8^+$ T cell responses in BALB/c and C57BL/6J mice respectively (Grant et al., 2012). Moreover, PEI complexed DNA improved antigen-specific Th1 type of cells and humoral mediated immunity substantially. Apart from their adjuvant properties, PEI also has the ability to protect DNA from degradation. Another cationic lipopolymer based on liposome-polyethylene glycol-polyethyleneimine complex (LPPC) proved to be a promising adjuvant, which can strongly adsorb the antigen on its surface leading to enhanced immune responses. LPPC has enhanced the antigen uptake and surface marker expression on immune cells with an increased activation of Th1-type of immunity (Chen et al., 2012).

CONCLUSIONS

Polymers are having many promising applications in the formulation of new generation vaccines, implantation studies, therapeutics, development of new disease models and in the activation or suppression of immune responses. As an adjuvant, polymers can be efficiently used to deliver an antigen and fine tune the ensuing immune responses. Adjuvant properties of polymers are mainly dependent on its extrinsic and intrinsic properties such as polymer chemistry, format, molecular weight, charge and a fine balance between hydrophobicity and hydrophilicity. Polymers are proved to be promising candidates for antigen delivery and when mixed with an antigen can induce an antigen-specific immune response. But several questions remain to be explored further like mechanisms of antigen processing and presentation, type of cells recruited at the injection site and the nature of signaling pathways involved during the induction of immune responses involving polymeric adjuvants.

Acknowledgement

Supported by Swedish Rheumatism Association, King Gustaf V:s 80-years foundation, Swedish Research Council and KI (Fobi).

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