Nanoparticles: A Vaccine Adjuvant for Subcutaneous Administration

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Summary

Subcutaneous administration is the most appropriate route for delivering an antigen because the antigen can drain directly from the injection site to lymph nodes where immunocompetent cells reside. Nanoparticles, having a size ranging from 1-1,000 nm, are considered as the most promising adjuvant for subcutaneous immunization. This article is aimed to review the studies relating to the investigation of nanoparticles efficiency to elicit the immune response following subcutaneous administration and to determine if the nanoparticles are actually capable to enhance immune response. Factors influencing the achievement and the magnitude of immune induction are also included.

Introduction

Adjuvants are defined as substances used in combination with a specific antigen to enhance the immune response (Newman and Powell, 1995; O'Hagan, 1997). A variety of adjuvants have been identified and investigated over the years including 1) inorganic compounds such as aluminium hydroxide, aluminium phosphate and calcium phosphate, 2) oil-based or emulsion adjuvants such as Freund's adjuvants and MF59, 3) bacterial products and their derivatives such as lipopolysaccharides, diphosphoryl lipid A, monophosphoryl lipid A, trehalose dimycolate, cholera toxin, muramyl dipeptide, 4) cytokines including monocyte colony-stimulating factor, granulocyte colony-stimulating factor, interleukins and interferons 5) oligodeoxynucleotides containing CpG motifs (Krieg, 2001) and 6) particulate delivery systems such as liposomes, immune-stimulating complexes (ISCOMs), microparticles and nanoparticles. To enhance immunogenicity of an antigen, these adjuvants are required and they can be used either alone or in combination with the others. A discussion of details on other adjuvants apart from nanoparticles is, however, beyond the scope of this review.

Subcutaneous administration is considered as the most appropriate route for delivering an antigen to secondary lymphoid organs such as lymph nodes where the adaptive immunity is initiated (Oussoren and Storm, 2001). It has been long known that the fate of particles following subcutaneous administration has been shown to be size dependent (Figure 1) (Hawley et al., 1995; Higuchi et al., 1999; Ikomi et al., 1999). Large particles, having a size greater than 1 μm are unable to access and drain into the lymphatics and may not be phagocytosed readily. Hence, they are retained at the injection site until they degrade to a sufficiently small size (Ikomi et al., 1995; Porter, 1997; Ikomi et al., 1999). Inversely, small particles having a size less than 1 μm enter lymphatic capillaries, which form a one-way drainage system connected to the lymphatic vessels, lymph nodes and lymphatic ducts, respectively. Transport of these sub-micron particles into draining lymphatic capillaries occurs via one of two mechanisms, again depending on particle size. Particles having a diameter of less than 100 nm enter the lymphatic capillaries through the gaps between the lymphatic endothelial cells (Figure 2). On the other hand, particles having a size between 100 nm and 1 μm are favorably phagocytosed by antigen presenting cells such as dendritic cells which subsequently passage into the lymphatic capillaries.

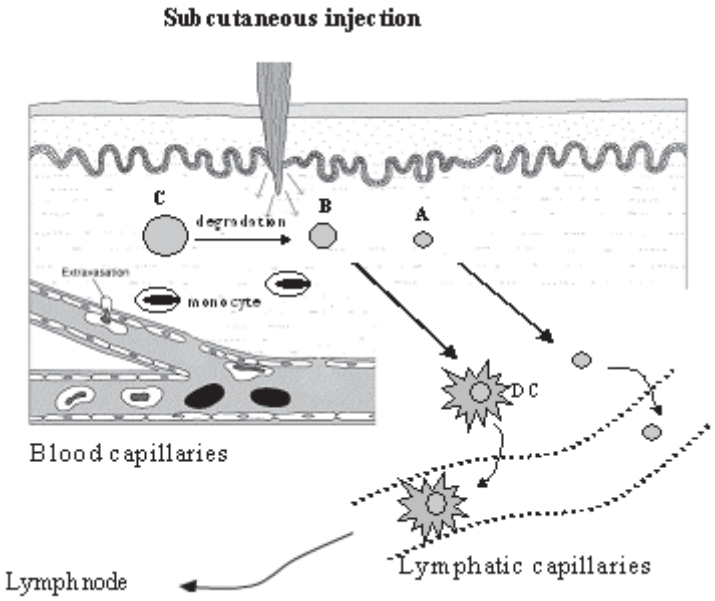

Figure 1 Schematic showing the fate of different sized particles following subcutaneous administration. A) Small particles (< 100 nm) drain directly into lymphatic capillary via the intercellular pathway. B) Particles having sizes 100 nm - 1 μm are phagocytosed by dendritic cells and are transported via the intracellular pathway. C) Large particles (> 1 μm) are retained at the injection site and slowly degraded
Sources: Modified from Potter (2002)

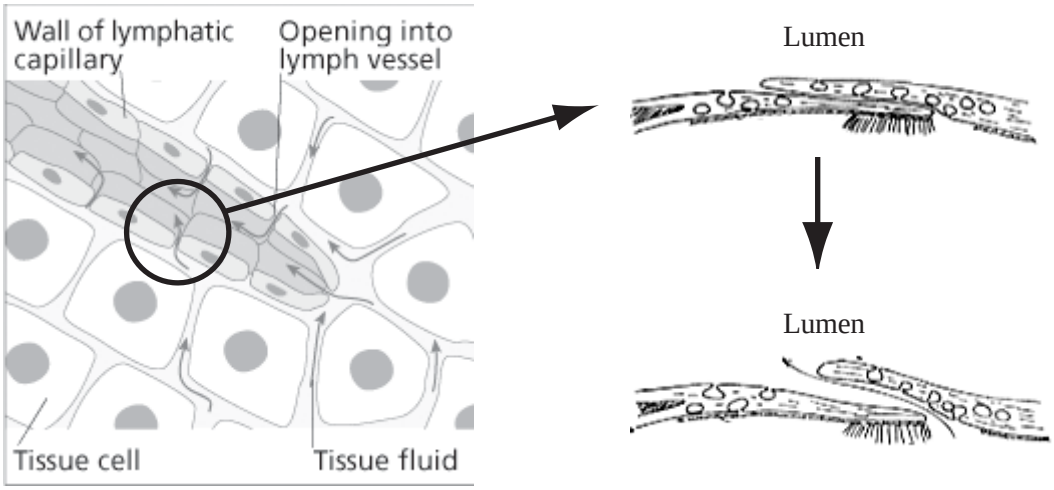

Figure 2 Schematic of a lymphatic capillary showing overlapping cells and direction of lymph flows (left panel). Enlarged pictures show the overlapping endothelial cells (right panel). The endothelial cells lining the walls of a lymphatic capillary are overlapping and forming flaplike minivalves which act as one-way valves to prevent backflow of lymph from inside out when the fluid pressure inside the lumen of capillary is higher than outside (top, right panel). Conversely, when the fluid pressure of the interstitial tissue surrounding the lymphatic capillary increases, these valves will be pushed inward allowing fluid to flow into the lumen (bottom, right panel)
Sources: Schmid-Schonbein and Zweifach (1994), Cancer Research UK (2001)

Due to their small particle size, nanoparticles are thought to be the most promising system for delivery of antigen to the draining lymph nodes following subcutaneous administration. Therefore, the aim of this review is to summarize studies relating to the investigation of the nanoparticles to provoke immune protection following subcutaneous injection, to determine if nanoparticles are

actually able to induce immune protection and to discuss what factors influence the achievement and the magnitude of immune response.

Nanoparticles as vaccine adjuvants

Nanoparticles are defined as particles with a size ranging from 1 - 1,000 nm. They can be classified into two types: nanospheres, having a matrix interior, and nanocapsules, having a central cavity surrounded by a polymer wall. A number of studies have investigated the potential of nanoparticles as vaccine adjuvants following subcutaneous administration. These studies are summarised in Table 1. These investigations have focused on poly(methyl methacrylate) (PMMA), poly(D,L-lactide-co-glycolide) (PLGA) and poly(alkylcyanoacrylate) (PACA) nanoparticles.

PMMA nanoparticles were shown to be the most effective adjuvant for inactivated HIV-2 split whole virus when compared to 23 other known adjuvants including alum and Freund's adjuvants (Stieneker et al., 1995). The PMMA nanoparticles have also been reported to enhance the immune response to various antigens including influenza virus (Kreuter and Speiser, 1976; Kreuter et al., 1976; Kreuter and Haenzel, 1978; Kreuter and Liehl, 1981; Kreuter, 1992), bovine serum albumin (BSA) and HIV-1 split vaccine (Kreuter, 1992).

PMMA nanoparticles can be prepared by dissolving methylmethacrylate (MMA) monomer in distilled water or buffer in concentrations up to 2% (w/v). Polymerisation can then be initiated either by gamma-radiation or by heating (65 - 85 ^{o}C) in the presence of a polymerisation initiator such as ammonium peroxodisulfate or potassium peroxodisulfate (Stieneker and Kreuter, 1994). The adjuvant effect of PMMA nanoparticles is affected by formulation variables such as MMA concentration used in preparation (Kreuter and Speiser, 1976), method of antigen incorporation (Kreuter and Liehl, 1981) and antigen concentration (Kreuter and Speiser, 1976). Kreuter and Speiser (1976) investigated the effect of MMA concentration (ranging from 0 to 2% w/v) on the level of antibody response against influenza virus. The authors found that increasing the amount of monomer up to a concentration of 0.5% w/v of the polymerisation medium resulted in an enhanced immune response. Use of concentrations greater than this resulted in a decreased response (Kreuter et al., 1976). The decrease in immune response to influenza virus when using high concentrations of monomer may be due to an increase in particle size of PMMA nanoparticles (from 100 - 200 nm to 500 nm) resulting from aggregate formation as suggested by Kreuter and co-workers (1978, 1986).

In general, an antigen can be incorporated in nanoparticles either by encapsulation (antigen is added prior to polymerisation) or by sorption (antigen is added after polymerisation). Using an MMA concentration 0.5% for the preparation of nanoparticles, encapsulation of influenza virus results in higher antibody responses than sorption (Kreuter and Liehl, 1981). This may be due to better protection of the antigen from degradation and/or a more sustained release following encapsulation (Kreuter and Haenzel, 1978).

Concentration of antigen has also been reported to have an effect on the antibody response (Kreuter and Speiser, 1976). It was found that increasing antigen concentration resulted in an increase in the level of antibody response (Kreuter and Speiser, 1976).

Nanoparticles prepared from synthetic polyesters such as PLGA polymers have also been investigated for their adjuvant effects and have been shown to enhance the immune responses to various antigens as summarised in Table 1. The release of antigen from PLGA nanoparticles can be manipulated by varying the ratio of lactide and glycolide polymers and/or molecular weight of the polymers (Coombes et al., 1996). The effects of these variables on the adjuvant effect of PLGA nanoparticles have been investigated. For instance, it was demonstrated that nanoparticles prepared from a 75:25 PLGA copolymer (a slow degrading polymer) gave a slower release rate of antigen than those prepared from a 50:50 PLGA copolymer (a fast degrading polymer)

(Coombes et al., 1996). It was subsequently demonstrated that the level of IgG antibody following subcutaneous immunization with OVA-loaded 75:25 PLGA nanoparticles was maintained for a longer period in comparison to OVA-loaded 50:50 PLGA nanoparticles (Coombes et al., 1996).

PLGA nanoparticles can be prepared by oil-in-water (o/w) or water-in-oil-in-water (w/o/w) emulsion/solvent evaporation (Lemoine and Preat, 1998). O/W emulsion/solvent evaporation is carried out by dissolving PLGA in a volatile organic solvent e.g. dichloromethane. The organic phase is then emulsified with an aqueous phase containing the antigen together with a stabilizer/surfactant such as polyvinyl alcohol (PVA) using a homogeniser or a sonicator. Following emulsification, the organic solvent in the resulting o/w emulsion is evaporated under reduced pressure (Lemoine and Preat, 1998). This method enables a high entrapment of hydrophobic bioactives (Bodmeier and Chen, 1990) but only a poor entrapment of hydrophilic bioactives (Bodmeier and Mc Ginity, 1987). To enhance entrapment of hydrophilic bioactives a w/o/w emulsion/solvent evaporation method has been proposed (Jeffery et al., 1993). A primary w/o emulsion is first prepared by emulsifying an aqueous phase containing the antigen in the organic solvent containing the polymer. The primary w/o emulsion is then emulsified in an outer aqueous phase containing a stabilizer/surfactant to obtain a w/o/w emulsion. The organic solvent is then evaporated under reduced pressure (Coombes et al., 1996; Lemoine and Preat, 1998). Besides emulsion/solvent evaporation, PLGA nanoparticles can also be prepared by coacervation by adding a solution of PLGA in for example an acetone-methanol mixture dropwise to an aqueous solution of antigen containing a stabilizer/surfactant. The system is stirred overnight to evaporate off the solvent (Conway et al., 2001).

Like PMMA, the incorporation of an antigen in PLGA nanoparticles can be performed by encapsulation or sorption. Again, the immune response elicited when an antigen is encapsulated inside PLGA nanoparticles has been shown to be higher than that elicited when the antigen is adsorbed onto the surface of the nanoparticles (Coombes et al., 1996). As discussed above, this may be due to better protection of antigen from degradation and/or a more sustained release following encapsulation (Kreuter and Haenzel, 1978). However, the addition of the antigen before the addition of PLGA polymer for encapsulation may result in its denaturation due to the exposure of antigen to organic solvents (e.g., dichloromethane) and high shear rates. This can lead to the loss of immunogenicity (Alonso et al., 1994).

The adjuvant effect of PLGA nanoparticles can be enhanced by combining the nanoparticles with other adjuvants such as alum (Raghuvanshi et al., 2001) and CpG oligodeoxynucleotide (Diwan et al., 2002). Raghuvanshi and associates (2001) reported that the combination of alum with PLGA nanoparticles enhanced the immune response to tetanus toxoid (TT) to a similar extent as two injections of TT-alum and to a higher extent than a single injection of TT-loaded PLGA nanoparticles. The authors suggested three possible mechanisms for the synergistic effect. Firstly, the combination offers both an initial dose (resulting from alum) and a booster dose (resulting from the nanoparticles). Secondly, alum is able to attract immune cells to the injection site. Thirdly, alum forms a gel at the injection site and thus may slow down the drainage of nanoparticles. As a consequence, the interaction between nanoparticles and antigen presenting cells (APCs) is enhanced. The adjuvant effect of PLGA nanoparticles can also be enhanced by increasing the hydrophobicity of the polymers through mixing PLGA polymers with other hydrophobic polymers such as poly- -caprolactone (Singh et al., 2001). CpG motifs have also been found to enhance immune response of C57BL/6 mice to tetanus toxoid (TT) when they are co-encapsulated in PLGA nanospheres (Diwan et al., 2002).

Table 1 Summary of the literature relating to the use of nanoparticles as vaccine adjuvants following s.c. or i.m. injection.

Type of particles	Preparation method	Size	Antigen	Year	References
PMMA	γ-radiation	50-300 nm	Influenza virus	1976	(Kreuter et al., 1976b and c)
PMA, PMMA, PEMA, PBMA	γ-radiation, heating in the presence of ammonium peroxodisulfate, heating in an autoclave	100-200 nm	Influenza virus	1978	(Kreuter et al., 1978)
PMMA	γ-radiation	n.m.	Influenza virus	1981	(Kreuter et al., 1981)
Polyacrylamide	Emulsion polymerisation	250-300 nm	L-asparaginase	1982	(Edman et al., 1982)
PMMA	Heating in the presence of potassium peroxodisulfate	60-310 nm.	Bovine serum albumin (BSA)	1986	(Kreuter et al., 1986)
MMA/HEMA copolymer ratio 2:1, 1:1 and 1:2	Heating in the presence of potassium peroxodisulfate	190-360nm .	BSA, influenza virus, HIV-1 and HIV-2 split vaccine	1988, 1992	(Kreuter et al., 1988; Kreuter, 1992)
PMMA	γ-radiation	60-260 nm			
PECA	Emulsion polymerisation	90 nm			
PBCA	Emulsion polymerisation	60 nm			
PS	-	100-270 nm			
PMMA	γ-radiation	120 nm	HIV-2	1995	(Stieneker et al., 1995)
PBCA	Emulsion polymerisation	443 nm			
PHCA	Emulsion polymerisation	961 nm			
PLGA ratio 50:50, 75:25	w/o/w emulsion/solvent evaporation	460-900 nm	Ovalbumin	1996	(Coombes et al., 1996)

Table 1 Summary of the literature relating to the use of nanoparticles as vaccine adjuvants following s.c. or i.m. injection. (continued)

Type of particles	Preparation method	Size	Antigen	Year	References
PLGA 50:50 mw 8000	o/w emulsion/solvent evaporation, w/o/w emulsion/solvent evaporation	200 nm	Hemagglutinin	1998	(Lemoine et al., 1998)
PLGA 50:50	w/o/w emulsion/solvent evaporation method	250-1000 nm	BSA	2000	(Hernandez et al., 2000)
PLGA 50:50	w/o/w emulsion/solvent evaporation method Coacervation	1.5-4.7 μm . 200-600 nm	Pertussis toxoid and filamentous hemagglutinin	2001	(Conway et al., 2001)
PLGA 50:50 mw 100,000	w/o/w emulsion solvent evaporation technique	240 nm	Tetanus toxoid	2001	(Raghuvanshi et al., 2001)
PLA PLGA 75:25 PLA + PCL PLGA + PCL	w/o/w emulsion/solvent evaporation method	185 nm 182 nm 181 nm 172 nm	Diphtheria toxoid	2001	(Singh et al., 2001)
PLGA 50:50	w/o/w emulsion/solvent evaporation method	300-308 nm	Tetanus toxoid	2002	(Diwan et al., 2002)
PECA	o/w interfacial polymerization of microemulsions	300 – 400 nm	Ovalbumin	2002	Pitaksuteepong et al., 2002)

Abbreviation: MMA/HEMA = methyl methacrylate-2-hydroxyethylmethacrylate copolymer; PBCA = poly(butyl cyanoacrylate); PECA = poly(ethyl cyanoacrylate); PBMA = poly(butyl methacrylate); PCL = poly- -caprolactone; PEMA = poly(ethyl methacrylate); PHCA = poly(hexyl cyanoacrylate); PLA = poly(D,L-lactide); PLGA = poly(D,L-lactide-co-glycolide); PMA = poly(methacrylate); PMMA = poly(methyl methacrylate); PS = polystyrene

Both PMMA and PLGA nanoparticles are slowly biodegradable, which can sustain the release of antigen over a period of months (Kreuter, 1994). Recently, it has been suggested that the antigen presentation by APCs to naive and effector T cells may only be required over the first few days for an efficient induction of T cell expansion and differentiation and that the prolonging of antigen presentation can lead to T cell death and tolerance (Jelley-Gibbs et al., 2000). Hence, nanoparticles, which release antigen over a period of days, may be better delivery systems for vaccine antigens than those which release antigen very slowly. The release of antigen over such a short time period can be obtained from PACA nanoparticles. However, a limited number of studies have investigated the potential of PACA nanoparticles in eliciting an immune response following subcutaneous administration (Kreuter et al., 1988; Stieneker et al., 1995). In these studies, PACA nanoparticles were prepared by the emulsion polymerisation method which alkylcyanoacrylate monomer is added dropwise into an acidic solution containing surfactant. An antigen (bovine serum albumin or inactivated whole human immunodeficiency virus type 2 (HIV-2)) was incorporated in the resulting nanoparticles by sorption. The adjuvant effect of PACA nanoparticles reported from these studies was shown to be low. This might be due to the incorporation method of the antigen which was carried out by sorption. As discussed above, the sorption method offers poor protection and/or cannot successfully prolong the release of an antigen. In addition, following mixing PACA nanoparticles with antigen solution (phosphate buffer solution), the adsorption was facilitated by either gently agitating for 2 days (Kreuter et al., 1988) or sonicating for 2 hrs on three consecutive days (Stieneker et al., 1995). The dispersion of nanoparticles in an aqueous phase may result in the degradation of the PACA polymers, which are known to be fast degrading. Furthermore, the sonication process may as well disrupt the structure of nanoparticles although in these studies the temperature was kept below 20^{o}C. The loading efficiency of the antigens onto PACA nanoparticles in these studies was not reported.

Pitaksuteepong and colleagues (2002) has evaluated the potential of the poly(ethyl cyanoacrylate) (PECA) nanocapsules prepared by interfacial polymerisation of water-in-oil biocompatible microemulsions for vaccine delivery following subcutaneous administration. The preparation method of PECA nanocapsules based on this technique was chosen because it offered several advantages over the other methods. Firstly, encapsulation efficiency of hydrophilic bioactives such as insulin in the nanocapsules was found to be high, i.e., 80-95% at a monomer concentration of 1.5% (w/v) of the polymerisation medium (Watnasirichaikul et al., 2000). Secondly, since microemulsions are spontaneously forming, high shear force as used in the preparation of PMMA and PLGA can be avoided. Thirdly, the polymerisation of PECA is simply initiated by hydroxyl ions resulting from the dissociation of water and thus no additional energy input such as radiation or heating is required. Using a murine dendritic D1 cell line, it was found that PECA nanocapsules enhance delivery of antigen to dendritic cells compared to formulation of antigen as a solution (Pitaksuteepong et al., 2004). The potential of nanocapsule formulations to initiate an immune response was evaluated using a Balb/c mouse model. The results suggested that the immune response in mice could be enhanced when OVA was encapsulated in PECA nanocapsules. The difference in the magnitude of the immune response between mice receiving the two types of nanocapsules (i.e., those prepared using 1% and 6% PECA monomer) was observed. The immune response which was obtained from the nanocapsules prepared using 6% monomer appeared more sustainable and the difference was more pronounced following boosting.

Conclusions

Several studies detailed in this review have demonstrated that nanoparticles are the promising adjuvant systems for subcutaneous sub-unit antigen delivery. This is due to particles in the nano-size range are shown to be efficiently taken up by APCs. An enhanced uptake of nanoparticles by APCs facilitates the drainage of particles from the injection site to draining lymph nodes where immunocompetent cells including T cells reside and thus lead to a strong adjuvant effect. However, type of monomer/polymer employed, the preparation technique, the incorporation method of an antigen and antigen concentration are needed to be considered in order to obtain high level of immune response.

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