Expert Opinion

1. Introduction
2. Colloidal delivery systems
3. Expert opinion

Immunostimulatory colloidal delivery systems for cancer vaccines

Anne Saupe†, Warren McBurney, Thomas Rades & Sarah Hook
†*School of Pharmacy, University of Otago, PO Box 913, Dunedin, New Zealand*

Cancer vaccine delivery is a multidisciplinary scientific field that is currently undergoing rapid development. An important component of cancer vaccines is the development of novel vaccine delivery strategies, such as colloidal immunostimulatory delivery systems. The importance of formulation strategies for cancer vaccines can be explained by the poor immunogenicity of tumour antigens. Colloidal vaccine delivery systems modify the kinetics, body distribution, uptake and release of the vaccine. This review explores recent research that is directed towards more targeted treatments of cancer through to colloidal vaccine delivery systems. Widely investigated carrier systems include polymeric micro- and nanoparticles, liposomes, archaeal lipid liposomes (archaeosomes), immune-stimulating complexes and virus-like particles. These systems are evaluated in terms of their formulation techniques, immunological mechanisms of action as well as the potential and limitations of such colloidal systems in the field of cancer vaccines.

Keywords: archaeosomes, cancer vaccine delivery, colloidal systems, immune stimulating complexes, liposomes, polymeric microparticles, polymeric nanoparticles, virus-like particles

Expert Opin. Drug Deliv. (2006) 3(3):345-354

1. Introduction

The three standard therapies for the treatment of cancer are the surgical removal of tumours, chemotherapy and radiation. Immunotherapy, which includes treatment with cancer vaccines, is now emerging as a fourth viable alternate approach to cancer therapy. For the treatment of cancer by vaccination, the aim of the therapy is to stimulate a therapeutic antitumour immune response. This is generated by immunisation with tumour-specific or tumour-related antigens. Such antigens are proteins or peptides that are expressed only by the tumour or by a known range of tissues, including the tumour. A wide range of such proteins have been identified and vaccines that contain either the entire protein, peptides from the proteins, or DNA that encodes the peptides or protein are being used as cancer therapies. However, the major limitation with vaccines that use such an approach is the inherently low immunogenicity of peptides and proteins. This is largely attributable to the fact that they do not contain any of the molecular patterns or danger signals that are necessary for activating antigen-presenting cells (APC), such as dendritic cells (DCs) [1]. DNA-based vaccines are intrinsically more immunostimulatory as these vaccines usually contain bacterial plasmids, which in turn contain unmethylated C poly G motifs (CpG) that interact with Toll-like receptors present in APCs, providing the signals that are necessary to stimulate APCs [2,3].

Due to this low immunogenicity, tumour antigens that are delivered on their own do not generate therapeutic immune responses. Indeed, soluble peptide and protein vaccines can induce an immune tolerance, which is an undesirable outcome where there is downregulation of the immune response against the cancer [4]. Cancer vaccines also present the additional problem where the antigens that are being used to stimulate the antitumour immune response are often self-antigens (or are closely

informa
healthcare

related to self-antigens), which means that the vaccine must overcome or bypass the regulatory mechanisms that prevent the development of immune responses to self-antigens [5]. Finally, tumours are themselves often immune suppressive, creating yet more obstacles for the development of a therapeutic anti-tumour immune response. For example, melanoma tumours have been demonstrated to produce IL-10 [6] and TGF-β [7], which are two powerful anti-inflammatory and regulatory cytokines that can downregulate antitumour T-cell responses.

Therefore, the development of suitable adjuvants to provide the necessary danger signals to stimulate immunity, and delivery systems to protect and target peptide, protein and DNA cancer vaccines, is of critical importance. Adjuvants are a diverse group of compounds that can have a variety of effects. They can enhance the magnitude of an immune response, stimulate a particular type of immune response (e.g., a cytotoxic immune response [T helper cell type 1 (T_H1)] as compared with a humoral or antibody-mediated immune response [T_H2]) [1,8], protect antigens from chemical or physical degradation [9], or they can provide targeted and/or controlled release of the vaccine antigen to APC [10,11]. All of these features are important when designing or choosing an optimal vaccine adjuvant. Traditionally, the adjuvants used in human vaccines have been based on aluminium salts (often termed alum). Although the exact mechanism of action of these has never been fully elucidated, it is thought to be partly due to the creation of a retained repository of antigen (or depot) that persists over time with a sustained and gradual release of the vaccine antigen with subsequent prolonged stimulation of immunity [12]. However, this concept has been challenged [13]. Furthermore, incorporation into such a depot may increase the colloidal nature of the antigen, leading to a more effective uptake by APCs, such as DCs [12,14,15]. However, there are potential drawbacks that are associated with the use of aluminium salts for improving immunological responses to vaccines. Perhaps the most limiting factor in the use of aluminium salts is the bias this adjuvant shows towards the generation of T_H2-type or antibody-mediated immunity, at least in animal models [12,16,17]. Whilst this arm of the immune system is advantageous in treating extracellular infections, the successful eradication of cancer requires a cell-mediated (or $CD8^+$ and T_H1-type) immune response. Consequently, aluminium-based adjuvants are not satisfactory for the generation of strong anticancer immune responses, although co-administration with cytokines such as IL-12 and IL-18 has demonstrated the potential for switching aluminium salt responses to T_H1 [18,19]. Furthermore, aluminium salt-based adjuvants are limited to parenteral use, being ineffective as adjuvants for the mucosal delivery of antigens [20]. In recent years, concerns have also been raised over the safety of using aluminium-based adjuvants, especially with regard to the potential for triggering allergic responses and syndromes such as macrophagic myofasciitis [15,21-23].

As a consequence of concerns over the efficacy and toxicity of aluminium-based adjuvants, a great amount of research has gone into the development of alternative, novel vaccine strategies in which the adjuvant is often directly incorporated into the delivery system. These new adjuvants can be grouped into several broad categories including immune-stimulating molecules, depot-forming reagents or formulations, emulsions and colloidal delivery systems. This review focuses on colloidal delivery systems, such as microparticles, nanoparticles, liposomes, archaeal lipid liposomes (archaeosomes), immune-stimulating complexes (ISCOMs) and virus-like particles (VLPs) (Table 1).

2. Colloidal delivery systems

2.1 Micro- and nanoparticles

Polymeric micro- and nanoparticles are comprised of biodegradable polymers and show promise for antigen-based vaccines due to their capacity for high antigen loading and enhanced antigen presentation efficiency. Microparticles are of limited use when given parenterally. For safety reasons, the particle size is limited to 5 µm as larger particles may lead to capillary blockage. Biocompatibility is also an essential feature for potential application as a new vaccination strategy. Synthetic polymers such as polycyanoacrylate [24], poly(D, L-lactide) as well as related polymers such as poly(lactide-co-glycolide) (PLGA) and poly(lactic acid) can be used to produce micro- and nanoparticles [25,26]. Natural polymers such as chitosan [27,28], gelatine [29] and sodium alginate [9] have also been investigated to overcome some of the toxicological problems that are associated with synthetic polymers. Particles can be prepared by different techniques, such as spray drying, freeze drying or solvent evaporation [30,31].

Small particles possess a very high surface:volume ratio and, depending on the particle charge and surface properties, particles can be designed to adsorb onto organs and tissues [32]. Antigen loading into particles can be achieved by two methods: by incorporating the antigen at the time of particle production or by adsorbing the antigen after the formation of particles by incubation in the drug solution [33]. Encapsulating the antigen in particles provides protection for agents that are susceptible to degradation and offers the possibility of prolonged release. Polymer-coated particles are being investigated for the delivery of vaccines to the intestine as these particles are able to protect them from adverse conditions that could affect their biological activity, such as the acidic environment of the stomach and the presence of enzymes [9]. It has been shown that increased MHC I antigen presentation can be achieved by priming APC with antigen-encapsulating, acid-degradable nanoparticles, which degrade in the acidic environment of the endosome following phagocytosis, thus releasing the antigen into the cytoplasm [34,35].

Another advantage of using polymeric particles as delivery carriers is that they can be targeted to APC by adjusting their size as it is known that APCs phagocytose particles that are 0.5 – 3 µm in diameter [36,37]. Microparticles are avidly taken up by APC, which subsequently mature and present the

Table 1. Summary of colloidal delivery used for cancer vaccines.

Delivery system	Mechanism of action and example of clinical trials	Advantages	Limitations
Micro- and nanoparticles	APC targeting, prolonged release, absorbance onto organs, tissues, mucosal surfaces, possibility of continued stimulation Phase I/IB study in 16 cancer patients [51]	Biodegradable polymers available High antigen loading Enhanced antigen protection Enhanced antigen presentation efficiency	Size limits use for parental application Toxicity issues due to use of organic solvents during production process
Liposomes	Modified liposomes can target APC and APC activation Phase I/II trial in 171 patients [68]	Versatile Non-toxic Biodegradable Biocompatible Enhanced antigen protection	Physical instabilities Low entrapment rates Scaling-up problems
Archaeosomes	APC targeting and activation	High physicochemical stability Highly immune stimulatory	Experimental Low entrapment rates Biological production
Immune-stimulating complexes	APC activation Placebo-controlled clinical trial in 46 patients [93]	Highly immune stimulatory	Toxicity Antigen must contain lipophilic domain
Virus-like particles	APC targeting and activation Phase II study in 277 women [91]	Highly immune stimulatory	Biological production

APC: Antigen presenting cell.

antigen following migration to draining lymph nodes [38]. Moreover, because formulations can be designed to have protracted release profiles, prolonged exposure of the cells of the immune system to the antigen is another possible mechanism by which micro- and nanocolloidal systems may enhance immune responses [39]. Depending on the particle charge, surface properties and relative hydrophobicity, micro- and nanoparticles can be designed to adsorb preferentially to organs, tissues or mucosal surfaces [32]. This offers the possibility for a potentially wide range of applications for cancer vaccination [40].

PLGA particles have been studied most extensively for the controlled delivery of peptide drugs due to their proven safety record and established use in marketed products [26,38]. In recent years, PLGA particles have been used as carriers for several vaccines that contain either protein antigens [41] or plasmid DNA [42].

Recent studies have investigated the ability of injectable biodegradable polymeric PLGA particles to control the release of vaccine antigens, to reduce the number of doses in the immunisation schedule and to optimise the desired immune response via selective targeting of the antigen to APCs. A formulation was developed that was able to deliver proteins for extended periods of time from PLGAs with minimal protein instability. Persistent levels of neutralising antibodies and immunological memory were exhibited in response to vaccination [25].

Luby *et al*. examined the ability of microparticle-encapsulated DNA to stimulate an antitumour immune response in mice and humans [43]. The duration of the immune response, the effect on the immune response of multiple injections and the safety of repeated injections were studied. The results demonstrated that the PLGA-encapsulated DNA elicited durable immune responses, that the responses are dependent on repeated immunisation and that the formulation was well tolerated.

The immune-stimulating activity of two new chitosan-based formulations (one a colloidal formulation and one an emulsion) was compared in the study by Seferian and Martinez [44]. Chitosan is a naturally occurring polysaccharide material that is biodegradable and non-toxic [45]. Chitosan suspensions or micro- and nanoparticles have been reported to exhibit immune-stimulating activity such as increasing accumulation and activation of macrophages and polymorphonuclear cells, suppressing tumour growth and enhancing cytotoxic T-lymphocyte responses and delayed type hypersensitivity [46]. A zinc–chitosan particle adjuvant formulation and an emulsion formulation containing chitosan were effective in sensitising mice and guinea-pigs for the production of antigen-specific delayed type hypersensitivity responses, indicating that these adjuvants stimulate both B and T lymphocytes [44].

Non-degradable particles of gold, latex, silica or polystyrene have also been used as efficient antigen carriers and some have demonstrated adjuvant effects [47,48]. An antigen that is coupled to these particles induces long-term reactivity, possibly because the particles persist in the tissues, providing a reservoir of antigen for continued stimulation. Colloidal gold is known to change APC function although the mechanism has not yet been determined [49].

In addition, micro- and nanoparticles can be used in vaccine production to transfect or deliver the antigen and adjuvants to

whole-cell vaccines (e.g., tumour cell vaccines) [50]. A Phase I/IB study in 16 cancer patients was carried out to determine the safety of GM-CSF transfected autologous tumour vaccine that was administered by intradermal injection. The vaccine consisted of irradiated autologous tumour cells transfected with GM-CSF DNA-coated gold particles by gene gun. The transfected cells were then injected back into the patients who were monitored for evidence of antitumour immune response. The results suggested that clinically relevant levels of gene expression were only apparent in a minority of the patients [51].

2.2 Liposomes

Another approach to enhance the immunogenicity of vaccine antigens is the use of lipidic vesicles (liposomes) [1]. Liposomes are non-toxic, biodegradable and biocompatible phospholipid vesicles. The bilayers are separated from one another by aqueous domains and enclose an aqueous core. Most commonly used lipids are egg-phoshatidylcholine and egg-phoshatidylglycerol. The alternating hydrophilic and hydrophobic structure enables liposomes to entrap compounds from a wide spectrum of solubility. The versatility of liposomes also allows for the loading of adjuvants as well as vaccine antigens into the particles [52,53]. Liposomes have been used as vaccine delivery systems against bacterial [54] and viral infections [55,56] as well as tumours [57]. Liposomes are commonly produced using a film hydration method or alternatively using reverse-phase evaporation of an organic/aqueous phospholipid emulsion [58]. Active ingredients can be added to the lipid film or to the hydrating agent, depending on their chemical properties. Liposomes are regarded as non-toxic when they are prepared using phospholipids that are identical to those that occur in mammalian cell membranes. These kind of vesicles are used as carriers of antigens as the composition is inherently non-immunogenic but can be modified by the addition of other compounds to act as adjuvants for the immune system response [59].

Despite the large volume of research that has been undertaken on liposomes, only a small number of liposomal products have been approved for human use. This could be due to many reasons, including physical instabilities, low entrapment rates of molecules and compounds into liposomes and the high cost of liposome production especially on a large commercial scale [60].

After peripheral subcutaneous application, liposomes have been shown to distribute preferentially via the lymph nodes and reach local organised lymphoid tissues [61]. Proteins that are encapsulated in liposomes can reach DCs *in vivo* and induce primary cytotoxic T-lymphocyte responses [62]. Another advantage of liposomes is that they can be easily modified to allow the incorporation of molecules to specifically target cells of the immune system (e.g., APC) by the inclusion of specific sugar moieties [1,50,63,64] or antibodies [65]. Liposomes have been used to provide a cytokine supplement in tumour cell vaccines by providing a cytokine reservoir at the antigen presentation site [66]. Liposomal delivery of cytokines has the advantage that they may provide a prolonged cytokine presence at the vaccination site by virtue of their established potential for the slow release of the entrapped agents [67].

Van Slooten *et al.* examined the adjuvanticity of murine INF-γ (mINF-γ) liposomes compared with that achieved by *INF-γ* gene transfection of B16 tumour cells in an established tumour cell vaccination protocol in the murine B16 melanoma model [66]. Radiolabelling studies indicated that free mIFN-γ rapidly cleared from the subcutaneous injection site. Association of [^{125}I]-mIFN-γ with liposomes substantially increased the local residence time (a fourfold increase of the AUC). Moderate but significant $CD8^+$ T-cell activity against tumour cells was found for mice that were immunised with irradiated cells, supplemented with mIFN-γ liposomes, compared with untreated control animals.

In the study of Neelapu *et al.*, 10 patients with advanced-stage lymphoma were treated with a liposomal vaccine formulation that contained a tumour immunoglobulin protein (Id) following chemotherapy-induced clinical remission [57]. Autologous tumour response and specific T_H1 cytokine response were induced by vaccination in ten and nine patients, respectively. Antitumour immune responses were mediated by both $CD4^+$ and $CD8^+$ T cells, were human lymphocyte antigen class I and II associated and persisted for a period of 18 months following vaccination. Specific anti-Id antibody responses were detected in 4 patients. After a median follow up of 50 months, 6 of the 10 patients remained in continuous first complete remission.

In a clinical Phase IIB trial, the effect of a liposomal vaccine containing L-BLP25 plus best supportive care was compared with best supportive care alone in patients with stage IIIB and IV non-small cell lung cancer [68]. L-BLP25 is a lyophilised preparation that consists of BLP25 lipopeptide, immunoadjuvant monophosphoryl lipid A and three lipids (cholesterol, dimyristerol phoshatidylglcycerol and dipalmitoylphoshatidylcholin), forming a liposomal product. Results from the study suggested a potential survival advantage for patients who were randomly assigned to the L-BLP25 treated group. However, only 16 of 78 assessable patients in the L-BLP25 arm developed an immune response, as measured by a T-cell proliferation result [68].

2.3 Archaeosomes

The plasmid membrane of an archaeobacteria contains a unique polar lipid structure, which is characterised by fully saturated phytanyl chains attached via ether bonds to the glycerol backbone carbons [69]. Ether glycerolipids can be extracted from various archaeobacteria and formulated into archaeosomes. Briefly, archeal strains are grown and the lipids are extracted from the frozen and thawed biomass using methanol, chloroform and water. The total polar lipid fraction is collected by cold acetone precipitation [70]. Lipid vesicles are produced by hydrating the polar lipids in aqueous solution of the antigen [71,72]. The advantages of this carrier system include the ability to provide

an antigenic depot (as with microspheres and conventional liposomes) and, more importantly, for vaccine delivery and immune-stimulating capacities [71]. Compared with liposomes, archaeosomes demonstrate relatively higher stabilities to oxidative stress, high temperature, alkaline pH, action of phospholipases, bile salts and serum proteins. [72,73]. In initial studies, archaeosomes were shown to augment the potent antibody and T_H1/T_H2 responses to the entrapped antigen [74].

Krishnan *et al.* compared the differential ability of various archaea to evoke a $CD8^+$ T-cell response and protection to tumour challenge with the model antigen, ovalbumin [71]. Immunisation of mice with ovalbumin that was entrapped in the archaeal lipid vesicles evoked a strong $CD8^+$ cytotoxic T-lymphocyte response to the entrapped antigen. Tumour protection was also noted when ovalbumin–archaeosomes were injected concurrently with the tumour challenge.

2.4 Immune-stimulating complexes

ISCOMs were first described by Morein *et al.* in 1984 [75]. They are cage-like spherical complexes of ~ 40 nm (the size range of viruses), consisting of saponin adjuvant (Quil A), cholesterol and phospholipids. These lipophilic particles are rapidly incorporated into the membranes of cells and may promote endocytosis of the antigen by DCs, monocytes and macrophages [76]. Quil A is a toxic mixture of crystalline triterpenoid sapogenins obtained by hydrolysis of saponin from the Chilean tree *Quillaja saponaria* Molina. So far, it has been limited to use in veterinary vaccines, mainly due to concerns over its haemolytical activity and reactions at the site of injection. The toxicity is decreased following the incorporation of Quil A into lipidic particles [77]. Quil A imparts a strong negative charge to the particles and also serves as a built-in adjuvant, through an aldehyde group on the triterpene aglycone of saponin, forming a Schiff base with the amino groups on co-stimulatory T-cell receptors [63]. In contrast to liposomes that have an enclosed phospholipid bilayer (Figure 1A), ISCOMs have an open lattice structure due to the presence of the hydrophilic saponins. The addition of Quil A disrupts the liposome bilayer and regular spherical cage-like structures of ~ 40 nm in diameter are formed (Figure 1B) [63]. This open cage-like structure has major implications for the types of antigens that can be loaded onto this delivery system. The absence of an enclosed aqueous space means that antigens must be incorporated into the lattice structure. Therefore, the chemical structure of the antigen must contain, or be modified to contain, a lipophilic domain. ISCOMs can be prepared by a number of methods including dialysis [78], hydration [63,79] and ethanol injection [80].

ISCOMs are reported to associate with intracellular lipid membranes and localise with the cytosolic and vesicular compartments in APCs [81]. They are more rapidly distributed from the site of injection to draining lymph nodes and the spleen than soluble protein, as well as persisting in these tissues for longer [76]. ISCOMs are versatile adjuvants and have been administered by oral, intranasal and parenteral routes to induce mucosal immunity in mice [82].

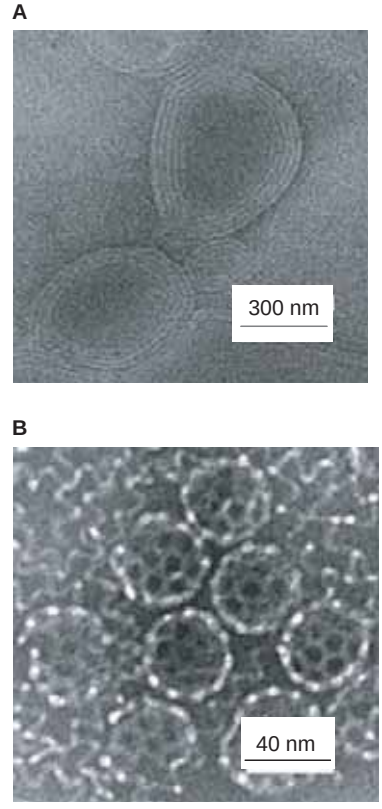

Figure 1. A) Liposomes with an enclosed phospholipid bilayer; B) immune-stimulating complexes with an open lattice structure.

Lenarczyk used murine models and a model cancer antigen to show that ISCOM vaccines induced potent $CD8^+$ T-cell responses, mediated protection in three different tumour models, promoted T_H1-biased immunity and induced $CD8^+$ T-cell responses in the absence of aid from $CD4^+$ T cells. These activities were found to be substantially improved when the vaccine antigen was associated with the ISCOM structure. Furthermore, the presence of pre-existing antibodies *in vivo* against the vaccine antigen did not inhibit $CD8^+$ T-cell induction by the ISCOM vaccine [83].

Phase I clinical trials have been carried out in which NY-ESO-1 protein, a cancer-testis antigen that is expressed in many tumours, was formulated with the ISCOM adjuvant and administered to patients. Higher antibody titres were observed in patients who were immunised with the protein in ISCOMs compared with the protein alone. $CD8^+$ T-cell responses were also observed. Some injection-site reactions were present in patients who were immunised with higher doses of the ISCOM-protein formulation [84].

2.5 Virus-like particles

A variety of viral proteins spontaneously assemble into structures that closely resemble virions. VLPs are an assembly of structural proteins that organise into substructures, which further organise into the final particle [85]. Due to the repetitive structure of VLPs, these are highly immunogenic and have been shown to induce strong and long-lasting B- [86] and T-cell responses [87] in the absence of adjuvants. Contact between human DCs or monocytes and VLPs induces DC maturation and rapid secretion of inflammatory cytokines [88].

VLPs are not infectious and have similar properties to naive virions, enabling them to be used as both particulate carriers and an adjuvant. These can be formed by the transfection of mammalian [89] or insect cells [90], with plasmids encoding the viral structural proteins.

A VLP vaccine was used in a double-blind, placebo-controlled Phase II trial in order to assess the efficacy of a prophylactic quadrivalent VLP vaccine that targeted the human papillomavirus types associated with 70% of cervical cancers [91]. A total of 227 young women were randomly assigned to the vaccine group whilst 275 received placebo formulations. The primary end point was the combined incidence of infection with the human papillomavirus, or cervical or external genital disease. The vaccine was highly immunogenic with all of the women who received the active vaccine developing a high detectable antibody response to the virus by month 7 [91].

3. Expert opinion

At present, there are a wide variety of immunostimulatory colloidal delivery systems available for use to deliver cancer vaccines. However, there are a number of problems that are associated with the use of such novel vaccine delivery systems for cancer therapy that must be addressed. First, as reported in this review, only a few clinical trials that use immunostimulatory colloidal delivery systems have been undertaken. In order to increase the use of colloidal delivery systems in cancer vaccine trials, collaborative research between oncologists, immunologists and pharmaceutical scientists is critical. The immunologist will, in general, use whatever adjuvants and formulations are readily available when designing a new cancer vaccine. If they do not have access to colloidal delivery systems or to researchers who can manufacture, characterise and tailor make these to the specifications required by the immunologist, then they will not use them. This reflects the current situation.

The second problem with the use of novel immunostimulatory colloidal delivery systems for cancer vaccines is one that is common to all new cancer vaccines; demonstrating vaccine efficacy. Whilst there are numerous tools that allow the demonstration of vaccine efficacy in animal models, it has proven difficult to measure vaccine efficacy in cancer clinical trials. In addition, although it is often easier to gain approval for clinical trials of vaccines in cancer patients than for other patient groups, the patient population will be one that has failed conventional therapies and has a poor prognosis [92]. Therefore, although useful data may be collected on vaccine safety and toxicity, vaccine efficacy is generally low. In a review of cancer vaccine trials carried out by Rosenberg in 2004 that examined 440 patients who were treated with a variety of cancer vaccines, the response rate was only 2.6% [92]. Furthermore, the criteria for measuring responses can be variable across trials and may include such diverse parameters as measurable decreases in tumour mass, increases in antitumour antibody or $CD8^+$ T cells, increased time to relapse or even symptom improvement. This, when combined with differences in patient and tumour characteristics, makes the comparison of the efficacy of different vaccine approaches very difficult.

The authors have reviewed a number of different types of collodial systems, each with different strengths and weaknesses (Table 1). Which type of system will be used in the clinic will ultimately depend on what characteristics are required; for example, the importance of sustained delivery, protection of the antigen or tolerability. The most important characteristic for a vaccine delivery system is immunogenicity and the ability to stimulate an antitumour immune response. The colloidal vaccines that show the most promise at present are those that contain built-in adjuvants. Other colloidal delivery systems will need to be modified in order to achieve maximal activation of the immune response. This is the area that will be the focus of much research in the next few years. The inclusion of adjuvants that interact with Toll-like receptors or other pattern recognition receptors on APCs into the particles is one potential way of achieving this aim.

Therefore, in summary, although there are a wide variety of immune-stimulating colloidal vaccine delivery systems in development at present, few of these have been used in clinical cancer trials. Those cancer vaccines that use colloidal delivery systems do not seem to be very effective, but data on responses are difficult to obtain and are often variable. The challenge is for pharmaceutical scientists, immunologists and oncologists to work together to design vaccine delivery systems that can overcome tolerance to self-antigens and tumour- and/or therapy-induced immune suppression as well as to stimulate effective therapeutic immune responses. The advantages of colloidal delivery systems with regard to protection of the antigen, controlled release and the delivery of multiple antigens and immune-activating signals to the cells of the immune system have been discussed. Once we have vaccines that produce measurable responses, then issues such as the timing of vaccination, route of delivery or if the vaccine should be used as an adjuvant to traditional cancer therapy can be addressed. The field of therapeutic cancer vaccines is still very much in its infancy but, due to the potential of colloidal vaccine delivery systems, such research would be well worth pursuing.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. COPLAND MJ, RADES T, DAVIES NM, BAIRD MA: Lipid based particulate formulations for the delivery of antigen. *Immunol. Cell Biol.* (2005) 83(2):97-105.
2. PRUD'HOMME GJ: DNA vaccination against tumors. *J. Gene Med.* (2005) 7(1):3-17.
3. TUDOR D, DUBUQUOY C, GABORIAU V *et al.*: TLR9 pathway is involved in adjuvant effects of plasmid DNA-based vaccines. *Vaccine* (2005) 23(10):1258-1264.
4. TOES R, BLOM R, OFFRINGA R, KAST W, MELIEF C: Enhanced tumor outgrowth after peptide vaccination. Functional deletion of tumor-specific CTL induced by peptide vaccination can lead to the inability to reject tumors. *J. Immunol.* (1996) 156(10):3911-3918.
5. HOUGHTON AN, GUEVARA-PATINO JA: Immune recognition of self in immunity against cancer. *J. Clin. Invest.* (2004) 114(4):468-471.
6. SATO T, MCCUE P, MASUOKA K *et al.*: Interleukin 10 production by human melanoma. *Clin. Cancer Res.* (1996) 2(8):1383-1390.
7. VAN BELLE P, RODECK U, NUAMAH I, HALPERN AC, ELDER DE: Melanoma-associated expression of transforming growth factor-beta isoforms. *Am. J. Pathol.* (1996) 148(6):1887-1894.
8. MESA C, FERNANDEZ LE: Challenges facing adjuvants for cancer immunotherapy. *Immunol. Cell Biol.* (2004) 82(6):644-650.
9. BORGES O, BORCHARD G, VERHOEF JC, DE SOUSA A, JUNGINGER HE: Preparation of coated nanoparticles for a new mucosal vaccine delivery system. *Int. J. Pharm.* (2005) 299(1-2):155-166.
10. PASHINE A, VALIANTE NM, ULMER JB: Targeting the innate immune response with improved vaccine adjuvants. *Nat. Med.* (2005) 11(4 Suppl.):S63-S68.

•• An excellent overview of adjuvants and delivery systems; describes approaches for the discovery and development of immunostimulatory compounds and vaccine formulations that target innate immune responses.

11. GAMVRELLIS A, LEONG D, HANLEY JC *et al.*: Vaccines that facilitate antigen entry into dendritic cells. *Immunol. Cell Biol.* (2004) 82(5):506-516.

• This paper outlines specific immune activation by describing different approaches for providing more specific immune regulation.

12. LINDBLAD EB: Aluminium compounds for use in vaccines. *Immunol. Cell Biol.* (2004) 82(5):497-505.
13. FLAREND RE, HEM SL, WHITE JL *et al.*: *In vivo* absorption of aluminium-containing vaccine adjuvants using 26Al. *Vaccine* (1997) 15(12-13):1314-1318.
14. LINDBLAD EB: Aluminium adjuvants – in retrospect and prospect. *Vaccine* (2004) 22(27-28):3658-3668.
15. GUPTA RK, ROST BE, RELYVELD E, SIBER GR: Adjuvant properties of aluminium and calcium compounds. In: *Vaccine Design: the Subunit and Adjuvant Approach*. MF Powell, MJ Newman (Eds), Plenum Press, New York, NY, USA (1995):229-248.
16. COTTREZ F, HURST SD, COFFMAN RL, GROUX H: T regulatory cells 1 inhibit a Th2-specific response *in vivo*. *J. Immunol.* (2000) 165(9):4848-4853.
17. BREWER JM, CONACHER M, SATOSKAR A, BLUETHMANN H, ALEXANDER J: In interleukin-4-deficient mice, alum not only generates T helper 1 responses equivalent to freund's complete adjuvant, but continues to induce T helper 2 cytokine production. *Eur. J. Immunol.* (1996) 26(9):2062-2066.
18. JANKOVIC D, CASPAR P, ZWEIG M *et al.*: Adsorption to aluminum hydroxide promotes the activity of IL-12 as an adjuvant for antibody as well as type 1 cytokine responses to HIV-1 gp120. *J. Immunol.* (1997) 159(5):2409-2417.
19. POLLOCK KG, CONACHER M, WEI XQ, ALEXANDER J, BREWER JM: Interleukin-18 plays a role in both the alum-induced T helper 2 response and the T helper 1 response induced by alum-adsorbed interleukin-12. *Immunology* (2003) 108(2):137-143.
20. PETROVSKY N, AGUILAR JC: Vaccine adjuvants: current state and future trends. *Immunol. Cell Biol.* (2004) 82(5):488-496.
21. GHERARDI RK, COQUET M, CHERIN P *et al.*: Macrophagic myofasciitis lesions assess long-term persistence of vaccine-derived aluminium hydroxide in muscle. *Brain* (2001) 124(Pt 9):1821-1831.
22. GHERARDI RK, AUTHIER FJ: Aluminum inclusion macrophagic myofasciitis: a recently identified condition. *Immunol. Allergy Clin. North Am.* (2003) 23(4):699-712.
23. GOTO N, KATO H, MAEYAMA J, ETO K, YOSHIHARA S: Studies on the toxicities of aluminium hydroxide and calcium phosphate as immunological adjuvants for vaccines. *Vaccine* (1993) 11(9):914-918.
24. KRAUEL K, DAVIES NM, HOOK S, RADES T: Using different structure types of microemulsions for the preparation of poly(alkylcyanoacrylate) nanoparticles by interfacial polymerization. *J. Control. Release* (2005) 106(1-2):76-87.
25. JIANG W, GUPTA RK, DESHPANDE MC, SCHWENDEMAN SP: Biodegradable poly(lactic-co-glycolic acid) microparticles for injectable delivery of vaccine antigens. *Adv. Drug Deliv. Rev.* (2005) 57(3):391-410.
26. ZHOU S, LIAO X, LI X, DENG X, LI H: Polylactide-co-poly(ethylene glycol) microspheres as potential vaccine delivery systems. *J. Control. Release* (2003) 86(2-3):195-205.
27. ILLUM L, JABBAL-GILL I, HINCHCLIFFE M, FISHER AN, DAVIS SS: Chitosan as a novel nasal delivery system for vaccines. *Adv. Drug Deliv. Rev.* (2001) 51(1-3):81-96.
28. AGNIHOTRI SA, MALLIKARJUNA NN, AMINABHAVI TM: Recent advances on chitosan-based micro- and nanoparticles in drug delivery. *J. Control. Release* (2004) 100(1):5-28.
29. SAXENA A, SACHIN K, BOHIDAR HB, VERMA AK: Effect of molecular weight heterogeneity on drug encapsulation efficiency of gelatin nano-particles. *Colloids Surf. B Biointerfaces* (2005) 45(1):42-48.
30. SCHOLL I, BOLTZ-NITULESCU G, JENSEN-JAROLIM E: Review of novel particulate antigen delivery systems with special focus on treatment of type I allergy. *J. Control. Release* (2005) 104(1):1-27.
31. CLELAND JL: Solvent evaporation processes for the production of controlled release biodegradable microsphere formulations for therapeutics and vaccines. *Biotechnol. Prog.* (1998) 14(1):102-107.

32. TROSTER SD, WALLIS KH, MULLER RH, KREUTER J: Correlation of the surface hydrophobicity of ^{14}C-poly(methyl methacrylate) nanoparticles to their body distribution. *J. Control. Release* (1992) 20(3):247-260.

33. CHESKO J, KAZZAZ J, UGOZZOLI M, O'HAGAN DT, SINGH M: An investigation of the factors controlling the adsorption of protein antigens to anionic PLG microparticles. *J. Pharm. Sci.* (2005) 94(11):2510-2519.

34. KWON YJ, STANDLEY SM, GOH SL, FRECHET JM: Enhanced antigen presentation and immunostimulation of dendritic cells using acid-degradable cationic nanoparticles. *J. Control. Release* (2005) 105(3):199-212.

35. MURTHY N, XU M, SCHUCK S *et al.*: A macromolecular delivery vehicle for protein-based vaccines: acid-degradable protein-loaded microgels. *Proc. Natl. Acad. Sci. USA* (2003) 100(9):4995-5000.

36. KAWAGUCHI H, KOIWAI N, OHTSUKA Y, MIYAMOTO M, SASAKAWA S: Phagocytosis of latex particles by leucocytes. I. Dependence of phagocytosis on the size and surface potential of particles. *Biomaterials* (1986) 7(1):61-66.

37. FOGED C, BRODIN B, FROKJAER S, SUNDBLAD A: Particle size and surface charge affect particle uptake by human dendritic cells in an *in vitro* model. *Int. J. Pharm.* (2005) 298(2):315-322.

38. LUTSIAK MEC, ROBINSON DR, COESTER C, KWON GS, SAMUEL J: Analysis of poly(D, L-lactic-co-glycolic acid) nanosphere uptake by human dendritic cells and macrophages *in vitro*. *Pharm. Res.* (2002) 19(10):1480-1487.

39. O'HAGAN DT, MACKICHAN ML, SINGH M: Recent developments in adjuvants for vaccines against infectious diseases. *Biomol. Eng.* (2001) 18(3):69-85.

40. BRANNON-PEPPAS L, BLANCHETTE JO: Nanoparticle and targeted systems for cancer therapy. *Adv. Drug Deliv. Rev.* (2004) 56(11):1649-1659.

41. SINGH M, KAZZAZ J, CHESKO J *et al.*: Anionic microparticles are a potent delivery system for recombinant antigens from *Neisseria meningitidis* serotype B. *J. Pharm. Sci.* (2004) 93(2):273-282.

42. JONES DH, CORRIS S, MCDONALD S, CLEGG JCS, FARRAR GH: Poly(D, L-lactide-co-glycolide)-encapsulated plasmid DNA elicits systemic and mucosal antibody responses to encoded protein after oral administration. *Vaccine* (1997) 15(8):814-817.

43. LUBY TM, COLE G, BAKER L *et al.*: Repeated immunization with plasmid DNA formulated in poly(lactide-co-glycolide) microparticles is well tolerated and stimulates durable T cell responses to the tumor-associated antigen cytochrome P450 1B1. *Clin. Immunol.* (2004) 112(1):45-53.

44. SEFERIAN PG, MARTINEZ ML: Immune stimulating activity of two new chitosan containing adjuvant formulations. *Vaccine* (2000) 19(6):661-668.

45. BANERJEE T, MITRA S, KUMAR SINGH A, KUMAR SHARMA R, MAITRA A: Preparation, characterization and biodistribution of ultrafine chitosan nanoparticles. *Int. J. Pharm.* (2002) 243(1-2):93-105.

46. NAGAMOTO T, HATTORI Y, TAKAYAMA K, MAITANI Y: Novel chitosan particles and chitosan-coated emulsions inducing immune response via intranasal vaccine delivery. *Pharm. Res.* (2004) 21(4):671-674.

47. GRANUM B, GAARDER PI, GROENG E-C *et al.*: Fine particles of widely different composition have an adjuvant effect on the production of allergen-specific antibodies. *Toxicol. Lett.* (2001) 118(3):171-181.

48. SELLBORN A, ANDERSSON M, HEDLUND J *et al.*: Immune complement activation on polystyrene and silicon dioxide surfaces: Impact of reversible IgG adsorption. *Mol. Immunol.* (2005) 42(5):569-574.

49. ZHANG L, WIDERA G, RABUSSAY D: Enhancement of the effectiveness of electroporation-augmented cutaneous DNA vaccination by a particulate adjuvant. *Bioelectrochemistry* (2004) 63(1-2):369-373.

50. HOCKERTZ S: Present and future of cancer vaccines. *Toxicology* (2005) 214(1-2):151-161.

51. MAHVI DM, SHI FS, YANG NS *et al.*: Immunization by particle-mediated transfer of the granulocyte-macrophage colony-stimulating factor gene into autologous tumor cells in melanoma or sarcoma patients: report of a Phase I/IB study. *Hum. Gene Ther.* (2002) 13(14):1711-1721.
- Provides information on essential features such as APC targeting, prolonged release, absorbance onto organs, tissues and mucosal surfaces, possibility of continued stimulation for particulate systems and describes a Phase I/IB study in 16 cancer patients.

52. DRUMMOND DC, MEYER O, HONG K, KIRPOTIN DB, PAPAHADJOPOULOS D: Optimizing liposomes for delivery of chemotherapeutic agents to solid tumors. *Pharmacol. Rev.* (1999) 51(4):691-744.

53. FELNEROVA D, VIRET J-F, GLUCK R, MOSER C: Liposomes and virosomes as delivery systems for antigens, nucleic acids and drugs. *Curr. Opin. Biotechnol.* (2004) 15(6):518-529.

54. IRIE T, WATARAI S, IWASAKI T, KODAMA H: Protection against experimental *Aeromonas salmonicida* infection in carp by oral immunisation with bacterial antigen entrapped liposomes. *Fish Shellfish Immunol.* (2005) 18(3):235-242.

55. AMBROSCH F, WIEDERMANN G, JONAS S *et al.*: Immunogenicity and protectivity of a new liposomal hepatitis A vaccine. *Vaccine* (1997) 15(11):1209-1213.

56. OKADA E, SASAKI S, ISHII N *et al.*: Intranasal immunization of a DNA vaccine with IL-12- and granulocyte- macrophage colony-stimulating factor (GM-CSF)-expressing plasmids in liposomes induces strong mucosal and cell-mediated immune responses against HIV-1 antigens. *J. Immunol.* (1997) 159(7):3638-3647.

57. NEELAPU SS, BASKAR S, GAUSE BL *et al.*: Human autologous tumor-specific t-cell responses induced by liposomal delivery of a lymphoma antigen. *Clin. Cancer Res.* (2004) 10(24):8309-8317.

58. LIANG MT, DAVIES NM, TOTH I: Encapsulation of lipopeptides within liposomes: effect of number of lipid chains, chain length and method of liposome preparation. *Int. J. Pharm.* (2005) 301(1-2):247-254.

59. GUPTA PN, MISHRA V, RAWAT A *et al.*: Non-invasive vaccine delivery in transfersomes, niosomes and liposomes: a comparative study. *Int. J. Pharm.* (2005) 293(1-2):73-82.

60. KAYSER O, OLBRICH C, CROFT SL, KIDERLEN AF: Formulation and biopharmaceutical issues in the development of drug delivery systems for

antiparasitic drugs. *Parasitol. Res.* (2003) 90(0):S63-S70.

61. OUSSOREN C, STORM G: Lymphatic uptake and biodistribution of liposomes after subcutaneous injection. III. Influence of surface modification with poly(ethylene glycol). *Pharm. Res.* (1997) 14(10):1479-1484.
62. NAIR S, ZHOU F, REDDY R, HUANG L, ROUSE B: Soluble proteins delivered to dendritic cells via pH-sensitive liposomes induce primary cytotoxic T lymphocyte responses *in vitro*. *J. Exp. Med.* (1992) 175(2):609-612.
63. DEMANA PH, DAVIES NM, BERGER B, RADES T: Incorporation of ovalbumin into ISCOMs and related colloidal particles prepared by the lipid film hydration method. *Int. J. Pharm.* (2004) 278(2):263-274.
64. WHITE K, RADES T, FURNEAUX R, TYLER P, HOOK S: Mannosylated liposomes as antigen delivery vehicles for targeting to dendritic cells. *J. Pharmacy Pharmacol.* (2005). In press.
65. FUKASAWA M, SHIMIZU Y, SHIKATA K *et al.*: Liposome oligomannose-coated with neoglycolipid, a new candidate for a safe adjuvant for induction of $CD8^+$ cytotoxic T lymphocytes. *FEBS Lett.* (1998) 441(3):353-356.
66. VAN SLOOTEN ML, STORM G, ZOEPHEL A *et al.*: Liposomes containing interferon-gamma as adjuvant in tumor cell vaccines. *Pharm. Res.* (2000) 17(1):42-48.
67. VAN DER VEEN AH, EGGERMONT AM, SEYNHAEVE AL, VAN TIEL, TEN HAGEN TL: Biodistribution and tumor localization of stealth liposomal tumor necrosis factor-alpha; in soft tissue sarcoma bearing rats. *Int. J. Cancer* (1998) 77(6):901-906.
68. BUTTS C, MURRAY N, MAKSYMIUK A *et al.*: Randomized Phase IIB trial of BLP25 liposome vaccine in stage IIIB and IV non-small-cell lung cancer. *J. Clin. Oncol.* (2005) 23(27):6674-6681.
- Provides the mechanism of action and an example of clinical trial for modified liposomes (Phase I/II trial in 171 patients).
69. KOGA Y, MORII H: Special methods for the analysis of ether lipid structure and metabolism in archaea. *Anal. Biochem.* (2006) 348(1):1-14.
70. LELKES PI, GOLDENBERG D, GLIOZZI A *et al.*: Vesicles from mixtures of bipolar archaebacterial lipids with egg phosphatidylcholine. *Biochim. Biophys. Acta- Biomembranes* (1983) 732(3):714-718.
71. KRISHNAN L, DICAIRE CJ, PATEL GB, SPROTT GD: Archaeosome vaccine adjuvants induce strong humoral, cell-mediated, and memory responses: comparison to conventional liposomes and alum. *Infect. Immun.* (2000) 68(1):54-63.
72. SPROTT GD, PATEL GB, KRISHNAN L: Archaeobacterial ether lipid liposomes as vaccine adjuvants. *Methods Enzymol.* (2003) 373:155-172
73. CHOQUET CG, PATEL GB, BEVERIDGE TJ, SPROTT GD: Stability of pressure-extruded liposomes made from archaeobacterial ether lipids. *Appl. Microbiol. Biotechnol.* (1994) 42(2-3):375-384.
74. SPROTT GD, TOLSON DL, PATEL GB: Archaeosomes as novel antigen delivery systems. *FEMS Microbiol. Lett.* (1997) 154(1):17-22.
75. MOREIN B, SUNDQUIST B, HOGLUND S, DALSGAARD K, OSTERHAUS A: ISCOM, a novel structure for antigenic presentation of membrane proteins from enveloped viruses. *Nature* (1984) 308(5958):457-460.
76. VILLACRES MC, BEHBOUDI S, NIKKILA T, LOVGREN-BENGTSSON K, MOREIN B: Internalization of iscom-borne antigens and presentation under MHC class I or class II restriction. *Cell. Immunol.* (1998) 185(1):30-38.
77. BARR IG, SJOLANDER A, COX JC: ISCOMs and other saponin based adjuvants. *Adv. Drug Deliv. Rev.* (1998) 32(3):247-271.
78. DEMANA PH, DAVIES NM, BERGER B, VOSGERAU U, RADES T: A comparison of pseudo-ternary diagrams of aqueous mixtures of Quil A, cholesterol and phospholipid prepared by lipid-film hydration and dialysis. *J. Pharm. Pharmacol.* (2004) 56(5):573-580.
79. DEMANA PH, DAVIES NM, HOOK S, RADES T: Quil A-lipid powder formulations releasing ISCOMs and related colloidal stuctures upon hydration. *J. Control. Release* (2005) 103(1):45-59.
80. LENDEMANS DG, MYSCHIK J, HOOK S, RADES T: Immuno-stimulating complexes prepared by ethanol injection. *J. Pharm. Pharmacol.* (2005) 57(6):729-733.
81. BUNGENER L, HUCKRIEDE A, WILSCHUT J, DAEMEN T: Delivery of protein antigens to the immune system by fusion-active virosomes: a comparison with liposomes and ISCOMs. *Biosci. Rep.* (2002) 22(2):323-338.
82. MOREIN B, HU K-F, ABUSUGRA I: Current status and potential application of ISCOMs in veterinary medicine. *Adv. Drug Deliv. Rev.* (2004) 56(10):1367-1382.
83. LENARCZYK A, LE TT, DRANE D *et al.*: ISCOM based vaccines for cancer immunotherapy. *Vaccine* (2004) 22(8):963-974.
84. CEBON JS, DAVIS ID, PARENTE PA: Phase I clinical trial of NY-ESO-1 protein formulated with immunostimulatory complexes (ISCOM adjuvant) in patients with minimal residual disease. *Cancer Immun.* (2003):19.
85. BLUTT SE, WARFIELD KL, O'NEAL CM, ESTES MK, CONNER ME: Host, viral, and vaccine factors that determine protective efficacy induced by rotavirus and virus-like particles (VLPs). *Vaccine* (2006) 24(8):1170-1179.
86. ROSENBERG SA, SHERRY RM, MORTON KE *et al.*: Tumor progression can occur despite the induction of very high levels of self/tumor antigen-specific $CD8^+$ T cells in patients with melanoma. *J. Immunol.* (2005) 175(9):6169-6176.
87. WARFIELD KL, OLINGER G, DEAL EM *et al.*: Induction of humoral and $CD8^+$ T cell responses are required for protection against lethal ebola virus infection. *J. Immunol.* (2005) 175(2):1184-1191.
88. LENZ P, THOMPSON CD, DAY PM *et al.*: Interaction of papillomavirus virus-like particles with human myeloid antigen-presenting cells. *Clin. Immunol.* (2003) 106(3):231-237.
89. KRAUSSLICH HG, OCHSENBAUER C, TRAENCKNER A-M *et al.*: Analysis of protein expression and virus-like particle formation in mammalian cell lines stably expressing HIV-1 gag and env gene products with or without active HIV proteinase. *Virology* (1993) 192(2):605-617.
90. ADAMSON CS, DAVIES A, SONEOKA Y *et al.*: A block in virus-like particle maturation following assembly of

murine leukaemia virus in insect cells. *Virology* (2003) 314(2):488-496.

91. VILLA LL, COSTA RL, PETTA CA *et al.*: Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre Phase II efficacy trial. *Lancet Oncol.* (2005) 6(5):271-278.
92. ROSENBERG SA, YANG JC, RESTIFO NP: Cancer immunotherapy: moving beyond current vaccines. *Nat. Med.* (2004) 10(9):909-915.
93. DAVIS ID, CHEN W, JACKSON H *et al.*: Recombinant NY-ESO-1 protein with ISCOMATRIX adjuvant induces broad integrated antibody and $CD4^+$ and $CD8^+$ T cell responses in humans. *Proc. Natl. Acad. Sci. USA* (2004) 101(29):10697-10702.
- Describes new delivery system (ISCOMS) as an adjuvant in a placebo-controlled clinical trial in 46 patients.

Affiliation

Anne Saupe[†1], Warren McBurney[2], Thomas Rades[3] & Sarah Hook[4]
[†]Author for correspondence
[1]Formulation scientist, School of Pharmacy, University of Otago, PO Box 913, Dunedin, New Zealand
Tel: +64 3 4797272; Fax: +64 3 4797034;
E-mail: anne.saupe@stonebow.otago.ac.nz
[2]Immunologist, School of Pharmacy, University of Otago, PO Box 913, Dunedin, New Zealand
[3]Formulation scientist, School of Pharmacy, University of Otago, PO Box 913, Dunedin, New Zealand
[4]Immunologist, School of Pharmacy, University of Otago, PO Box 913, Dunedin, New Zealand