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Enhanced immune response induced by BSA loaded in hydroxyethylstarch microparticles

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Abstract

Microparticles and nanoparticles represent promising carriers for the *in vivo* delivery of peptides, proteins or deoxyribonucleic acid (DNA). In this study, new hydroxyethylstarch (HES) microparticles were obtained by interfacial cross-linking with terephtaloyl chloride. These microparticles exhibit the characteristics required to improve antigen release and presentation to antigen presentating cells compared to free antigens. The adjuvant activity of HES microparticles as vaccine carrier was investigated in mice using bovine serum albumin (BSA) as model antigen. We showed HES microparticles were phagocyted by peritoneal mononuclear cells. The immunization with BSA-microparticles induced antibody synthesis that was predominantly immunoglobulin G1 (IgG1). Aluminium hydroxide remained more efficient to induce IgG synthesis. The analysis of the cytokine profile from spleen cells revealed that BSA-microparticles induced the secretion of both interferon-γ (IFN-γ) and interleukin-4 (IL-4). However, the immune responses induced by BSA-microparticles were qualitatively and quantitatively affected by the route of injection. Taken together, these results demonstrate that HES microparticles induce a mixed T helper 1/T helper 2 response against BSA and may be a suitable delivery and presentation system in the field of vaccine development.
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Keywords: Hydroxyethylstarch microparticles; Vaccine adjuvant; Th1/Th2 response; Immunization route

1. Introduction

Historically, vaccine design is a largely empirical process based on the use of attenuated microorganisms. However, the increasing emphasis for improved vaccine safety has led to the use of highly purified products such as protein subunits or naked DNA. Although these compounds offer greater safety than live-attenuated or killed pathogens, they are poorly immunogenic when administered without adjuvants. Adjuvants were originally described as substances used in combination with a specific antigen that produced a more robust immune response than the antigen alone (Ramon, 1924; Sanchez et al., 1980; Vogel and Powell, 1995). Three kinds of the most frequently used adjuvants can be distinguished: (i) particulate; (ii) non-particulate (such as saponins and lipid A); and (iii) combined adjuvant composition, each exerting a specific immune modulation (Lovgren-Bengtsson, 1998). The only adjuvant licensed for human use is aluminium hydroxide (alum) (Glenny et al., 1926; Sesardic and Dobbelaer, 2004). However, alum does not potentiate the immune response against many antigens and is not efficient to generate a cell-mediated immunity (Gupta and Siber, 1995; Gupta, 1998). Consequently, over the last decade, significant research efforts have been undertaken for the development of new, improved vaccine adjuvants and antigen delivery and presentation systems (O'Hagan and Valiante, 2003).

Particulate adjuvants act as delivery systems that promote the presentation of antigens to the immune system and can differ in chemical composition: liposomes, virosomes, ISCOMS, emulsions, or biopolymers nano- and microparticles (Kersten and Crommelin, 2003; O'Hagan et al., 2006). These delivery systems facilitate antigen uptake, transport or presentation by antigen presentating cells (APCs) which are thought to directly activate T cells (Reddy et al., 2006). The biodegradable and biocompatible polyesters, poly-lactide-*co*-glycolides (PLG), are

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the primary candidates for the development of microparticles as vaccine or delivery systems, since they have been use in humans for many years as resorbable suture material and as controlled release drug delivery systems (Walter et al., 2001; Elamanchili et al., 2004; Waeckerle and Groettrup, 2005). Similarly, our hydroxyethylstarch (HES) microparticles were prepared from the biodegradable and biocompatible molecule, hydroxyethylstarch (Voluven®), which is widely used in humans as artificial colloids for intravascular volume replacement (Langeron et al., 2001; Jungheinrich et al., 2002).

In a previous study, we have characterized and demonstrated that HES microparticles developed in our laboratory exhibited properties required for antigen delivery systems, especially to enhance the presentation of antigens, such as *in vivo* biocompatibility, shelf-life stability, high loading capacity, *in vivo* biodegradability, the controlled release and the localization of the loaded proteins (Devy et al., 2006).

In this work, we assessed the ability of HES microparticles in inducing an immune response in mice against the bovine serum albumin (BSA) protein in comparison with the alum adjuvant. We showed that the immunization of mice with BSA loaded in HES microparticles triggered an immune response against BSA and that both magnitude and type of the response were affected depending on the route of administration. The capacity of HES microparticles to be phagocyted by APCs was also assessed.

2. Materials and methods

2.1. Mice

Female B6D2F1 mice, 6 weeks old, weighing 19–22 g, were purchased from Charles River Laboratories (Iffa Credo, l'Arbresle, France) and housed at the animal maintenance facility under controlled conditions ($24 \pm 1\,^{\circ}C$, humidity of $50 \pm 10\%$, and a 12/12 h light/dark cycle). All experiments were conducted according to the animal care and use of the European Community.

2.2. Materials

Hydroxyethylstarch (Voluven®) was purchased from Fresenius Kabi (Sèvres, France) and terephtaloyl chloride from Acros Organics (Noisy-le-grand, France); Ovalbumin (Ova), BSA, tween 20®, 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), HEPES buffer, trypan blue and cytochalasin D were from Sigma (St Quentin Fallavier, France); chloroform, cyclohexane, ethanol, were provided by SDS (Peypin, France); sorbitan trioleate (Span® 85) was from Seppic (Sepiprod, Castres, France); RPMI 1640 medium, fetal calf serum (FCS), phosphate buffer saline (PBS) were from Gibco-Invitrogen (Cergy-Pontoise, France); 3,3′,5-tetramethylbenzidine (TMB) and lymphoprep™ 1.077 (Ficoll) from AbCys (Paris, France); horse radish peroxidase (HRP) goat-anti-mouse IgG was from Interchim (Montluçon, France); horse radish peroxidase rat-anti-mouse IgG1 and rat-anti-mouse IgG2a antibodies were from Pharmingen (San Diego, CA, USA) and alhydrogel 1.3% (Alum) was from Brenntag Biosector (Frederikssund, Danemark).

2.3. Preparation of HES microparticles

The microparticles were prepared by the interfacial cross-linking method according to our protocol (Levy and Andry, 1990; Devy et al., 2006). A 20% (w/v) HES solution was prepared in carbonate buffer pH 9.8. This aqueous phase (6 mL) was emulsified under mechanical agitation in 30 mL cyclohexane containing 5% (v/v) Span® 85. After 5 min, 40 mL of a 5% (w/v) solution of terephtaloyl chloride in chloroform/cyclohexane (1/4) were added to the emulsion and stirring was prolonged for 30 min. The reaction was stopped by dilution with 40 mL of chloroform/cyclohexane (1/4). Microparticles were successively washed in cyclohexane, 95% ethanol containing 2% Tween 20®, 95% ethanol and water. Finally, microparticles were suspended in water and lyophilized. Particles were sized by a laser diffraction technique (Coulter Particle Sizer, type LS 200 Coultronics, France). Size distribution was displayed in terms of volume versus particle size. The size of these HES microparticles was found to range from 4 to 15 μm with an average of 8.3 ± 1.9 μm. Scanning electron microscopy was used to study the shape and the surface of HES microparticles and to check total removal of terephtaloyl chloride in microparticle wall.

For *in vitro* phagocytosis assay, fluorescent microparticles were prepared by incorporating 30 mg of fluoresceinamine in the HES solution before the emulsification step.

BSA loading was obtained according to our protocol (Devy et al., 2006). Five micrograms of HES microparticles were incubated in 0.5 mL of 0.15% (w/v) BSA diluted in 0.9% NaCl for 3 h under shaking at 37 °C, and were then centrifuged to remove unloaded BSA. BSA-loaded HES microparticles (100 μg of BSA encapsulated in 5 mg of microparticles) were resuspended in saline and were immediately inoculated in mice.

2.4. In vitro phagocytosis assay

Mice were sacrificed and peritoneal cells were collected by flushing the peritoneal cavity with 10 mL of 0.9% NaCl. Mononuclear cells were isolated using a ficoll gradient and resuspended in RPMI 1640 supplemented with 10% FCS, 100 U/mL penicillin and 100 μg/mL streptomycin (complete medium). 10^6 cells were incubated in culture chambers (Nunc). After 12 h, the medium was replaced by 200 μL HEPES buffer (0.2 M, pH 7.4) fluoresceinamine-labelled microparticles for 4 h at 37 °C. The microparticles suspension was replaced by 200 μL of trypan blue solution (250 μg/mL in citrate buffer pH 4.4) to quench extracellular fluorescence. After 1 min, phagocytosis was observed using a fluorescence microscope (Olympus IX70).

For control experiments, phagocytosis was inhibited by adding cytochalasin D (10 μg/mL) to the culture medium 1 h prior to the addition of microparticles and to the microparticles suspensions (Wan et al., 1993).

2.5. Immunization protocol

On days 1 and 21, each group of mice was immunized with saline, 100 μg BSA, 100 μg BSA in Alum (130 μg per injection) or 100 μg BSA loaded in HES microparticles. Each mouse received 200 μL of suspension per injection for *i.p.* route and 100 μl for *s.c.* route. Blood samples were collected from the tail artery on days 7, 11, 15, 21, 30, 38, 48 and 77 after the first immunization. Each sample was processed and serum was collected and stored at −20 °C for further experiments.

2.6. Anti-BSA antibody measurement

An enzyme-linked immunosorbent assay (ELISA) was carried out to assess anti-BSA antibody synthesis. A 96-well microtiter plate (Nunc-immunoplate Maxisorp) was coated with 50 μg of BSA in PBS per well overnight at 4 °C. After washing, the plate was blocked with a 1% OVA in PBS/Tween 20® (PBS/T) for 1 h to avoid non-specific binding. The plate was washed and diluted serum samples were added to the wells for 2 h at RT. Serum from mice immunized with saline was used as a control. One hundred microlitres of HRP-conjugated goat-anti-mouse IgG, rat-anti-mouse IgG1 or rat-anti-mouse IgG2a (1:1000 dilution) were added to each well for 2 h at RT. After washing with PBS/T, TMB was added for 10 min and the reaction was stopped with 1N H_2SO_4. Absorbance was determined with a microplate reader (Multiskan Ascent, Labsystems) at 450 nm. The normal serum absorbance value was subtracted of all absorbance values. Results are given as (absorbance values) × ($-\log_2$(dilution × 10)).

2.7. Splenocyte proliferation assay

Five weeks after the last immunization, spleens from treated (BSA, BSA/Alum or BSA-microparticles) or control (saline-immunized) mice were removed and cell suspensions were prepared in complete growth medium. Red blood cells were depleted using ficoll centrifugation and 5×10^5 cells were incubated in a 96-well plate in the presence of 2.5 mg/mL BSA. After incubation for 60 h at 37 °C, 50 μL of MTT (2 mg/mL) were added to each well during 4 h. The plates were centrifuged (1400 × *g*, 5 min) and 200 μL of DMSO were added to each well. Absorbance was measured using a microplate reader at 540 nm. The stimulation index (SI) was calculated according to the following formula: SI = absorbance (treated mice splenocytes)/absorbance (control mice splenocytes).

2.8. Cytokine assays

IFN-γ and IL-4 were quantified by a sandwich enzyme immunoassay in the supernatants collected from treated or control splenocytes cultured with BSA. Microtiter plates were coated with anti-mouse IFN-γ (R&D systems) or IL-4 (AbCys) overnight in a moist chamber at 4 °C. After blocking, the supernatants from splenocytes culture were added for 2 h at RT. IFN-γ or IL-4 were diluted to appropriate concentrations in assay buffer to create appropriate standard curves. The plates were washed and a secondary antibody conjugated with biotin was added, followed by avidine-peroxidase for 2 h at RT. The plates were washed and TMB was added for 10 min at RT. The reaction was stopped with H_2SO_4 and absorbance was measured 450 nm. Cytokine concentrations were determined in each sample from curves established with standard dilutions of purified IFN-γ and IL-4.

2.9. Statistical analysis

Results were expressed as mean ± SEM. The statistical significance was estimated with a Student's *t*-test for unpaired observations. A P value <0.05 was considered to be significant.

3. Results

3.1. Phagocytosis of HES microparticles by mononuclear cells

The capacity of HES microparticles to be phagocyted by peritoneal mononuclear cells was first examined. Phagocytosis of fluoresceinamine-labelled microparticles was monitored by fluorescence microscopy. In the absence of trypan blue, fluorescence emitted from microparticles in the culture medium was observed (Fig. 1a). Trypan blue does not quench intracellular fluorescence of phagocyted HES microparticles in mononuclear cells. Therefore, only phagocyted microparticles were detectable in the presence of trypan blue (Fig. 1b). The addition of cytochalasin D, a potent inhibitor of actin polymerization, resulted in complete inhibition of phagocytosis (Fig. 1c). These results show that peritoneal mononuclear cells can take HES microparticles up. Fig. 1d shows a HES microparticle internalized into a mononuclear cell.

3.2. Anti-BSA IgG responses

To investigate the effect of HES microparticles on the induction of a humoral immune response against BSA, mice were immunized twice with BSA, BSA/alum or BSA-microparticles by *i.p.* or *s.c.* routes and antibody titers were determined by ELISA. When injected *i.p.*, the three formulations did not induce antibody synthesis during the primary immune response, i.e. after the first immunization (Fig. 2a). The loading of BSA into HES microparticles elicited anti-BSA IgG during the secondary response (after the second injection), with a maximal titre at day 38. However, *i.p.* immunization with BSA-microparticles remained less efficient than BSA/alum. When injected *s.c.*, the antibody response was increased in the three groups following the first injection (Fig. 2b). However, BSA-microparticles and BSA/alum were more efficient than free BSA. After reboosting, the highest antibody titres were observed at day 30 and remained stable until day 77. No significant differences in immunization with BSA-microparticles and free BSA were observed during the secondary IgG response. Therefore, the injection of BSA in *s.c.* resulted in a stronger IgG response than in *i.p.*

To determine whether the immunization with BSA loaded in HES microparticles induced a Th1- or a Th2-mediated immune

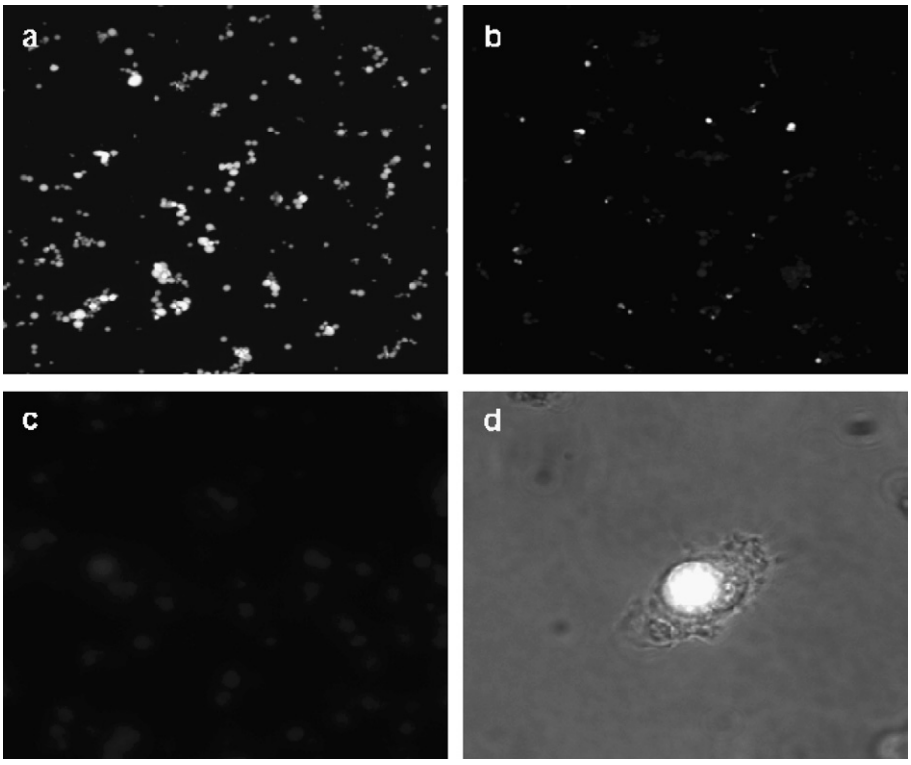

Fig. 1. Phagocytosis of fluoresceinamine-labelled HES microparticles by mononuclear cells observed under fluorescence microscopy: (a) fluorescence emitted by microparticles in culture supernatant or internalized by mononuclear cells; (b) fluorescence emitted after trypan blue quenching; (c) cells were treated with cytochalasin D before incubation with microparticles; (d) detail of a phagocyted microparticle into a cell using simultaneous phase contrast and fluorescence microscopy. Magnification 20× (a, b, c) and 60× (d).

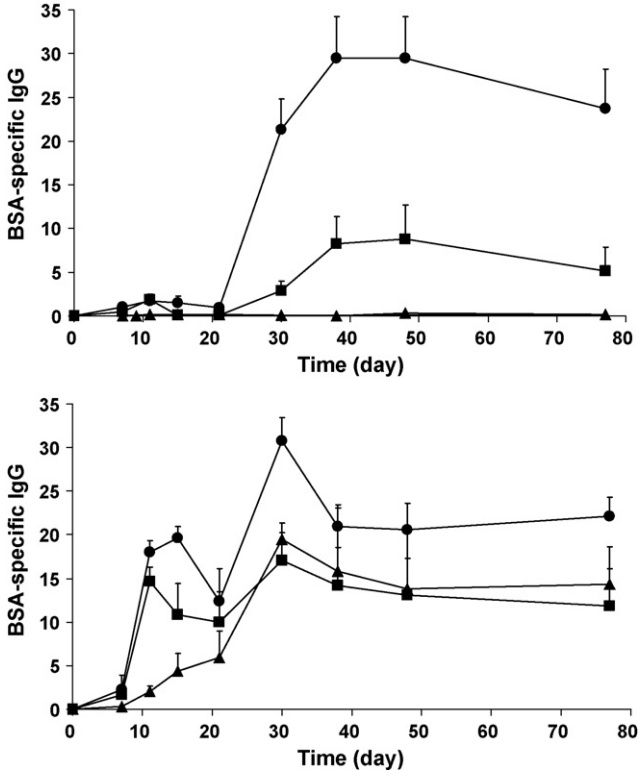

Fig. 2. Serum IgG levels after immunization with BSA (▲), BSA/alum (●) or BSA-microparticles (■) by *i.p.* (a) or *s.c.* (b) route. Values represent means ± SEM. ($n = 3$).

response, the titres of IgG1 and IgG2a subsets were measured for each group. As shown in Fig. 3a, alum, and to a lesser extent HES microparticles, enhanced the anti-BSA IgG1 levels in mice immunized by *i.p.* route compared to the injection of free BSA. Alum and microparticles also enhanced IgG2a titres compared to the injection of free BSA (Fig. 3b), but no significant differences were observed between the two groups. The immunization with BSA-microparticles elicited a higher IgG1 than IgG2a titre, as demonstrated by a positive IgG1/IgG2a ratio (Fig. 3c). However, this ratio was more elevated in mice immunized with BSA/alum, indicating that the alum adjuvant was more efficient than HES microparticles in triggering a Th2-type immune response against BSA.

The loading of BSA in HES microparticles did not improve both IgG1 and IgG2a responses compared to the injection of free BSA by s.*c.* route (Fig. 4a and b), although a predominating IgG1 response was observed (Fig. 4c). On the contrary, the alum adjuvant induced both a sustained IgG1 response against BSA (Fig. 4a), and an increasing IgG2a response from days 30 to 77 (Fig. 4b).

3.3. Splenocytes proliferation assay and cytokine profiles

Spleen cell proliferation was measured in mice immunized with BSA, BSA/alum or BSA-microparticles. As shown in Fig. 5, the loading of BSA in microparticles did not significantly enhance splenocyte proliferation compared to free BSA.

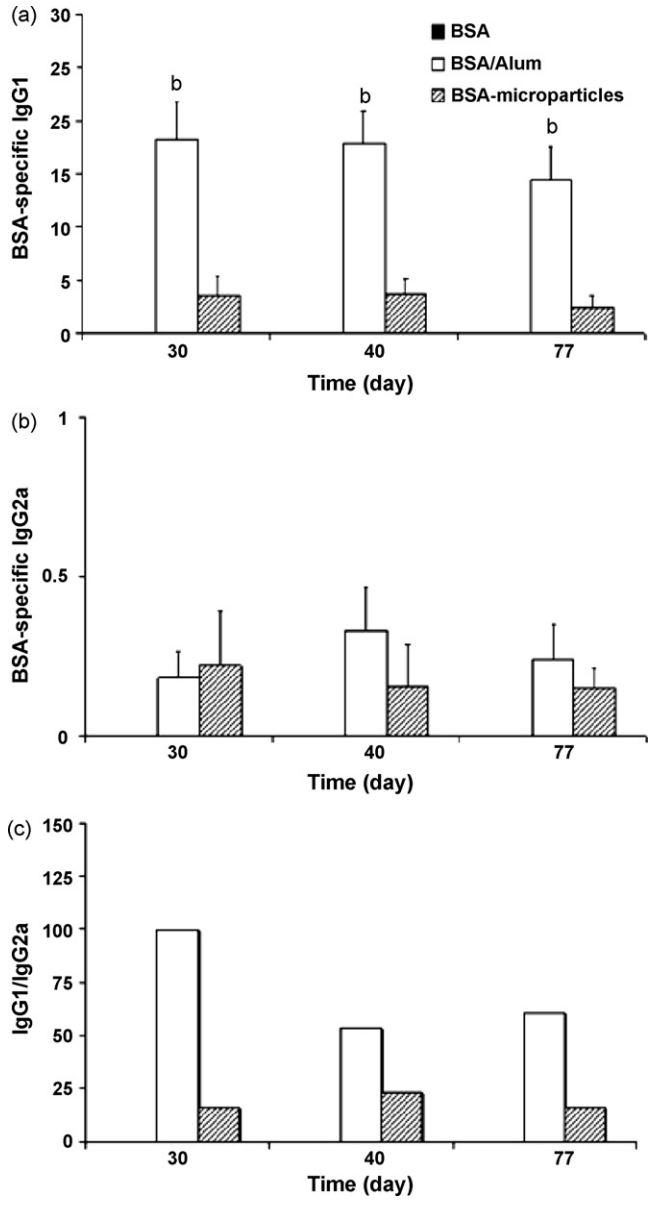

Fig. 3. Serum IgG1 and IgG2a levels in mice immunized with BSA, BSA/alum or BSA-microparticles by *i.p.* route. Values represent means ± SEM. ($n = 3$). [a] $P < 0.05$ between BSA and BSA/alum, [b] $P < 0.05$ between BSA/alum and BSA-microparticles.

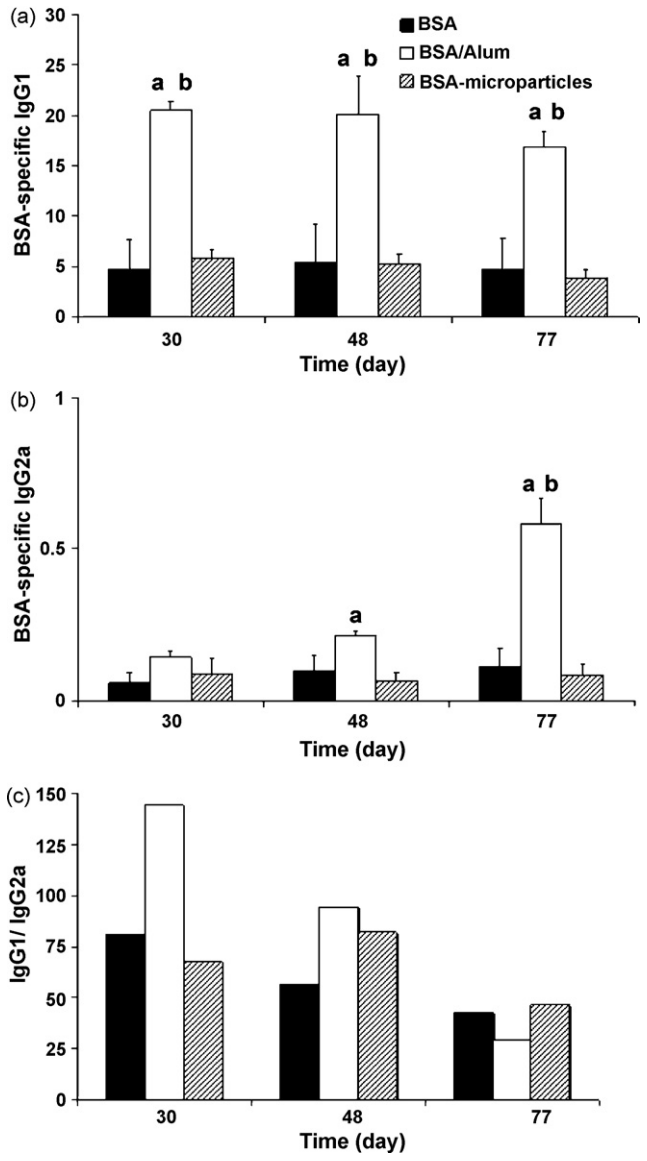

Fig. 4. Serum IgG1 and IgG2a levels in mice immunized with BSA, BSA/alum or BSA-microparticles by *s.c.* route. Values represent means ± SEM ($n = 3$). [a] $P < 0.05$ between BSA and BSA/alum, [b] $P < 0.05$ between BSA/alum and BSA-microparticles.

The strongest proliferation was obtained with the *s.c.* injected BSA/alum formulation.

The cytokine profile of splenocytes can also be used to distinguish the Th1/Th2 type of immune responses. The supernatants from splenocytes stimulated with BSA were used for IL-4 and IFN-γ assays. In mice immunized *i.p.*, no detectable production of IFN-γ was found in BSA- and BSA/alum-immunized mice (Table 1). In contrast, the level of IFN-γ was dramatically increased in mice immunized *i.p.* with BSA-microparticles. The levels of IL-4 were unchanged in BSA and BSA/alum groups, whereas it was significantly increased in BSA-microparticles. When injected *s.c.*, BSA-microparticles were more efficient to induce IFN-γ secretion by spleen cells.

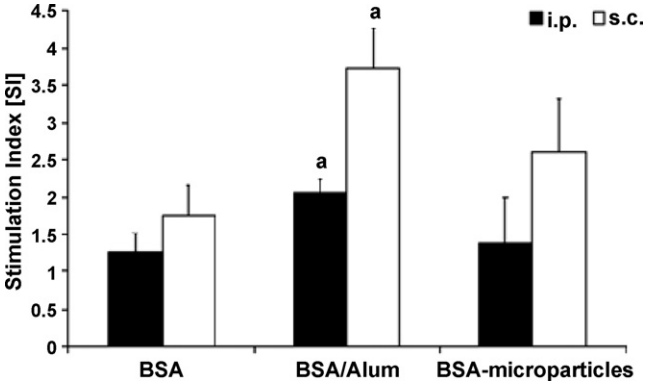

Fig. 5. Proliferation of spleen cells following immunization with BSA, BSA/alum or BSA-microparticles by *i.p.* or *s.c.* route. Values represent means ± SEM. ($n = 3$). [a] $P < 0.05$ between BSA and BSA/alum.

Table 1
Levels of IL-4 and IFN-γ released from splenocytes from mice immunized with BSA, BS/alum or BSA/microparticles[a]

	Cytokines (pg/mL)			
	i.p.		*s.c.*	
Groups	IL-4	IFN-γ	IL-4	IFN-γ
BSA	27 ± 13	N.D.	55 ± 22	47 ± 26
BSA/alum	35 ± 5	N.D.	101 ± 43	150 ± 65
BSA/microparticles	77 ± 15[b,c]	333 ± 115[b,c]	67 ± 16	172 ± 56[b]

N.D.: not detected.
[a] Results expressed as mean ± S.E.M. ($n = 3$).
[b] $P < 0.05$ between BSA and BSA/microparticles.
[c] $P < 0.05$ between BSA/alum and BSA/microparticles.

4. Discussion

Development of new adjuvant, particularly for peptides and proteins, is growing in importance and attracting increased interest (Sinha and Trehan, 2003; Degim and Celebi, 2007). Protein- or peptide-based vaccines need to be formulated in appropriate adjuvants and administrated through the adequate route to elicit either a Th1 or a Th2 type of immune response. In this study, HES microparticles were used as a delivery and antigen presentation system to induce immune responses against BSA. Several microspheres such as poly(D,L-lactide-*co*-glycolide) (PLGA) and poly(D,L-lactide) (PLA) microparticles and nanoparticles have been used in humans as antigen delivery systems (Walter et al., 2001; Elamanchili et al., 2004; Waeckerle and Groettrup, 2005). Particulate adjuvants (e.g. microparticles, ISCOMs, liposomes, virosomes, and virus-like particles) offer several attributes for their use as vaccine delivery systems (O'Hagan et al., 2006). They have a size similar to pathogens that the immune system has evolved to combat and consequently, are efficiently internalized by APCs (Elamanchili et al., 2007). We have shown that HES microparticles exhibited a size which allows their internalization by peritoneal mononuclear cells. This phenomenon was totally abolished when cells were pre-incubated with cytochalasin D. The internalization of epitope-containing microparticles by APCs such as dendritic cells (DCs) and their subsequent migration to lymphe nodes is required to present antigens to T cells (Banchereau and Steinman, 1998; Thiele et al., 2002). Audran et al. (2003) have reported that biodegradable microspheres whose size allows them to be phagocyted (below 10 μm), prolonged *in vitro* peptide presentation to APCs. Consequently, HES microparticles with a diameter lower than 10 μm could be phagocyte by APCs, and the others would allow a progressive release of loaded BSA after biodegradation. These two phenomena would allow a prolongation of BSA presentation to APCs.

We previously showed that BSA was contained into HES microparticles, and was also presented at the surface of the microparticles (Devy et al., 2006). The loading of BSA into HES microparticles enables the presentation of several antigens on their surface, which have been shown to be optimal for B cell activation. Organized arrays of surface-bound antigens efficiently cross-link B cell receptors and constitute a strong activation signal (Bachmann et al., 1993; Fehr et al., 1998).

On the contrary, the immunostimulatory effects of HES microparticles do not appear to be associated with an antigen release. In a previous study we showed that after an initial release of 20% BSA during the first 30 min, no BSA entrapped in HES microparticles was released for the following 4 days. Moreover, HES microparticles exhibited a smooth surface which minimizes the antigen release contrary to a porous structure (Alonso et al., 1993) and it is generally believed that the antigen depot effect contributes to the adjuvant effects of many antigen delivery vehicles (Liang et al., 2006).

In mice *i.p.* immunized with 100 μg of BSA, no IgG response was obtained. Contrary to, a long-lasting IgG secondary immune response against BSA was obtained in mice *i.p.* immunized with the same quantity of BSA-loaded microparticles. However, in mice *s.c.* immunized, the secondary immune response was the same for BSA or BSA-microparticles, but, the primary immune response was higher with BSA-loaded microparticles. These suggesting that the route of injection may affect the extent of the antibody response. DCs, the professional APCs involved in the establishment of the immune response may be activated in a different manner following *i.p.* or *s.c.* injection, as there are differents subsets of DCs depending on their localization. Moreover the influence of the immunization route to induce an IgG response against proteins loaded in microparticles has been demonstrated by several studies (Gutierro et al., 2002). In all cases, HES microparticles elicited a weaker antibody response compared to the commonly used alum adjuvant which is known to induce strong humoral immune responses (Petrvsky and Aguilar, 2004).

Adjuvants have been used to shift the immune response toward a Th1 or a Th2-mediated immune response depending on their mode of action. The Th1 immune response, which is mediated by Th1 cells, is characterized by the production of IL-2, TNF-α and IFN-γ and by an enhanced production of IgG2a, IgG2b and IgG3 subclasses. A Th1 immune response is a requisite for cytotoxic T lymphocyte (CTL) production. The Th2 response is characterized by the production of IL-4, IL-5 and IL-10, and by an enhanced production of IgG1 subset and secretory IgA. The Th1 response is required for protective immunity against intracellular infectious agents, such as viruses, bacteria and protozoa, and against cancer cells. Th2 immunity is effective for a protection against most bacteria as well as against certain viral infections (Cox and Coulter, 1997). The currently available adjuvants mainly stimulate the Th2 type immune response, which is frequently ineffective against intracellular pathogens and malignant cells.

We have shown here that HES microparticles induce a mixed Th1/Th2 response. Several studies reported the induction of a predominant Th1 response by microparticles, mediated by the production of the IgG2a subset (Vordermeier et al., 1995). Immunization by *i.p.* route with BSA-microparticles enhanced both IgG1 and IgG2a production, although the predominant subset was IgG1, as demonstrated by an elevated IgG1/IgG2a ratio. The use of alum as adjuvant elicited high IgG1 titres compared to HES microparticles. The production of high IgG1 levels associated with secretion of IgE has been involved in inflammation (Relyveld et al., 1998; Erazo et al., 2007). However, the *s.c.* injec-

tion of BSA loaded in microparticles did not enhance neither IgG1 nor IgG2a secretion compared to free BSA, demonstrating the importance of the choice of the route of administration.

The *i.p.* immunization with HES microparticles enhanced the production of Th1 and Th2-related cytokines. The levels of IFN-γ and IL-4 released from splenocytes were significantly increased in comparison to mice immunized with BSA or BSA/alum. Only IFN-γ secretion was increased when BSA-microparticles were *s.c.* injected. Our results on the induction of an important humoral immune response by BSA/Alum, in spite of the no production of Th2-related cytokine are in agreement with Brewer et al. (1999), who have reported that Alum can induce Th2 response independently of IL-4 mediated signalling. The proliferation of spleen cells was significantly higher in BSA/alum group. Alum, may be more efficient to expand the number of splenocytes rather than activate splenocytes. On the contrary, the proliferation of spleen cells was not significantly different in BSA and in BSA-microparticles groups. Therefore, HES microparticles may be more efficient to activate rather than expand the number of splenocytes.

The immunization with HES microparticles did not induce any change in the behaviour of mice. Peritoneal granulomas were found after *i.p.* immunization of mice with BSA/alum, but not with BSA-microparticles. Several other studies reported the appearance of post-vaccination granulomas in animals or children immunized with aluminium-adsorbed vaccines (Bordet et al., 2001; Valtulini et al., 2005).

In conclusion, this study shows that the loading of BSA in HES microparticles can induce both a Th1 and a Th2 immune response. Such a mixed Th1/Th2 response is critical for the control of malignant cells as well as infectious pathogens. In addition, HES-microparticles are industrially easy to prepare, use, manufacture and applicable to a wide range of vaccines. The physical loading of the antigen by HES-microparticles is an advantage compared to other conjugation procedures. Indeed, the conjugation procedure is not without difficulties and may involve modification of peptide epitope as well as complication in respect to the characterization and reproductibility of conjugate structures (Kazzaz et al., 2000; Singh et al., 2006). Therefore, HES microparticles may be used as a suitable drug delivery system for a broad range of antigens to generate vaccines useful in the field of tumor immunotherapy.

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