Subcutaneous inverse vaccination with PLGA particles loaded with a MOG peptide and IL-10 decreases the severity of experimental autoimmune encephalomyelitis

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**A B S T R A C T**

“Inverse vaccination” refers to antigen-specific tolerogenic immunization treatments that are capable of inhibiting autoimmune responses. In experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS), initial trials using purified myelin antigens required repeated injections because of the rapid clearance of the antigens. This problem has been overcome by DNA-based vaccines encoding for myelin autoantigens alone or in combination with “adjuvant” molecules, such as interleukin (IL)-4 or IL-10, that support regulatory immune responses. Phase I and II clinical trials with myelin basic protein (MBP)-based DNA vaccines showed positive results in reducing magnetic resonance imaging (MRI)-measured lesions and inducing tolerance to myelin antigens in subsets of MS patients. However, DNA vaccination has potential risks that limit its use in humans. An alternative approach could be the use of protein-based inverse vaccines loaded in polymeric biodegradable lactic–glycolic acid (PLGA) nano/microparticles (NP) to obtain the sustained release of antigens and regulatory adjuvants. The aim of this work was to test the effectiveness of PLGA-NP loaded with the myelin oligodendrocyte glycoprotein (MOG)35–55 autoantigen and recombinant (r) IL-10 to inverse vaccine mice with EAE. In vitro experiments showed that upon encapsulation in PLGA-NP, both MOG35–55 and rIL-10 were released for several weeks into the supernatant. PLGA-NP did not display cytotoxic or proinflammatory activity and were partially endocytosed by phagocytes. In vivo experiments showed that subcutaneous prophylactic and therapeutic inverse vaccination with PLGA-NP loaded with MOG35–55 and rIL-10 significantly ameliorated the course of EAE induced with MOG35–55 in C57BL/6 mice. Moreover, they decreased the histopathologic lesions in the central nervous tissue and the secretion of IL-17 and interferon (IFN)-γ induced by MOG35–55 in splenic T cells in vitro. These data suggest that subcutaneous PLGA-NP-based inverse vaccination may be an effective tool to treat autoimmune diseases.

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Abbreviations: BCA, bicinchoninic acid; CHO, Chinese hamster ovary cells; CNS, central nervous system; CTI, cytotoxic T lymphocytes; DCM, dichloromethan; DLS, dynamic light scattering; EAE, experimental autoimmune encephalomyelitis; FDA, food and drug administration; H&E, hematoxylin&eosin; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; MCF-7, human breast adenocarcinoma cell line; MBP, myelin basic protein; MRI, magnetic resonance imaging; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Ni-NTA, nickel-nitritotriacetic agarose; NIRF, near-infrared fluorescence; NK, natural killer; NP, nano/microparticles; OD, optical density; PBMCs, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; PDI, polydispersity index; PLGA, polymeric biodegradable lactic–glycolic acid; PLP, proteolipid protein; r, recombinant; PVA, poly/vinyl alcohol; RR, remitting relapsing form; RT, room temperature; s.c., subcutaneous injections; SEM, scanning electron microscopy; SE, standard error; TH, T helper; TREG, effector T; TGF, transforming growth factor; TNF, Tumor Necrosis Factor; TREG, regulatory T.

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1. Introduction

Autoimmune diseases may be ascribed to a disturbed balance between the activated effector T (T<sub>eff</sub>) cells that recognize self antigens and the regulatory T (T<sub>reg</sub>) cells that suppress T<sub>eff</sub> activation and control both the immune and autoimmune reactions [1–5]. Several immunoregulatory molecules, such as IL-10, TGF-β, and indoleamine 2,3-dioxygenase (IDO), can support T<sub>reg</sub> function [3,4,6,7]. In particular, IL-10 plays a role in the development and immunosuppressive function of several T<sub>reg</sub> cell types [2] and is a crucial protective cytokine in several autoimmune diseases [3].

Adoptive transfer of several types of T<sub>reg</sub> cells results in protective activity in animal models of autoimmune diseases, but the translation of these studies to humans has been hampered by several issues [8,9]. In particular, the low frequency of these cells, their poor growth in culture, and their high functional plasticity in vivo have made their use a significant challenge. Antigen-specific strategies may potentially circumvent these limitations in vivo [10,11], but effective approaches to restore the T<sub>eff</sub>/T<sub>reg</sub> balance in vivo remain largely undefined [9].

Multiple sclerosis (MS) is characterized by an autoimmune response against the axons and myelin sheaths of the central nervous system (CNS) that results in axonal loss and demyelination [12]. Several myelin proteins, such as myelin basic protein (MBP), proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG), have been implicated as targets of autoreactive T cells in MS and its animal model, experimental autoimmune encephalomyelitis (EAE) [12]. According to the “epitope spreading” model, few epitopes and antigens play a role in the initiation or perpetuation of the disease, but this reactivity tends to spread during the disease course [13].

“Inverse vaccination” refers to antigen-specific tolerogenic immunization treatments that restore the T<sub>eff</sub>/T<sub>reg</sub> balance and inhibit autoimmune responses. Inverse vaccination may be obtained by the prolonged treatment with high doses of the autoantigens. In EAE, systemic administration of myelin antigens either prevented or treated EAE, but the delivery of repeated massive doses of these proteins were required for the therapeutic effect [14–16]. This problem has been overcome by DNA-based vaccines encoding the myelin antigens alone or in combination with immunomodulatory molecules, such as IL-4 or IL-10, that act as “adjuvants” for T<sub>reg</sub> function [17,18]. The EAE results were so promising that they prompted a first clinical trial in MS patients, that showed some positive results in reducing the MRI-measured disease activity and inducing antigen-specific tolerance to myelin antigens in patients with relapse-remitting (RR) MS [19]. However, DNA vaccination is still controversial in MS since a second phase 2 trial showed no significant improvement in magnetic resonance imaging lesion parameters and reduction of new CNS lesions only in the patients displaying high concentrations of anti-MBP antibodies [20]. Moreover while DNA-based vaccines allow for the sustained expression of both the antigens and the adjuvant molecules, it is not possible to control their dose or expression kinetics, and this may be a problem in the case of adverse reactions.

An alternative method to obtain the sustained release of antigens and adjuvants may be to load them in solid biodegradable particles. Polymeric biodegradable lact–glycolic acid (PLGA) particles are attractive carriers due to their biodegradability, bio-compatibility and approval by the Food and Drug Administration (FDA) [21]. They maintain effective concentrations of the loaded proteins for prolonged periods of times by trapping them in a hydrated polymer network that enables their slow release [21]. PLGA allows for the fine-tuning of the degradation rate and subsequent antigen release from several days to more than one year by modulating the polymer lactide–glycolide ratio, the molecular weight, and the crystal profile [22]. The controlled and/or sustained release of antigens and immune modulators from polymeric materials has been used previously to induce the proper immune effector response [23]. The use of PLGA-NP can enhance tolerance induction in some delivery settings, such as nasal vaccination [24]. Moreover, a recent study showed that the intravenous infusion of either polystyrene or PLGA particles chemically coupled with an encephalitogenic peptide can prevent and cure EAE [25]. That strategy stemmed from previous observations showing that autoantigenic peptides coupled to apoptotic leukocytes can induce antigen-specific tolerance in models of autoimmune diseases, allergy, and transplantation by inducing the depletion and anergy of the antigen-specific lymphocytes. However, a key limitation of this approach was that it required systemic (intravenous) injection of the vaccine and was not effective upon local (such as subcutaneous) injection [25]. This implies that the intrinsic risks of life-threatening adverse reactions (such as systemic anaphylaxis) might discourage use of these vaccines in humans.

The aim of the present study was to develop a novel approach for inverse vaccination based on the sustained release of autoantigens and adjuvants from PLGA-NP delivered subcutaneously.

2. Materials and methods

2.1. PLGA production and characterization

PLGA NP were prepared by a modified double solvent evaporation method [26]. Nanoparticles containing MOG<sub>35-55</sub> (MOG–PLGA), IL-10 (IL10–PLGA), 6-coumarin (C6–PLGA) or IR783 (IR783–PLGA) fluorescent dyes were produced by adding MOG<sub>35-55</sub> (200 μg), IL-10 (200 μg), 6-coumarin (C6) (6 mg) or IR783 (6 mg) to the PLGA solution during the preparation. The release of MOG<sub>35-55</sub> and IL-10 were evaluated by dialysis in PBS at 37 °C, and the proteins released were quantified by the BCA assay (for MOG<sub>35-55</sub>) and by ELISA (for rIL-10). Details are given in Supplementary information.

2.2. In vitro biological analyses

Cell toxicity was assessed in human breast adenocarcinoma cell line (MCF-7) using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.

Proinflammatory activity was assessed on human peripheral blood mononuclear cells (PBMCs) that were cultured with PLGA particles and/or bacterial lipopolysaccharide (LPS; 1 μg/ml) for 48 h. Then, Tumor Necrosis Factor (TNF)-α secretion in the supernatant was measured by ELISA.

The cellular uptake of PLGA–NP was investigated in MCF-7 cells incubated with C6–PLGA–NP at 37 °C for 4 h. Then, the cells were analyzed by confocal laser microscopy and flow cytometry.

Lymphocyte activation was assessed in EAE mouse spleen cells cultured in the presence and absence of 10 μg/ml MOG<sub>35-55</sub> for 5 d. Then, the supernatants were collected, and the levels of IFN-γ, IL-10, and IL-17 were evaluated by ELISA.

Histologic analysis of the EAE lesions was performed in the spinal cords fixed in 10% formalin. Sections were stained with hematoxylin&eosin (H&E) and with Luxol fast blue dye for myelin staining. Details of these methods are given in Supplementary information.

2.3. In vivo analyses

Female C57BL/6 mice were purchased from Harlan (Horst, The Netherlands) and were housed under specific pathogen-free conditions at the animal facility at the University of Eastern Piedmont “A. Avogadro” (Novara). The experiments were performed in accordance with the guidelines of the Institutional Ethical Committee for Animal Experimentation.
The biodistribution of PLGA-NP was assessed in C57BL/6 mice after a subcutaneous (s.c.) injection of IR783-PLGA or PBS. After 7 days, the mice were euthanized, and their organs were analyzed with an IVIS® imaging system (Perkin Elmer).

Prophylactic and therapeutic vaccination was performed in female C57BL/6 mice with EAE induced as previously described [27]. Mice (4–8 weeks old) were vaccinated by s.c. injection of PLGA-NP loaded with either 2 μg MOG35-55 or mouse IL-10. The NP were dissolved in PBS, and the injections were given in one flank either (i) on days 30 and -15 (prophylactic vaccination) from EAE induction (day 0) or (ii) on days 8 and 22 after EAE induction (therapeutic vaccination). Each experiment involved 5–10 mice/group. Details are given in Supplementary information.

2.4. Statistical analysis

The statistical analyses were performed using the Mann–Whitney U-test calculated with GraphPad Instat software (GraphPad Software, San Diego, CA).

3. Results

3.1. Physical and chemical characterization of PLGA-NP

We produced PLGA 65:35 lyophilized nanoparticles that were unloaded (blank-PLGA) or loaded with either MOG35-55 (MOG-PLGA) or rIL-10 (IL10-PLGA). PLGA-NP were dispersed in distilled water and analyzed for size distribution and zeta potential; the particles were also imaged using a scanning electron microscope (SEM). Similar results were obtained using blank-PLGA particles and MOG-PLGA or IL10-PLGA particles. The SEM showed a spherical shape with a smooth surface lacking any evident irregularities (Fig. 1A). The size distribution was evaluated by dynamic light scattering (DLS) and ranged 100–1000 nm with a peak approximately 200 nm and a polydispersity index (PDI) of approximately 0.2 for all formulations (Fig. 1B). This behavior can be related to the negative charge density (ζ-potential = -19 mV for PLGA; -21.1 mV for IL-10 and -24.9 mV for MOG35-55) exposed on the surface of the particles as a consequence of the presence of carboxylates moieties in the structure.

The loading efficiency of MOG35-55 and IL-10 was evaluated by calculating the amount of protein recovered after dissolving MOG-PLGA and IL10-PLGA in NaOH (1 M) and neutralizing by HCl (1N). The results showed that the loading efficiency was approximately 23% for rIL-10 and 90% for MOG35-55. This difference may be ascribed to the different sizes of these molecules (19 kDa for rIL-10 and 2.5 kDa for MOG35-55).

The in vitro release of human IL-10 or MOG35-55 from their respective PLGA preparations was assessed by incubating these particles in PBS at 37 °C and sampling the supernatant weekly. The in vitro release profiles showed that PLGA provided sustained release of each protein for several weeks with similar kinetics for each protein (Fig. 1C).
3.2. Biological characterization of the PLGA-NP

The cell toxicity of the PLGA preparations was assessed by performing an MTT assay on MCF-7 cells incubated with titrated amounts (0.6, 6, 60, and 600 μg/ml) of blank-PLGA. The results showed no significant toxicity at any dose (data not shown).

The pro-inflammatory activity of blank-PLGA and IL10-PLGA was evaluated by assessing the ability of the particles to induce the secretion of TNF-α in human PBMCs. Human PBMCs were incubated with titrated amounts (0, 6, 60, 600 μg/ml) of each PLGA preparation in the presence and absence of LPS (1 μg/ml), and TNF-α secretion was measured in the culture supernatant at 48h by ELISA. At concentrations ≥60 μg/ml, the blank-PLGA induced TNF-α secretion and increased the response to LPS. By contrast, none of the IL10-PLGA doses induced TNF-α secretion, and each dose inhibited that induced by LPS (Fig. 2).

The cellular uptake of the PLGA nanoparticles was assessed by incubating MCF-7 cells for 4h with either coumarin 6 (C6), a fluorescent lipophilic dye that emits in the FL-1 channel, or C6-PLGA. The cellular uptake of the fluorescent substance was then assessed by both confocal microscopy and flow cytometry. The results showed that C6-PLGA was partially taken up by phagocytes in a dose-dependent manner, and no substantial uptake was detected with C6 alone (Fig. 3A and B).

The in vivo biodistribution of the PLGA preparations was assessed by injecting mice subcutaneously with PLGA-NP loaded with the IR783 infrared dye (IR783-PLGA) or PBS (control). After 7 d, mice were euthanized, and their organs were analyzed by fluorescence imaging. As shown in Fig. 3C, strong signals were detected in the brain, heart, kidneys and, especially, the lungs. Mild signals were detected in the liver, spleen and lymph nodes.

3.3. Inverse vaccination of mice with EAE

We evaluated the in vivo effect of treatments with MOG35-55-based PLGA inverse vaccines in EAE induced in C57BL/6 mice.
Mice (n = 5–10/group) were vaccinated via s.c. injection of either blank-PLGA, MOG-PLGA, IL10-PLGA or MOG-PLGA plus IL10-PLGA in one flank on days-31 and -15 prior to EAE induction (prophylactic inverse vaccination). The results from two independent experiments showed that, at several time points, treatment with MOG-PLGA plus IL10-PLGA significantly inhibited EAE development compared to the other treatments (Fig. 4). Moreover, the average daily disease score calculated on the cumulative data from both experiments was significantly lower in mice treated with MOG-PLGA plus IL10-PLGA (mean ± SE, 1.3 ± 0.17) than in those treated with blank-PLGA (1.8 ± 0.23, p < 0.05) or IL10-PLGA (1.8 ± 0.22, p < 0.05). A small effect was displayed also by MOG-PLGA alone (1.5 ± 0.19) which inhibited EAE compared to blank-PLGA and IL-10-PLGA alone at some time points, but no significant differences were detected at the level of average disease scores (Fig. 4). By contrast, IL10-PLGA alone had no effect. No vaccination delayed the disease onset.

The effect of the vaccination on the immune response and CNS lesions was evaluated by comparing the anti-MOG spleen lymphocyte response and the histopathologic lesions in mice treated with MOG-PLGA plus IL10-PLGA and those treated with blank-PLGA. The anti-MOG response was assessed by incubating spleen lymphocytes collected at day 21 (the disease peak) after EAE induction with MOG35-55 and assessing cell proliferation (by 3H-thymidine incorporation) and the secretion of IFN-γ, IL-17, and IL-10 in the culture supernatants (by ELISA) after a 5 d culture. The results showed that vaccination with MOG-PLGA plus IL10-PLGA significantly inhibited the secretion of IL-17 and IFN-γ compared to vaccination with blank-PLGA, whereas no significant effect on cell proliferation was detected (Fig. 5A). The secretion of IL-10 was always undetectable (data not shown); this observation is in accordance with data reported by other authors [28].

The histopathologic analysis was performed on the spinal cords of mice collected at day 21 after EAE induction. Light microscopy analysis revealed that the mice treated with blank-PLGA displayed a higher inflammatory cell infiltrate than those treated with IL10-PLGA plus MOG-PLGA, but this difference was not statistically significant (Fig. 5B). However, detection of CD3+ T cells by immunofluorescence showed that they were strikingly more abundant in the former than in the latter group. Moreover, myelin staining showed a significantly dramatic loss of myelin in the mice treated with blank-PLGA, whereas mice treated with MOG-PLGA plus IL10-PLGA showed mild myelin damage only in the subpial myelin (Fig. 5B).

Finally, we assessed the effect of treatment with the different PLGA preparations delivered after EAE induction (therapeutic inverse vaccination). Mice (n = 8–10/group) were injected s.c. with either blank-PLGA, MOG-PLGA, IL10-PLGA or MOG-PLGA plus IL10-PLGA on days 8 and 22 after EAE induction. The results from two independent experiments showed that, at most time points, treatment with MOG-PLGA plus IL10-PLGA significantly inhibited EAE development compared to treatment with blank-PLGA or IL10-PLGA (Fig. 6). Moreover, the average daily disease score calculated on the cumulative data from both experiments was significantly lower in the mice treated with MOG-PLGA plus IL10-PLGA (mean ± SE, 1.2 ± 0.15) compared to those treated with blank-PLGA (1.8 ± 0.24, p < 0.05) or IL10-PLGA (1.8 ± 0.23, p < 0.05) or MOG-PLGA (1.6 ± 0.20, p < 0.05) (Fig. 6). By contrast, MOG-PLGA and IL-10-PLGA had no substantial effect. No vaccination delayed the disease onset.
4. Discussion

This work shows that s.c. inverse vaccination with a mixture of PLGA loaded with MOG$_{35,55}$ and IL-10 is effective at decreasing disease severity in mouse EAE.

Polymeric materials have been investigated for their ability to induce the proper immune effector responses, and in some cases, to promote tolerance. Because the induction and maintenance of immune tolerance is influenced by the persistence of the antigen and can be supported by tolerogenic cytokines, the use of biodegradable micro-nanoparticles is ideal for inverse vaccination because they can support a sustained release of several entrapped molecules [23].

An ideal nanoparticle for inverse vaccination should display low cell toxicity, low proinflammatory activity, sustained release of the vaccine active components, and slow elimination by phagocytes to allow their persistence in vivo to promote regulatory rather than effector immune functions. The MTT assay on MCF-7 cells showed that our particles were not toxic, which is in accordance with data reported by other groups [29].

Regarding the proinflammatory activity of PLGA, the data reported in the literature are controversial. Nicolete et al. showed that PLGA nano/microparticles per se can activate the inflammatory response; in particular, microparticles (used at 1 mg/ml) with a size ranging from 5 to 7 μm induced the production of higher amounts of TNF-α in murine macrophages than those induced by nanoparticles [30,31]. In our experimental setting, blank-PLGA induced TNF-α production at doses ≥60 μg/ml, but this proinflammatory effect was not detected when using IL10-PLGA; instead, these particles inhibited the TNF-α secretion induced by LPS. However, it must be noted that the in vivo experiments showed that the EAE onset was earlier in the prophylactic vaccination than in the therapeutic vaccination which suggests that pretreatment with the PLGA-NP may precondition the induction of EAE by stimulating the innate immune system. Therefore, it is likely that PLGA-NP may be used to improve the induction of EAE.

Fig. 5. T cell response and histopathologic lesions in EAE mice vaccinated with blank-PLGA or MOG-PLGA plus IL10-PLGA. (A) Spleen lymphocytes were collected 21 d after EAE induction and cultured with MOG$_{35,55}$; cell proliferation and the secretion of IFN-γ and IL-17 in the culture supernatants were assessed after a 5 d culture period. (B) Histopathologic analysis of the spinal cords collected from mice 21 d after EAE induction; data are shown as representative stainings and quantitative analysis performed in multiple experiments (bar graphs). H&E staining: (a) blank-PLGA, (b) MOG-PLGA plus IL10-PLGA (arrows mark the inflammatory infiltrate). Immunofluorescence staining of CD3+ T cells: (c) blank-PLGA, (d) MOG-10 plus IL-10 PLGA; KB (myelin) staining: (e) blank-PLGA, (f) MOG-PLGA plus IL10-PLGA; arrows indicate myelin vacuoles. Magnification 20×. The data are the mean ± SE of the results from 6 to 8 mice. Statistical analysis was performed with the Mann–Whitney U-test (*p < 0.05).

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immunity. This is in line with works showing that PLGA particles may support immunization in classical vaccination settings [32].

The release of MOG35-55 and IL-10 from the PLGA-NP was sustained, and both molecules were released for several weeks in vitro. The particles were partially endocytosed, an effect that is beneficial for antigen presentation, but this did not substantially affect their in vivo biodistribution.

The in vivo experiments in EAE showed that vaccination with a mixture of MOG-PLGA and IL10-PLGA displayed a significant protective effect and decreased the severity of the disease. This effect was detected not only when vaccination was performed in a prophylactic setting, i.e., before EAE induction, but also when it was performed in a therapeutic setting, i.e., after the disease onset. Thus, it may be a potential treatment for patients with MS.

The protective effect of IL10-PLGA and MOG-PLGA was accompanied by decreased histopathologic lesions in the spinal cord and decreased lymphocyte production of IFN-γ and IL-17 in response to MOG35-55 in vitro. These results suggest that this inverse vaccination inhibits the autoantigen-specific TH1 and TH17 responses that are crucial in the pathogenesis of MS and EAE. By contrast, MOG-PLGA had a minimal effect that was detectable only in the prophylactic treatment. Moreover, IL10-PLGA had no protective effect in any protocol, which is in accordance with previous data from Cannella et al. showing that treatment with IL-10 does not protect from EAE [33]. These results are in line with those recently reported by Peine et al. showing effectiveness of EAE treatment by codelivery of MOG and dexamethasone in acetated dextran microparticles [34]. Therefore, delivery of the autoantigen together with immunosuppressive agents, such as IL-10 or dexamethasone, is crucial to suppress the autoimmune response. Data obtained with tolerogenic DNA vaccines indicate that these immunosuppressive agents may act as “inverse adjuvants” supporting the induction of tolerance. Lack of these “inverse adjuvants” may explain the failure of clinical trials using autoantigens or “altered peptide ligands” alone which were terminated because of lack of substantial clinical effects and development of hypersensitivity reactions [35].

It is noteworthy that our vaccination was effective by the s.c. route. This marks a key difference from the inverse vaccination performed by Gets et al. with MOG35-55 covalently linked to the surface of either polystyrene beads or PLGA particles [25]. These authors obtained a striking protective effect using both prophylactic and therapeutic treatments, but this effect strictly depended on the intravenous delivery of the vaccine, which may restrict its use in humans because of the potential risk of anaphylactic reactions.

The effect of this intravenous vaccine seemed to be mediated by the induction of T cell anergy, as indicated by a strong inhibition of lymphocyte proliferation; this was possibly driven by tolerogenic macrophages taking up the antigen after scavenger receptors recognized the nanoparticles covalently linked to the self peptide. By contrast, our vaccine uses particles that slowly release both the autoantigen and IL-10. It does not inhibit T cell proliferation, but instead inhibits the secretion of IFN-γ and IL-17. Therefore, it seems to induce immune deviation instead of anergy and opens the way to further studies aiming to ameliorate the mixture of autoantigens and immunosuppressive adjuvants. Our data do not support the possibility that the protective effect was mediated by CD4+CD25+FoxP3+ TREG cells since their numbers were similar in the spleens of mice treated with blank-PLGA and those treated with IL10-PLGA plus MOG-PLGA (data not shown). This is in line with other reports [34], but it does not rule out the involvement of other populations of regulatory cells or CD4+CD25+FoxP3+ TREG cells compartmentalized in other tissues [36,37].
The in vivo biodistribution of the PLGA-NP was intriguing because substantial amounts were detectable in several organs one week after the injection. This biodistribution is in accordance with works showing that particles ≤ 50 nm rapidly diffuse from the injection site, whereas those > 1–5 μm tend to stay at the injection site [38]. Because the sizes of our particles ranged from 100 nm to 1 μm, it is conceivable that the smallest of these readily able to diffuse. It is noteworthy that among the other organs, the strongest signal was detected in the lungs. The lungs have been shown to play a key role in licensing T cells to enter CNS [39]. The signal in the brain is also intriguing because it suggests that the vaccine might exert an immunoregulatory effect directly at the site of the autoimmune aggression. This effect was not simply ascribable to an aspecific immunosuppressive activity of IL-10 because no effect was detected using IL10-PLGA alone. Because changing the particle size would profoundly affect the biodistribution, further work is needed to assess the role of the PLGA particle size in the immunosuppressive effect.

A key problem for translating this approach to humans is that, in MS, the target autoantigens are not fully known and may involve molecules different from MBP, MOG and PLP known to play a role in EAE and MS. Moreover, the autoreactivity is heterogeneous in different patients and changes during the disease course due to epitope spreading [12]. However, several works showed that tolerogenic vaccination, too, may induce an epitope spreading of the tolerance and may gradually involve also autoantigens not included in the vaccine. Moreover, amelioration of the vaccines might be obtained using mixtures of autoantigens [40, 41].

In conclusion, this work showed that PLGA-based inverse vaccination delivered subcutaneously may be effective in treating autoimmune diseases. Current treatments for autoimmune diseases (i.e., MS) are based on immunosuppressive therapies that affect the global immune response and display significant toxicity, especially when chronic therapy is needed. Even novel biotechnological drugs do not solve this problem but instead add the further problem of a dramatic increase in the economic costs of the therapy. Effective therapies with low cost and low toxicity are needed by patients and by the public health systems. Inverse vaccination would meet these criteria because it is capable of inducing an autoantigen-specific immunosuppression with the potential of being low cost and long lasting.

Conflict of interest

The authors state that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2014.08.016.

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