PLGA nanoparticles for 'inverse vaccination' in Experimental Autoimmune Encephalomyelitis (EAE)

G. Cappellano[1], AD. Woldetsadik[1], Y.Shivakumar[1], M.Rizzi[2], F.Carniato[3], E. Orilieri[1], A.Chiocchetti[1], L.Battaglia[4], M.Gallarate[4], R.Cavalli[4] , C. Dianzani[4], F. Renò[2], U. Dianzani[1].

[1]IRCAD and Department of Health Sciences, University of Eastern Piedmont A. Avogadro, Novara;
[2]IRCAD and Laboratorio di Ricerca Innovativa per il Wound Healing Department of Health Sciences, University of Eastern Piedmont A. Avogadro, Novara;
[3]Department of Science and Technology, University of Eastern Piedmont A. Avogadro, Alessandria;
[4]Department of Pharmaceutical Sciences and Technology, University of Turin, Turin, Italy.

The term 'inverse' (or tolerogenic) vaccination is used to indicate antigen-specific immunization protocols inducing regulatory/suppressive immune functions to inhibit autoimmune responses. In experimental autoimmune encephalomyelitis (EAE), an animal model of Multiple Sclerosis (MS), initial trials used administration of purified myelin antigens, but they needed massive doses and repeated injections due to their rapid clearance. This problem has been overcome by using DNA-based vaccines encoding for myelin autoantigens alone or in combination with 'adjuvant' molecules, such as IL-4 or IL-10, to support development of regulatory immune cells. This inverse vaccination can trigger a suppressive loop spreading to epitopes different from those used for vaccination, which is intriguing for MS where multiple autoantigens are involved. Moreover, recent phase I and II clinical trials with MBP-based DNA vaccines showed positive results in reducing MRI-measured disease activity and inducing tolerance to myelin antigens in MS patients. However, DNA vaccination has potential risks limiting its use in humans. Protein vaccination, which can be easily suspended in case of adverse reactions, would be preferred but it requires repeated injections of massive doses of the autoantigens and the adjuvants because of their rapid clearance. An alternative approach could be use of protein vaccines released from polymeric biodegradable lactic-glycolic acid (PLGA) particles (PLGA-NP), approved by FDA, to sustain release of antigens and regulatory adjuvants for extended periods. PLGA-NP maintain effective concentrations of the loaded protein for prolonged periods of times by trapping them in a hydrated polymer-network that enable slow-release. Aim of this work was to develop PLGA-NP-based tolerogenic vaccines containing myelin autoantigens and Treg adjuvants to treat EAE mice. Nanoparticles were formulated from poly D-L-lactide-co-glycolide polymer by solvent evaporation method. We produced PLGA-NP loaded with either the MOG_{35-55} autoantigen or rIL-10 used as an adjuvant for regulatory cells. The morphology of these particles was evaluated by Scanning Electron Microscope (SEM) and Dynamic light scattering (DLS), which detected a mean diameter of 591.7 nm and an -15 ± 2.12 mV zeta potential. PLGA-NP were not toxic to cells and did not induce secretion of proinflammatory cytokine (TNF-a) on monocytes. The encapsulated proteins were released in vitro for up to 2 months and the released rIL-10 maintained its functional activity, detected as ability to inhibit LPS-induced secretion of TNF-α on monocytes. Confocal microscopy experiments showed that minimal amounts of PLGA-NP, i.e. those with the smallest sizes, were taken up by phagocytes. Finally, *in vivo* preliminary experiments showed that subcutaneous treatment with MOG_{35-55}- and rIL-10-loaded PLGA-NP significantly inhibited development of EAE in C57/B6 mice without detectable toxic effects. These data suggest that PLGA-NP-based inverse vaccination may be an effective tool to treat autoimmune diseases.