Encapsulation of insulin peptides in liposomes for preventing type 1 diabetes in NOD mice

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ABSTRACT SUMMARY
Liposomes formed by phosphatidylserine (PS) lipid and filled with insulin peptides were used to mimic apoptotic bodies with immunological tolerance and anti-inflammatory effect in the treatment of type 1 diabetes. In vitro and in vivo results have shown that this novel formulation is highly effective in the prevention of type 1 diabetes with Non Obese Diabetic (NOD) mice.

INTRODUCTION
Type 1 diabetes mellitus (also known as T1D) is a disease based on the autoimmune destruction of the insulin-producing β-cells in the pancreas. Currently, T1D treatments are based on exogenous insulin administration. Last decades, the use of insulin has led to the emergence of significant secondary complications of diabetes, which is of great economic and health impact. In previous works, Vives-Pi et al. have demonstrated that the administration of apoptotic β-cells loaded into autologous dendritic cells (DCs) prevents diabetes in mice. Apoptotic cells are a key factor in the maintenance of immunological tolerance. The uptake of apoptotic islet cells prevents maturation of DCs, and this process induces specific tolerance to β-cells rather than autoimmunity. The shortcoming of this immunotherapy is the difficulty in obtaining and standardizing β-cell apoptotic bodies. Therefore, there was the necessity of seeking an alternative source of apoptotic β-cells to re-establish peripheral tolerance and prevent T1D.

To that end, herein we report the ability of phosphatidylserine (PS)-liposomes filled with insulin peptides to mimic a natural apoptotic β-cell, to induce tolerogenic DCs, to impair autologous T cell proliferation and to prevent experimental T1D. The peptides used for encapsulation were peptide A and B from insulin A chain (21 aa) and insulin B chain (30 aa) respectively.

EXPERIMENTAL METHODS
Liposomes were designed to mimic apoptotic bodies from β-cells. The liposomes were prepared using the thin film hydration method from a lipid mixture (30 mM) of phosphatidylserine (PS), phosphatidylcholine (PC) and cholesterol (CH). Lipids were dissolved in chloroform and the solvent was removed by evaporation under vacuum and nitrogen. The lipids were hydrated with the appropriate solution of peptide (A or B), and the liposomes thus obtained were homogenized to 1 μm by means of an extruder (Lipex Biomembranes, Vancouver, Canada). Particle size distributions and stability of liposomes were measured by dynamic light scattering (DLS) using Malvern Zetasizer, (Malvern Instruments, UK) in undiluted samples. The liposome morphology was examined using cryogenic transmission electron microscopy (cryo-TEM) in a JEOL-JEM 1400 microscope. Autoantigen encapsulation efficiency was
determined after protein quantification by Pierce BCA protein assay kit (Thermo Fisher Scientific Inc., Rockford, IL, USA).

RESULTS AND DISCUSSION

PS-liposomes loaded with insulin peptides A or B were prepared with an optimal particle size over 500 nm -suitable for an efficient uptake by phagocytic cells- and a net surface charge of -30 mV (see Figure 1). Encapsulation yield oscillates between 41-87%, depending of encapsulated peptide. Cryo-TEM characterization revealed that liposomes present multivesicular vesicle (MVV) morphology (see Figure 2), and are 1 μm in size, which is advantageous in terms of liposome uptake by macrophages, antigen encapsulation and immunological activity.

In vitro experiments revealed important features of liposomes. PS-liposomes are non toxic and phagocytosed by DCs, inducing PGE2 secretion, a previously described tolerogenic mediator. To assess the efficacy of liposomes for preventing T1D, we treated prediabetic NOD mice with a single dose of immunotherapy (3.5 mg of lipid) during the pre-diabetic period (8 weeks old). As expected, animals from the sham-control group developed diabetes from 13 weeks of age and with a final incidence of 81.3% (n=16). Interestingly, immunotherapy with liposomes containing diabetogenic autoantigens resulted in disease prevention, with an incidence of 50% (n=12). In vivo biodistribution of liposomes in prediabetic mice was analyzed by bioimaging. The ex vivo analysis of the organs shows that at 24 hours post administration, liposome signal took place primarily in the pancreatic lymph nodes, pancreas, spleen and mediastinal lymph nodes.

CONCLUSION

PS-liposomes containing insulin peptides have shown to be effective for arresting autoimmunity and preventing T1D in NOD mice in a single dose by apoptotic mimicry. We also show that apoptotic mimicry using liposomes can offer a solution with benefits in terms of design –customizable, large-scale production, stability and uniformity-. The clinical relevance of the herein proposed immunotherapy could prove to be very important due its translational potential in human diseases that require the re-establishment of immunological tolerance.

REFERENCES

ACKNOWLEDGMENTS
This work was supported by INSTIT.GERMANS TRIAS I PUJOL-ICN2 researching contract 29012013, ICREA foundation and the Spanish Government under the grant FIS PI12/00195.