INDUCTION OF ANTIGEN-SPECIFIC TOLERANCE BY CARRIER-CONJUGATED PEPTIDES

J. Pfeil[1], U. Lauer[1], A. Rigby[1], B. Hust[2], R. Krähmer[2], F. Leenders[2] , A. Hamann[1], U. Hoffmann[1]

[1]Experimental Rheumatology, Charité University Medicine Berlin; [2]celares GmbH, Berlin-Buch, Germany

INTRODUCTION

How can we improve the efficiency of antigen specific tolerance induction?

Although very successful in experimental models, peptide based tolerogenic vaccines have largely failed to show efficacy in the clinic. One experimental approach to enhance the efficiency of peptide immunization has been to increase the bioavailability of tolerogenic peptides by coupling a relevant antigen to long repetitive sequences found in parasite derived proteins (in collaboration with K. Falk and O. Rötzschke). Based on the success of this approach, we propose to conjugate T cell epitopes to synthetic carriers as Polyethylene Glycol (PEG), thereby enhancing peptide availability and increasing the efficiency of peptide vaccination.

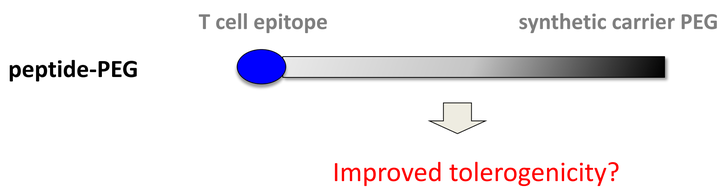

Fig. 1: Hypothesis. Conjugation of relevant T cell epitopes to PEG could increase antigen-specific tolerogenic potential.

METHODS

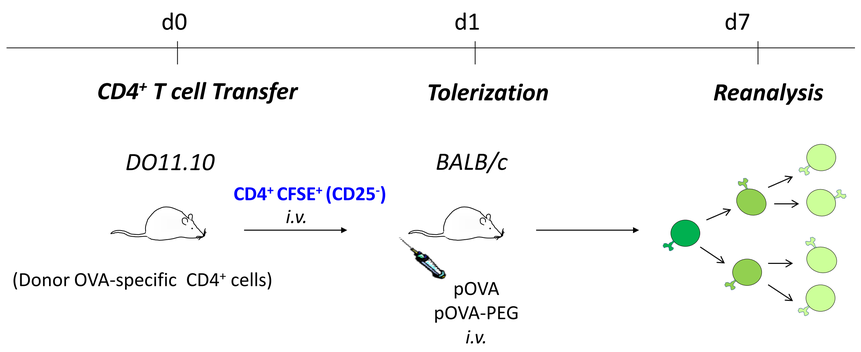

Fig. 2: Time scale for established tolerizing protocol (see fig. 5).

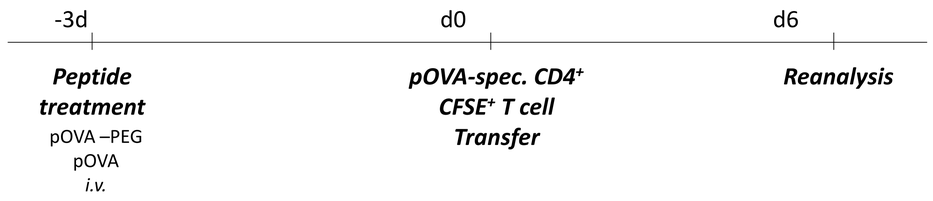

Fig. 3: Time scale for bioavailability test (see fig. 4).

RESULTS

Conjugation of pOVA to PEG extends peptide bioavailability depending on the size of PEG

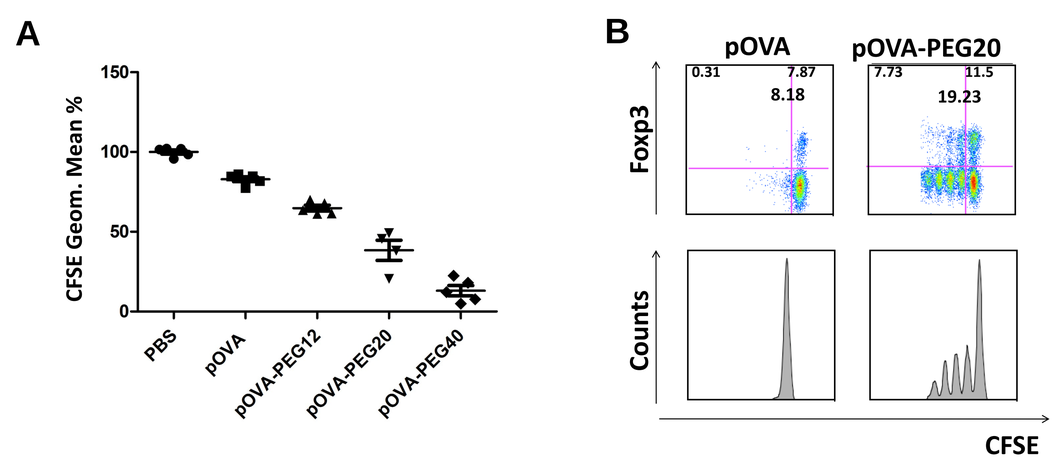

Fig. 4: PEGylated but not unconjugated pOVA is able to stimulate transferred CD4+ T cells and expand/induce Foxp3+ Tregs 3 days after peptide vaccination. OVA-specific CD4+ CFSE+ cells were transferred into BALB/c mice 3 days after peptide vaccination with free pOVA or pOVA conjugated to PEGs of different size. (A) Peripheral lymph node cells were gated on CD4+ OVA specific T cells and were analyzed for CFSE dilution. (B) Peripheral lymph node cells were gated on OVA-specific CD4+ T cells and were analyzed for CFSE and Foxp3.

RESULTS

Induction/expansion of Foxp3+ Tregs depends on dose and size of pOVA-PEG

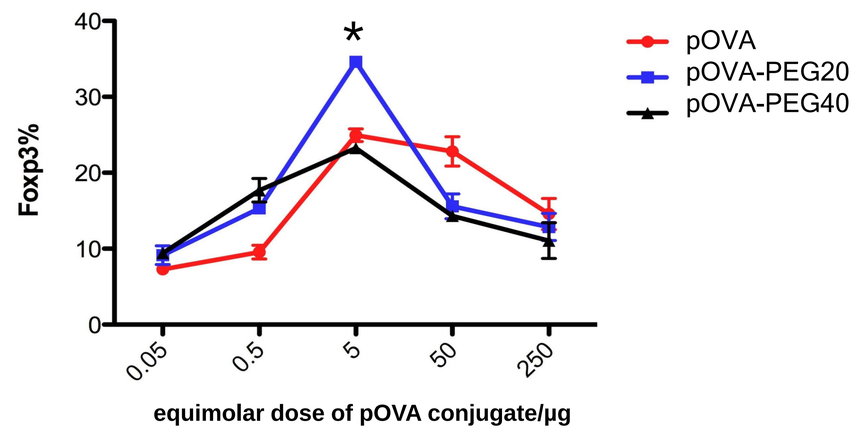

Fig. 5: Tolerization with 5µg of pOVA conjugated to 20kd PEG most efficiently induced/expanded Foxp3+ Tregs. OVA-specific CFSE labeled CD4+ cells were transferred into BALB/c mice (d0). Mice were tolerized the next day. Foxp3 expression was assessed by FACS analysis on day 7. Splenocytes were gated on OVA-specific CD4+ cells. * p<0.05

Tolerization with PEG conjugated peptides reduces frequency of pro-inflammatory T cells

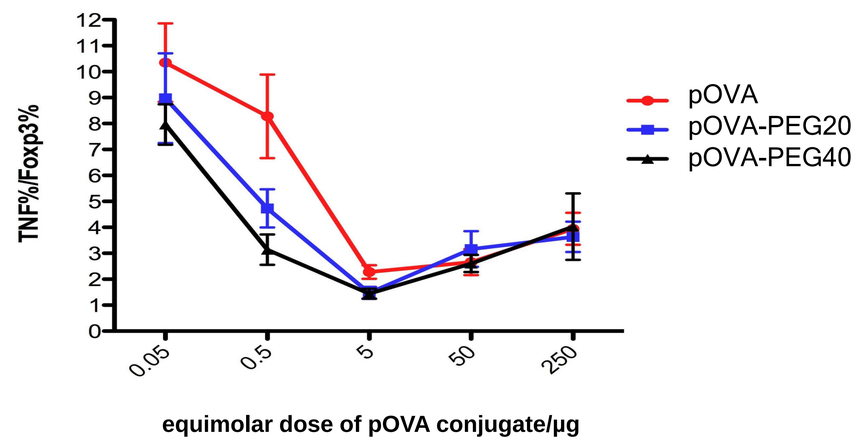

Fig. 6: Mice tolerized with PEGylated pOVA showed decreased TNF/Foxp3 ratio. OVA-specific CD4+ cells were transferred into BALB/c mice (d0). Mice were tolerized on d1 and TNF and Fox3 expression was assessed by FACS analysis on day 7. Splenocytes were gated on OVA-specific CD4+ cells.

Improved tolerogenic potential of PEG conjugated peptides in EAE

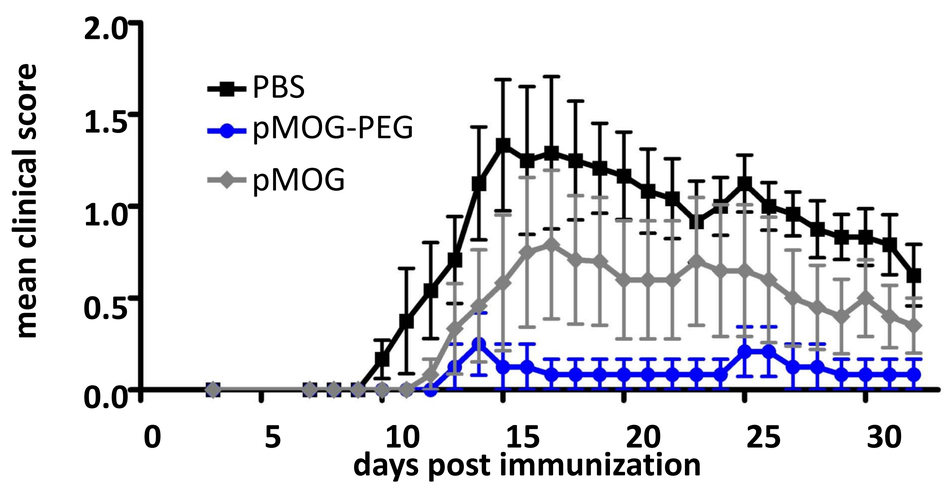

Fig. 7: Stronger suppression of EAE by pMOG-PEG compared to unconjugated peptide. EAE was induced in C57BL/6 mice with MOG_{35-55} in CFA on day 0 and pertussis toxin on day 0 and day 2. Mice were tolerized on day -7 prior to EAE induction with $pMOG_{35-55}$ (n=6) or equimolar amounts of $pMOG_{35-55}$-PEG 20 kd (n=6) or were treated with PBS (n=6). Mean clinical score per group and SEM is shown.

CONCLUSION

We here propose a novel approach to improve efficacy and feasibility of tolerogenic vaccination by conjugating immunodominant peptides to Polyethylene Glycol and believe this to be a promising therapeutic strategy for the treatment of allergies, autoimmunity and transplant rejection.

Contact: Jennifer Pfeil, Exp. Rheumatology, Charité University Medicine Berlin, Campus Mitte, c/o DRFZ, Charitéplatz 1, 10117 Berlin; ***pfeil@drfz.de***

CHARITÉ UNIVERSITÄTSMEDIZIN BERLIN | RCIS ResearchCenter for ImmunoSciences

celares

impulse ZIM für wachstum Zentrales Innovationsprogramm Mittelstand

Bundesministerium für Bildung und Forschung