

## Sustainability of Phenotype and Suppressive Activities of Tregs after Discontinuation of EPIT® but Not of OIT or SLIT Peanut Sensitized Mice

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**Rationale:** Allergen-specific immunotherapy aims to induce long term tolerance, possibly through the generation of regulatory T-cells (Tregs). This study analyzed the phenotype and suppressive activities of Tregs induced by EPIT, OIT or SLIT and their sustainability.

**Method:** BALB/c mice were orally sensitized to peanut and treated by EPIT, SLIT, OIT or left untreated (Sham). The proportion and phenotype of CD4+CD25+Foxp3+ cells (Tregs) were analyzed by flow cytometry after 8 weeks of treatment or 8 weeks after termination. The suppressive activities of Tregs were evaluated by the decrease of peanut-specific responses after adoptive transfer into sensitized mice.

**Results:** Tregs increased at the end of 8-week EPIT, OIT and SLIT vs Sham, significantly more with EPIT. Tregs induced by EPIT, and less so by OIT, increased expression of CTLA-4. Only SLIT induced IL-10+ Tregs. EPIT-induced Tregs were both CD62L+ and CD62L- contrasting with mainly CD62L- with OIT and SLIT. EPIT-induced Tregs expressed a larger repertoire of homing receptors compared to OIT and SLIT. Eight weeks after the discontinuation of EPIT, the level and phenotype of Tregs were maintained, but not after OIT or SLIT. Peanut-specific responses in mice adoptively transferred with Tregs isolated at the end of EPIT, OIT or SLIT were decreased compared to mice not receiving transferred cells. However, when adoptive transfer was done with Tregs isolated 8 weeks after terminating treatment, only EPIT-induced Tregs decreased peanut-specific responses.

**Conclusion:** EPIT, OIT and SLIT induced Tregs with different phenotypes. Only EPIT-induced Tregs were maintained after discontinuation of treatment, suggesting induction of tolerance.