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J Allergy Clin Immunol. Author manuscript; available in PMC 2015 February 01.

Published in final edited form as:

J Allergy Clin Immunol. 2014 February ; 133(2): 500–510. doi:10.1016/j.jaci.2013.12.1037.

Peanut oral immunotherapy results in increased antigen-induced Treg function and hypomethylation of *FOXP3*

Aleena Syed, BSc[1], **Marco A. Garcia, BSc**[1], **Shu-Chen Lyu, MSc**[1], **Robert Bucayu, BSc**[1], **Arunima Kohli, BSc**[1], **Satoru Ishida, PhD**[1], **Jelena P. Berglund, PhD**[4], **Mindy Tsai, DMSc**[2], **Holden Maecker, PhD**[3], **Gerri O'Riordan, RN**[1], **Stephen J. Galli, MD**[2,3], and **Kari C. Nadeau, MD, PhD**[1]

[1]Division of Allergy, Immunology and Rheumatology, Department of Pediatrics, Stanford University School of Medicine, Stanford, California 94305

[2]Department of Pathology, Stanford University School of Medicine, Stanford, California 94305

[3]Department of Microbiology & Immunology Stanford University School of Medicine, Stanford, California 94305

[4]Duke Translational Medicine Institute, Regulatory Affairs. Durham, North Carolina 27710

Abstract

Background—The mechanisms contributing to clinical immune tolerance remain incompletely understood. This study provides evidence for specific immune mechanisms that are associated with a model of operationally defined clinical tolerance.

Objective—Our overall objective was to study laboratory changes associated with clinical immune tolerance in antigen-induced T cells, basophils, and antibodies in subjects undergoing oral immunotherapy (OIT) for peanut allergy.

Methods—In a phase 1, single site study, we studied participants (n=23) undergoing peanut OIT and compared them to age-matched allergic controls (n=20) undergoing standard of care (abstaining from peanut) for 24 months. Participants were operationally defined as clinically immune tolerant (IT) if they had no detectable allergic reactions to a peanut oral food challenge after 3 months of therapy withdrawal (IT, n=7) while those that had an allergic reaction were categorized as non-tolerant (NT, n=13).

Results—Antibody and basophil activation measurements did not statistically differentiate between NT vs. IT. However, T-cell function and demethylation of *FOXP3* CpG sites in antigen-induced Treg were significantly different between IT vs. NT participants. When IT participants

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Address correspondence to: Kari C. Nadeau, Division of Allergy, Immunology and Rheumatology, Stanford University School of Medicine, 269 Campus Dr., CCSR Building, Room 3215, Stanford, California 94305, USA. Phone: 650.723.5227; Fax: 650-498-5560; knadeau@stanford.edu.

Authorship note: Aleena Syed and Marco A. Garcia share first authorship.

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were withdrawn from peanut therapy for an additional 3 months (total of 6 months); only 3 participants remained "immune tolerant" and 4 participants regained sensitivity along with increased methylation of *FOXP3* CpG sites in antigen-induced Treg.

Conclusion—In summary, modifications at the DNA level of antigen-induced T-cell subsets may be predictive of a state of operationally-defined clinical "immune tolerance" during peanut OIT.

Keywords

Food allergy; allergy; oral immunotherapy; peanut; T regulatory cells; desensitization; tolerance; epigenetics; Foxp3

INTRODUCTION

The mechanisms of clinical immune tolerance remain largely unknown. Studies of potential mechanisms of immunotherapy-induced tolerance to allergens have shown increases in allergen-specific blocking IgG antibodies [1], a shift from a T helper type 2 (Th2) response towards a T helper type 1 (Th1) response with increased IFN-γ production[2–6], reduction in specific IgE, reduced recruitment or increased anergy/deletion of T effector cells (Teff)[7–11] and induction of regulatory T cells (Treg)[12–19].

As key immune regulatory cells, Treg have been shown to play a pivotal role in maintaining immune tolerance with Treg deficiencies implicated in the development of allergies[20–24]. Moreover, several studies have demonstrated that epigenetic modifications in CpG-rich regions within the *FOXP3* locus of Treg are associated with stable Foxp3 expression and Treg suppressive function[25–27,69]. Treg subsets including natural Treg (nTreg, derived from the thymus) and induced Treg (iTreg, derived from the periphery from Teff cells) have been described[28–31]. iTreg can be characterized as CD4+CD25^{hi} cells that proliferate in response to specific antigens (i.e., CFSE^{lo} or CD40L/CD69+)[32–35]. iTreg can be Foxp3+ and/or TGF-β+ and/or IL-10+[32,34,36,37,49,68]. Type 1 regulatory T cells (Tr1) release IL-10[28,38,39] and express CD4, CD49, and LAG3 on their surface[28,38,39] which differs from nTreg expressing CD4, CD25^{hi}, CD127^{lo}, and perhaps by Helios on their surface[25–27,40–43].

We hypothesized that iTreg (CD4+CD25^{hi} cells proliferating in response to specific antigen) play a key role in mediating clinical immune tolerance and that assessing epigenetic modulation of the *FOXP3* locus within antigen-induced Treg might provide insight into mechanisms of clinical immune tolerance at the cellular and molecular level. Therefore, we conducted a study with peanut-allergic participants undergoing oral immunotherapy (OIT) to peanut protein over the course of 24 months (24mo), followed by withdrawal from therapy for 3mo, followed by oral food challenge (OFC) at 27mo. We operationally defined “immune tolerant” (IT) patients as those who were non-reactive to an OFC at 27mo and non-tolerant (NT) as those who reacted to an OFC at 27mo. IT participants were withdrawn from peanut another 3mo and rechallenged at 30mo. This work builds on our previous findings in aeroallergen immunotherapy[27], by showing that antigen-induced Treg can modulate Teff proliferation to peanut allergen during the course of OIT. We also show that

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the clinical phenotype of immune tolerance was associated with hypomethylation of *FOXP3* CpG sites in antigen-induced Treg (ai-Treg).

METHODS

The protocol for this study was reviewed and approved by the Institutional Review Board of Stanford University. Written informed consent was obtained for all participants before entering the study.

Study design and participants

Out of 81 screened, 43 peanut-allergic participants from the clinics at Stanford University Hospital were consented, passed screening, and enrolled in study (Online Repository (OR) Fig. E1). Double-blinded placebo-controlled food challenges (DBPCFC) occurred at screening (see OR for details on eligibility criteria and challenge dosing). Clinical reactivity is defined as any sign of allergic reaction (i.e., 1 or greater on Bock's criteria[44]). Subject demographics are summarized in OR Tables E1 and E2. The protocol was conducted in a hospital setting with trained staff and was performed similarly to Jones et al.[12]. The study outline is diagrammed in Fig. E1.

Collection and processing of samples

Blood was collected at baseline, 3, 6, 9, 12, 18, 24, 27, and 30mo. Lab personnel were blinded to participant treatment status. A complete blood count and differential was performed (Stanford Clinical Laboratories). Basophil activation assays were performed as previously described[45]. Specific IgE and IgG4 were measured (Stanford Clinical Laboratories). Treg, Teff, and DC subsets were phenotyped using flow cytometry (LSR II, BD Biosciences). Methylation site analysis was performed on cell subsets as previously described[46]. PBMCs were CFSE-labeled and cultured with peanut, egg, or timothy grass protein (see OR) to identify ai-Treg and Teff subsets. Ai-Treg were defined as Treg ($CD4+CD25^{hi}Foxp3^{+}$) that proliferated in the presence of peanut. Proliferation was measured by $CFSE^{lo}$ or CD40L/CD69+. nTreg (natural Treg) were defined by $CD4+CD25^{hi}Foxp3+$ with no proliferation to peanut. Additional information can be found in the OR.

Statistical analysis

Comparisons between pre- and post-treatment or between therapy (NT and/or IT) groups and control groups were evaluated with the nonparametric Mann-Whitney test, paired Wilcoxon test, and one-way and two-way ANOVA (GraphPad Prism Software 5.0), as appropriate. A P value of < 0.05 was considered statistically significant.

RESULTS

Participants

23 peanut-allergic patients underwent peanut OIT while 20 peanut-allergic, age-matched controls underwent standard of care (abstaining from peanut) (OR Table E1 and E2 for Demographics; Fig. E1 for schematic of study). Doses of peanut protein (Byrd Mill,

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Ashland, VA) were administered orally, with dose escalation every 2 weeks (as tolerated by the subject) up to 4000 mg protein by 24mo. Both active and control subjects underwent a graded oral food challenge (OFC) at 24mo. No subject in the control group successfully passed the OFC (i.e., all control subjects had signs of clinical reactivity) at 24mo. Participants with no reaction to OFC were defined as desensitized at 24mo (n=20) and abstained from therapy and avoided peanut-containing foods for 3mo. At 27mo, desensitized participants underwent another OFC. Patients who reacted (i.e., exhibited any signs of allergic response) were classified as non-tolerant (NT, n=13) and those who did not have any clinical allergic reaction were operationally defined as "immune tolerant" (IT, n=7). IT participants abstained from OIT and avoided all peanut-containing food for an additional 3mo (total of 6mo of avoidance) and were reassessed for "immune tolerance" with an OFC at 30mo (IT, n=3).

Humoral and basophil immune markers in IT vs. NT participants

To measure immune monitoring features, peripheral blood was collected longitudinally and analyzed for immunoglobulin levels and basophil activation. Levels of peanut-specific IgE showed no statistically significant differences between NT vs. IT (Fig. 1A). There was a trend for levels of peanut-specific IgG4 and peanut-specific IgG4:Ig E to increase over time in the active treatment groups undergoing OIT; however, differences among the 3 groups did not reach significance (p=0.24 and p=0.27, respectively) (Fig. 1B and Fig. 1C).

IgE binding can mediate basophil activation and several studies suggest basophils could serve as a possible tool to diagnose and monitor allergies[45,47,48]. To determine the effect of OIT on peripheral basophil activation, surface CD203c was after ex vivo stimulation with peanut allergen as per published techniques[45,47,48]. Basophil activation decreased at 3mo into OIT (Fig. 1D, *P<0.001) and at 9mo in both IT (mean MFI, 103 [± 46 SD]) and NT (mean MFI, 117 [± 41 SD]) groups, but levels in control participants remained constant (mean MFI at 9mo, 1837 [±140 SD]). Peanut-induced basophil response was most reduced in the IT group, although differences between IT and NT did not achieve statistical significance. Notably, basophils from each group retained high and statistically indistinguishable (P>0.99) levels of responsiveness to activation by anti-IgE (Fig. 1E).

Skin prick test (SPT) wheal diameter was decreased at 12mo for IT and NT participants compared to control (*P<0.05). No difference was observed in SPT results in NT vs. IT (NS, P=0.66) (Fig. 1F).

Antigen-induced Treg are induced during OIT and are functionally suppressive

We next investigated whether OIT induced allergen-specific Treg. Antigen--induced cells were identified by CFSE proliferation assays as $CD4^{+}CD25^{+hi}Foxp3^{+}CFSE^{lo}$ T cells and non-specific Treg (ns-Treg) were identified as $CD4^{+}CD25^{hi}Foxp3^{+}CFSE^{hi}$ T cells as previously described[32–34,49] (for gating, see Fig. 2A and 2B and OR). Initial studies characterizing T cell populations identified an increase in ai-Treg between baseline and 12mo after starting peanut OIT (TableI). The enhancement of antigen-induced Treg function appeared to be specific to peanut, as there were no changes in Treg specific for other offending allergens (OAA, specifically either egg or timothy grass allergen) not used in the

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OIT protocol (TableI). In addition, there was an increase in intracellular IL-10 in the ai-Treg population after therapy (TableI). We saw no significant differences at baseline in IT vs. NT vs. Control groups (Fig. E2) and grouped the baseline data together (n=43) in Fig. 2D. There were no differences in absolute counts of ns-Treg between control, IT, and NT subjects at any time point (Fig. 2C). Absolute counts of ai-Treg were significantly increased in IT vs. NT or control participants beginning at 6mo after start of therapy (Fig. 2C, *$P<0.002$) and remained so at 24mo (Fig. 2D, *$P<0.0001$).

The ai-Treg population identified in this study could be comprised of iTreg[49, 68]; in contrast, ns-Treg may represent a population of thymically-derived nTreg. While the phenotypic differences between iTreg and nTreg are subtle and highly debated[50–52,68], functionally, nTreg are thought to be nonspecific, and control systemic autoimmunity. Induced Treg or “activated” Treg were recently discussed by Sakaguchi and colleagues; activated Treg can be identified by $CD4^{+}CD25^{hi}CD45RA^{lo}Foxp3^{+}$; iTreg are hypothesized to be specific, and are implicated in allergic inflammation[53]. In order to further phenotype Treg populations, we performed flow cytometry using Helios. Our preliminary data suggest that ai-Treg express lower levels of Helios compared to ns-Treg (Fig. E3A and B), possibly indicating that the ai-Treg population are comprised of iTreg[25–27,40–42]; however the ability of levels of Helios expression to discriminate among different subset of Tregs is still under active investigation[43]. Given the increases in IL-10 in peanut-induced CD4+ T cells seen in our initial phenotyping studies (TableI), we investigated whether ai-Treg could represent Foxp3+ Tr1 cells. Our data suggest that the percentage of Tr1 cells is increased in patients on therapy compared to those without therapy (Fig. E4).

To study function, Treg were sorted by flow cytometry from all participants. The suppressive function of ai-Treg (defined as $CD4+CD25^{hi}Foxp3^{+}$ Treg proliferating to peanut) on ai-Teff responses was compared at baseline vs. 27mo. The data show no significant difference in the suppressive function at baseline for IT, NT, and Control groups (Fig. E2). In Fig. 2E, a Treg subpopulation ($CD4^{+}CD25^{hi}Foxp3^{+}CD45RO^{+}$) sorted by flow cytometry (FACS Aria, BD, San Jose, CA) was found to suppress Teff ($CD4+CD25^{lo/-}$, Foxp3-) proliferation to peanut (but not to other offending allergens or tetanus) (*$P<0.0001$). When naïve ($CD45RA^{+}$) $CD4^{+}CD25^{hi}Foxp3^{+}$ Treg cells were sorted out of the total Treg population used in the suppression assays, there was no loss in the ability to suppress Teff proliferation to peanut allergen (Fig. E5A) nor did purified naïve Treg cells exhibit any differences in Treg function pre or post-OIT (Fig. E5B), suggesting that enhanced suppressive function to peanut antigen during OIT was associated with allergen-specific $CD45RO^{+}$Treg. Of note, there was greater suppression by ai-Treg isolated from IT (95±5%) than NT (77±21%) participants ($P<0.0001$) (Fig. 2E). Suppressive function of ai-Treg from the control group at 27mo was similar to baseline (Fig. 2E). The increase in antigen-induced Treg function did not seem to be due to alterations in the antigen-induced Teff function, as antigen-induced Teff proliferation alone was unchanged between baseline and 27mo (i.e., 3mo after stopping OIT) for all treatment groups (Fig. E5A).

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OIT enhances the migratory activity of antigen-induced Treg

The functional migratory potential of Treg towards an intestinal epithelium cell line was analyzed *in vitro* by a chemotaxis assay. As shown in Fig. 3A, starting at 12mo, ai-Treg from IT or NT OIT participants showed increased migration activity compared to control (*P<0.001). Moreover, the migratory activity of ai-Treg from IT participants was significantly enhanced starting at 12mo compared to NT participants.

We next tested whether increased chemokine receptor expression was associated with the increase in migration activity. As shown in Fig. 3B and 3C, the expression levels of CCR8 increased at 24 and 27mo (i.e., at the end of OIT or 3mo after stopping OIT) as compared to CCR8 levels at baseline (*P<0.001) in both IT and NT participants. Soler et al. demonstrated that CCR8 is expressed by Th2 cells and Treg[83]; it is interesting that there is an increase in CCR8 in NT subjects, which could be due to expression in Treg and/or Th2. By contrast, CCR4 and CCR7 expression levels did not change significantly over the course of therapy. An example of chemokine receptor staining is shown in Fig. 3D.

Foxp3 is modified in antigen-induced Treg during OIT

Since Foxp3 is one of the indicators of Treg suppressive function and has been associated with maintenance of iTreg, and since Foxp3 is a transcription factor for CCR8[54–59], we measured expression of Foxp3 protein in ai-Treg using flow cytometry; there was significantly increased Foxp3 protein during OIT in ai-Treg from IT participants, but not in ai-Treg from NT or control participants (Fig. 4A, *P<0.001). Similarly, Foxp3 transcript levels were also increased at 24 and 27mo compared to baseline in ai-Treg from IT, but not in ai-Treg from NT or control participants (Fig. 4B).

We tested whether DNA methylation of the *FOXP3* gene in Treg decreased throughout the course of OIT. AiTreg were identified by double positive CD69/ CD40L expression (in addition to $CD4^{+}CD25^{hi}Foxp3^{+}$) upon peanut antigen incubation and were purified by flow cytometry. Compared to baseline, there was significantly decreased methylation of CpG sites in ai-Treg for all OIT participants at 24 and 27mo, with most pronounced hypomethylation in IT participants (Fig. 4C,*P<0.001). Participants that shifted from IT at 27mo to NT at 30mo were marked by an increase in methylation of CpG sites (Fig. 4C). Ai-Treg from control participants (Fig. 4C) and ns-Treg from all groups did not change (Fig. 4A).

Dendritic Cells (DCs) induced during OIT can influence epigenetic modifications in T cells

Since iTreg can be induced via interactions with DCs, we examined the effects of DCs studied before therapy (baseline) and at 27mo (3mo after stopping OIT) on modulation of *FOXP3* epigenetics in CD4+Teff IT vs. NT.. Teff cells and autologous DCs were purified by flow cytometry and cultured together (Fig. E6) in the presence of peanut protein for 3 days. Autologous cultures did not affect Teff viability. Fig. E7 shows that DCs obtained from either IT or NT participants at baseline did not alter *FOXP3* CpG methylation in Teff. However, DCs isolated post-therapy significantly decreased the percentage of *FOXP3* CpG methylation in Teff (*P<0.001). The percent of *FOXP3* methylation in Teff alone did not change over the course of the culture.

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DISCUSSION

In this study, 20/23 participants successfully completed 24mo of oral immunotherapy, tolerating up to 4 g of peanut protein after maintenance therapy. After 3mo of peanut avoidance, only 7/20 participants were defined as "immune tolerant"; these 7 avoided peanut for an additional 3mo (6mo total of avoidance) and only 3/7 remained clinically non-reactive (i.e., "immune tolerant"). We found that the IT participants had higher numbers of ai-Treg with greater suppressive function, and with higher levels of *FOXP3* hypomethylation, compared to NT and control participants. Thus, this study demonstrates a possible mechanism of IT in OIT involving: i) an increase in ai-Treg with enhanced chemotactic and suppressive behavior, and ii) epigenetic modifications within the *FOXP3* locus of such ai-Treg. None of the demographic or clinical features assessed at baseline affected the outcome of IT vs. NT. All subjects on OIT were on maintenance for at least 3mo (i.e., from 21mo to 24mo); number of months on maintenance did not distinguish IT vs. NT.

In this study, we focused on the possible role of Treg and whether antigen-induced Treg were associated with "immune tolerance" in OIT. The method of identification of iTreg was consistent with other publications[32–36,49,68] in which iTreg were defined as proliferating CD4+CD25^{hi}Foxp3+ cells (i.e. either defined through CFSE^{lo} or CD40L/CD69+ in response to specific antigen stimulation). Furthermore, our data show that the iTreg population was CD45RO+ (and CD45RA^{neg}), Helios$^{lo/neg}$, CD49b+/LAG3+, Foxp3+, and IL-10+; therefore, the iTreg we identified with these markers are consistent with other studies[32–36] and are possibly Foxp3+ Tr1 cells[28,34,38,39]. Moreover, we demonstrated that the iTreg population induced through peanut OIT was specific in suppression of autologous Teff proliferation in response to peanut, not other antigens. So as to prevent cross contamination, the effector T cell population we used in functional assays was purified as CD4+CD25$^{lo/neg}$ and found to be Foxp3−.

Previous studies have demonstrated the presence of increased Treg in patients who develop "immune tolerance"[12,14,15,60]. Antigen-induced Treg, despite being in relatively small numbers compared to other immune cell subsets, have been shown be associated with natural loss of food allergy[61]. In contrast to other OIT studies in which high doses of antigen are administered with concomitant omalizumab use[62], the present OIT protocol, like that developed by Burks, Jones, and others[12,63–65], started with a low initial dose of peanut followed by slow dose escalations.

Most previous immunotherapy studies have not proven whether Treg are functionally suppressive, whether they migrate towards intestines, or whether they are antigen-induced. The population of ai-Treg that we identified during OIT had a marked increase in Foxp3 expression, and associated increases in both chemotaxis toward intestinal epithelial cells and suppressive function toward antigen-induced Teff (Fig. 2–4) in IT participants. Our data therefore suggest that one possible mechanism of OIT involves modifications of ai-Treg to enhance their suppressor function, possibly in the intestinal tissues. Demethylation of *FOXP3* plays a crucial role in Treg plasticity and suppressive function[66–69] and could play a role in immune tolerance. Importantly, we have documented hypomethylation within the CpG locus of *FOXP3* in ai-Treg in IT vs. NT subjects (Fig. 4). We followed participants up

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to 30mo (i.e., until 6mo after stopping OIT) and saw that 4/7 IT participants (broken lines in Fig. 4C) were no longer “tolerant” at 30mo. Such “resensitization” was associated with increased methylation of CpG sites in the *FOXP3* locus (Fig. 4C).

Our results suggest possible Treg biomarkers that might be useful in predicting a state of “immune tolerance” ; however, many cells in addition to Treg are thought to contribute to IT. Our recent aeroallergen immunotherapy study by Swamy et al[27] reported epigenetic changes in the *FOXP3* locus following therapy. The present study performed novel detailed mechanistic studies on sorted antigen-induced T cells and identified Foxp3+ Tr1 cells as the major iTreg population associated with success of therapy. While the current study focuses on epigenetic studies of the *FOXP3* locus, our data suggest a potential role for DCs in modulating *FOXP3* epigenetics in Teff cells (Fig. E7). Data from our laboratory demonstrate that DCs expressing indoleamine 2,3 dioxygenase (IDO) may help promote the conversion of naïve CD4+ T cells to Treg and that this conversion may be mediated via epigenetic changes at the *FOXP3* locus[84]. Janson, et al. have shown that *FOXP3* promoter demethylation was associated with the appearance of a committed Treg population[69], and increased expression of IDO by DCs has been linked to enhanced tolerogenicity and the suppression of Teff proliferation[70].

In a recent clinical study by Burks et al.[71], participants underwent egg OIT for 22mo. The clinical data in that study and ours are similar, in that the % of subjects with loss of clinical reactivity after a period of food allergen avoidance after OIT in our study was 30% (7/23 after 3mo peanut avoidance) and, in the Burks, et al.[60] study was 28% (11/40 after 4–6 weeks egg avoidance). One of the differences between these studies was that the OFC was performed with up to 4.0g of peanut protein (the same dose as the maintenance dose) in our study whereas Burks et al. used 10g of egg protein (a much higher dose than the maintenance dose of 2g egg protein). The role of specific IgG4 is under investigation in OIT[64–66,72]; Our data demonstrated a trend but not statistical significance. Although many studies have begun to investigate sustained therapeutic responses after OIT, questions remain regarding the long-term sustainability and safety of desensitization vs. such operationally defined examples of “immune tolerance” and whether specific IgG4, IgG4:IgE ratio, basophil reactivity, T cell or other cellular markers could be used to predict an outcome of IT[72,74].

While our data suggest that larger phase 2 trials in OIT are justified and feasible, limitations of the present study should be mentioned. First, we realize that this was a small cohort study performed at a single site. Secondly, our mechanistic conclusions are limited to changes detectable by sampling the peripheral circulation. It will be of interest to examine mechanisms at the local organ level as well, as has been done using tonsillar tissue[74].

In summary, our study investigated the durability of “immune tolerance” in food allergy OIT after a minimum of 3mo off therapy. Food allergy OIT is under investigation and is still in experimental stages[64–66,73,74]. It is important to note that NT participants, after stopping OIT, were still able to ingest a relatively large amount of peanut protein (as compared to their threshold dose at baseline food challenge) prior to developing allergic symptoms (Table E2). Our data suggest that ai-Treg, rather than ns-Treg, are a key regulatory cell type

modulating the immune response during OIT, and that epigenetic regulation of these T cells might contribute to the induction of such "immune tolerance". However, none of our data in this small study demonstrate a complete dichotomy between IT vs. NT; instead, there were incremental differences (some statistically significant, others not) in the extent of mechanistic changes. An interesting goal for future studies will be to determine whether a composite set of values for immune indicators (involving T cells, B cells, basophils, or other cells, or plasma markers) might be strongly associated with clinical immune tolerance and its durability. Moreover, large studies will be needed to determine whether such immune indicators can be identified, and, if so, whether they will be useful in refining peanut OIT so that it can provide safe, sustained, and effective therapy for peanut allergy patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Drs. Rosa Bacchetta, A. Wesley Burks, Wendy Davidson, Mark Davis, Silvia Gregori, Anne Hiegel, Stacie Jones, David Lewis, Marshall Plaut, Pam Steele, Kinjal M. Hew, and Brian Vickery for their advice in the conductance of the study and/or input in the manuscript.

Declaration of all sources of funding: Food Allergy Research and Education, the Fund for Food Allergy Research at Stanford, National Institute of Allergy and Infectious Diseases R21 (1R21AI09583801), and the Children's Health Research Institute/Lucile Packard Foundation. This project was supported by the National Center for Research Resources and the National Center for Advancing Translational Science of the National Institutes of Health (UL1RR024128 and TR000093).

Abbreviations used

BL	Baseline
mo	Months
DBPCFC	Double Blind Placebo Controlled Food Challenge
DC	Dendritic Cell
Foxp3	Forkhead Box Protein 3
ns-Treg	Non-specific regulatory T cell
OFC	Oral Food Challenge
OIT	Oral Immunotherapy
ai-Treg	antigen-induced regulatory T cell
SPT	Skin Prick Test
Teff	Effector CD4+T cell
Treg	Regulatory T cell

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References

1. James LK, Shamji MH, Walker SM, Wilson DR, Wachholz PA, Francis JN, Jacobson MR, Kimber I, Till SJ, Durham SR. Long-term tolerance after allergen immunotherapy is accompanied by selective persistence of blocking antibodies. J Allergy Clin Immunol. 2011 Feb; 127(2):509–516. Pub Med PMID: 21281875. [PubMed: 21281875]
2. Akdis CA, Akdis M, Blesken T, Wymann D, Alkan SS, Muller U, et al. Epitope-specific T cell tolerance to phospholipase A2 in bee venom immunotherapy and recovery by IL-2 and IL-15 in vitro. The Journal of Clinical Investigation. 1996 Oct 1; 98(7):1676–1683. PubMed PMID: 8833918. Pubmed Central PMCID: 507602. [PubMed: 8833918]
3. Akdis CA, Blaser K. Role of IL-10 in allergen-specific immunotherapy and normal response to allergens. Microbes and infection / Institut Pasteur. 2001 Sep; 3(11):891–898. PubMed PMID: 11564436. [PubMed: 11564436]
4. Chen Y, Kuchroo VK, Inobe J, Hafler DA, Weiner HL. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. Science. 1994 Aug 26; 265(5176):1237–1240. PubMed PMID: 7520605. [PubMed: 7520605]
5. Powrie F, Correa-Oliveira R, Mauze S, Coffman RL. Regulatory interactions between CD45RBhigh and CD45RBlow CD4+ T cells are important for the balance between protective and pathogenic cell-mediated immunity. The Journal of Experimental Medicine. 1994 Feb 1; 179(2):589–600. PubMed PMID: 7905019. Pubmed Central PMCID: 2191378. [PubMed: 7905019]
6. Billingham RE, Brent L, Medawar PB. Actively acquired tolerance of foreign cells. Nature. 1953 Oct 3; 172(4379):603–606. PubMed PMID: 13099277. [PubMed: 13099277]
7. Tanabe S. Epitope peptides and immunotherapy. Current protein & peptide science. 2007 Feb; 8(1): 109–118. PubMed PMID: 17305564. [PubMed: 17305564]
8. Bousquet J, Maasch HJ, Hejjaoui A, Skassa-Brociek W, Wahl R, Dhivert H, et al. Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids. III. Efficacy and safety of unfractionated and high-molecular-weight preparations in rhinoconjunctivitis and asthma. The Journal of Allergy and Clinical Immunology. 1989 Oct; 84(4 Pt 1):546–556. PubMed PMID: 2677092. [PubMed: 2677092]
9. Suko M, Mori A, Ito K, Okudaira H. Oral immunotherapy may induce T cell anergy. International archives of allergy and immunology. 1995 May-Jun;107(1–3):278–281. PubMed PMID: 7613150. [PubMed: 7613150]
10. Rolland J, O'Hehir R. Immunotherapy of allergy: anergy, deletion, and immune deviation. Current opinion in immunology. 1998 Dec; 10(6):640–645. PubMed PMID: 9914222. [PubMed: 9914222]
11. Agrawal B, Krantz MJ, Reddish MA, Longenecker BM. Cancer-associated MUC1 mucin inhibits human T-cell proliferation, which is reversible by IL-2. Nature medicine. 1998 Jan; 4(1):43–49. PubMed PMID: 9427605.
12. Jones SM, Pons L, Roberts JL, Scurlock AM, Perry TT, Kulis M, et al. Clinical efficacy and immune regulation with peanut oral immunotherapy. The Journal of Allergy and Clinical Immunology. 2009 Aug; 124(2):292–300. e1–97. PubMed PMID: 19577283. Pubmed Central PMCID: 2725434. [PubMed: 19577283]
13. Vickery BP, Burks W. Oral immunotherapy for food allergy. Current opinion in pediatrics. 2010 Dec; 22(6):765–770. PubMed PMID: 20852423. [PubMed: 20852423]
14. Mobs C, Slotosch C, Loffler H, Jakob T, Hertl M, Pfutzner W. Birch pollen immunotherapy leads to differential induction of regulatory T cells and delayed helper T cell immune deviation. Journal of immunology. 2010 Feb 15; 184(4):2194–2203. PubMed PMID: 20048125.
15. Hellings N, Raus J, Stinissen P. T-cell-based immunotherapy in multiple sclerosis: induction of regulatory immune networks by T-cell vaccination. Expert review of clinical immunology. 2006 Sep; 2(5):705–716. PubMed PMID: 20477626. [PubMed: 20477626]
16. Francis JN, Till SJ, Durham SR. Induction of IL-10+CD4+CD25+ T cells by grass pollen immunotherapy. The Journal of Allergy and Clinical Immunology. 2003 Jun; 111(6):1255–1261. PubMed PMID: 12789226. [PubMed: 12789226]
17. Gardner LM, Thien FC, Douglass JA, Rolland JM, O'Hehir RE. Induction of T 'regulatory' cells by standardized house dust mite immunotherapy: an increase in CD4+ CD25+ interleukin-10+ T cells

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expressing peripheral tissue trafficking markers. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2004 Aug; 34(8):1209–1219. PubMed PMID: 15298560. [PubMed: 15298560]

18. Ma S, Jevnikar AM. Transgenic rice for allergy immunotherapy. Proceedings of the National Academy of Sciences of the United States of America. 2005 Nov 29; 102(48):17255–17256. PubMed PMID: 16301530. Pubmed Central PMCID: 1297701. [PubMed: 16301530]
19. Kim BS, Kim IK, Park YJ, Kim YS, Kim YJ, Chang WS, et al. Conversion of Th2 memory cells into Foxp3+ regulatory T cells suppressing Th2-mediated allergic asthma. Proceedings of the National Academy of Sciences of the United States of America. 2010 May 11; 107(19):8742–8747. PubMed PMID: 20421479. Pubmed Central PMCID: 2889331. [PubMed: 20421479]
20. Jutel M, Akdis CA. Immunological mechanisms of allergen-specific immunotherapy. Allergy. 2011 Jun; 66(6):725–732. PubMed PMID: 21466562. [PubMed: 21466562]
21. Akdis M, Verhagen J, Taylor A, Karamloo F, Karagiannidis C, Crameri R, et al. Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. The Journal of experimental medicine. 2004 Jun 7; 199(11):1567–1575. PubMed PMID: 15173208. Pubmed Central PMCID: 2211782. [PubMed: 15173208]
22. Fujita H, Soyka MB, Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy. Clinical and translational allergy. 2012; 2(1):2. PubMed PMID: 22409879. Pubmed Central PMCID: 3395833. [PubMed: 22409879]
23. Burks W, Bannon G, Lehrer SB. Classic specific immunotherapy and new perspectives in specific immunotherapy for food allergy. Allergy. 2001; 56(Suppl 67):121–124. PubMed PMID: 11298027. [PubMed: 11298027]
24. Umetsu DT. Understanding the immunological basis of asthma; immunotherapy and regulatory T cells. Arbeiten aus dem Paul-Ehrlich-Institut. 2006; (95):211–214. discussion 5–6. PubMed PMID: 17393742.
25. Lal G, Zhang N, van der Touw W, Ding Y, Ju W, Bottinger EP, et al. Epigenetic regulation of Foxp3 expression in regulatory T cells by DNA methylation. Journal of immunology. 2009 Jan 1; 182(1):259–273. PubMed PMID: 19109157.
26. Huehn J, Polansky JK, Hamann A. Epigenetic control of FOXP3 expression: the key to a stable regulatory T-cell lineage? Nature reviews Immunology. 2009 Feb; 9(2):83–89. PubMed PMID: 19114986.
27. Swamy RS, Reshamwala N, Hunter T, Vissamsetti S, Santos CB, Baroody FM, et al. Epigenetic modifications and improved regulatory T-cell function in subjects undergoing dual sublingual immunotherapy. The Journal of Allergy and Clinical Immunology. 2012 Jul; 130(1):215–224. e7. PubMed PMID: 22677046. [PubMed: 22677046]
28. Nouri-Aria KT. Foxp3 expressing regulatory T-cells in allergic disease. Advances in experimental medicine and biology. 2009; 665:180–194. PubMed PMID: 20429425. [PubMed: 20429425]
29. Verginis P, McLaughlin KA, Wucherpfennig KW, von Boehmer H, Apostolou I. Induction of antigen-specific regulatory T cells in wild-type mice: visualization and targets of suppression. Proceedings of the National Academy of Sciences of the United States of America. 2008 Mar 4; 105(9):3479–3484. PubMed PMID: 18299571. Pubmed Central PMCID: 2265184. [PubMed: 18299571]
30. Round JL, Mazmanian SK. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proceedings of the National Academy of Sciences of the United States of America. 2010 Jul 6; 107(27):12204–12209. PubMed PMID: 20566854. Pubmed Central PMCID: 2901479. [PubMed: 20566854]
31. Moon JJ, Dash P, Oguin TH 3rd, McClaren JL, Chu HH, Thomas PG, et al. Quantitative impact of thymic selection on Foxp3+ and Foxp3- subsets of self-peptide/MHC class II-specific CD4+ T cells. Proceedings of the National Academy of Sciences of the United States of America. 2011 Aug 30; 108(35):14602–14607. PubMed PMID: 21873213. Pubmed Central PMCID: 3167500. [PubMed: 21873213]
32. Mayer E, Bannert C, Gruber S, Klunker S, Spittler A, Akdis CA, et al. Cord blood derived CD4+ CD25(high) T cells become functional regulatory T cells upon antigen encounter. PloS one. 2012; 7(1):e29355. PubMed PMID: 22272233. Pubmed Central PMCID: 3260151. [PubMed: 22272233]

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33. Levitsky J, Leventhal JR, Miller J, Huang X, Chen L, Chandrasekaran D, et al. Favorable effects of alemtuzumab on allospecific regulatory T-cell generation. Human immunology. 2012 Feb; 73(2): 141–149. PubMed PMID: 22154724. [PubMed: 22154724]
34. Mittag D, Varese N, Scholzen A, Mansell A, Barker G, Rice G, et al. TLR ligands of ryegrass pollen microbial contaminants enhance Th1 and Th2 responses and decrease induction of Foxp3(hi) regulatory T cells. European journal of immunology. 2013 Mar; 43(3):723–733. PubMed PMID: 23238878. [PubMed: 23238878]
35. Chattopadhyay PK, Yu J, Roederer M. Live-cell assay to detect antigen-specific CD4+ T-cell responses by CD154 expression. Nature protocols. 2006; 1(1):1–6. PubMed PMID: 17406204.
36. Imai A, Sugita S, Kawazoe Y, Horie S, Yamada Y, Keino H, et al. Immunosuppressive properties of regulatory T cells generated by incubation of peripheral blood mononuclear cells with supernatants of human RPE cells. Investigative ophthalmology & visual science. 2012; 53(11): 7299–7309. PubMed PMID: 23036994. [PubMed: 23036994]
37. Ukena SN, Hopting M, Velaga S, Ivanyi P, Grosse J, Baron U, et al. Isolation strategies of regulatory T cells for clinical trials: phenotype, function, stability, and expansion capacity. Experimental hematology. 2011 Dec; 39(12):1152–1160. PubMed PMID: 21864487. [PubMed: 21864487]
38. Gagliani N, Magnani CF, Huber S, Gianolini ME, Pala M, Licona-Limon P, et al. Coexpression of CD49b and LAG-3 identifies human and mouse T regulatory type 1 cells. Nature medicine. 2013 Jun; 19(6):739–746. PubMed PMID: 23624599.
39. Pot C, Apetoh L, Kuchroo VK. Type 1 regulatory T cells (Tr1) in autoimmunity. Seminars in immunology. 2011 Jun; 23(3):202–208. PubMed PMID: 21840222. Pubmed Central PMCID: 3178065. [PubMed: 21840222]
40. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nature immunology. 2003 Apr; 4(4):330–336. PubMed PMID: 12612578. [PubMed: 12612578]
41. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science. 2003 Feb 14; 299(5609):1057–1061. PubMed PMID: 12522256. [PubMed: 12522256]
42. Thornton AM, Korty PE, Tran DQ, Wohlfert EA, Murray PE, Belkaid Y, et al. Expression of Helios, an Ikaros transcription factor family member, differentiates thymic-derived from peripherally induced Foxp3+ T regulatory cells. Journal of Immunology. 2010 Apr 1; 184(7): 3433–3441. PubMed PMID: 20181882.
43. Himmel ME, MacDonald KG, Garcia RV, Steiner TS, Levings MK. Helios+ and Helios- cells coexist within the natural FOXP3+ T regulatory cell subset in humans. The Journal of Immunology. 2013 Mar 1; 190(5):2001–2008. Pubmed PMID: 2335950. [PubMed: 23359504]
44. Sampson HA, Muñoz-Furlong A, Bock SA, Schmitt C, Bass R, Chowdhury BA, Decker WW, Furlong TJ, Galli SJ, Golden DB, Gruchalla RS, Harlor AD Jr, Hepner DL, Howarth M, Kaplan AP, Levy JH, Lewis LM, Lieberman PL, Metcalfe DD, Murphy R, Pollart SM, Pumphrey RS, Rosenwasser LJ, Simons FE, Wood JP, Camargo CA Jr. Symposium on the definition and management of anaphylaxis: summary report. J Allergy Clin Immunol. 2005 Mar; 115(3):584–591. Pub Med PMID: 113972. [PubMed: 15753908]
45. Gernez Y, Tirouvanziam R, Yu G, Ghosn EE, Reshamwala N, Nguyen T, et al. Basophil CD203c levels are increased at baseline and can be used to monitor omalizumab treatment in subjects with nut allergy. International archives of allergy and immunology. 2011; 154(4):318–127. PubMed PMID: 20975283. Pubmed Central PMCID: 3214954. [PubMed: 20975283]
46. Runyon RS, Cachola LM, Rajeshuni N, Hunter T, Garcia M, Ahn R, et al. Asthma discordance in twins is linked to epigenetic modifications of T cells. PloS one. 2012; 7(11):e48796. PubMed PMID: 23226205. Pubmed Central PMCID: 3511472. [PubMed: 23226205]
47. Buhring HJ, Streble A, Valent P. The basophil-specific ectoenzyme E-NPP3 (CD203c) as a marker for cell activation and allergy diagnosis. International archives of allergy and immunology. 2004 Apr; 133(4):317–329. PubMed PMID: 15031605. [PubMed: 15031605]
48. Kleine-Tebbe J, Erdmann S, Knol EF, MacGlashan DW Jr, Poulsen LK, Gibbs BF. Diagnostic tests based on human basophils: potentials, pitfalls and perspectives. International archives of allergy and immunology. 2006; 141(1):79–90. PubMed PMID: 16837789. [PubMed: 16837789]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

49. Miyara M, Yoshioka Y, Kitoh A, Shima T, Wing K, Niwa A, et al. Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor. Immunity. 2009 Jun 19; 30(6):899–911. PubMed PMID: 19464196. [PubMed: 19464196]
50. Schmetterer KG, Neunkirchner A, Pickl WF. Naturally occurring regulatory T cells: markers, mechanisms, and manipulation. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2012 Jun; 26(6):2253–2276. PubMed PMID: 22362896. [PubMed: 22362896]
51. Bilate AM, Lafaille JJ. Induced CD4+Foxp3+ regulatory T cells in immune tolerance. Annual review of immunology. 2012; 30:733–758. PubMed PMID: 22224762.
52. Josefowicz SZ, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. Annual review of immunology. 2012; 30:531–564. PubMed PMID: 22224781.
53. Josefowicz SZ, Niec RE, Kim HY, Treuting P, Chinen T, Zheng Y, et al. Extrathymically generated regulatory T cells control mucosal TH2 inflammation. Nature. 2012 Feb 16; 482(7385): 395–399. PubMed PMID: 22318520. Pubmed Central PMCID: 3485072. [PubMed: 22318520]
54. Sugimoto N, Oida T, Hirota K, Nakamura K, Nomura T, Uchiyama T, et al. Foxp3-dependent and -independent molecules specific for CD25+CD4+ natural regulatory T cells revealed by DNA microarray analysis. International immunology. 2006 Aug; 18(8):1197–1209. PubMed PMID: 16772372. [PubMed: 16772372]
55. Garcia G, Godot V, Humbert M. New chemokine targets for asthma therapy. Current allergy and asthma reports. 2005 Mar; 5(2):155–160. PubMed PMID: 15683617. [PubMed: 15683617]
56. Garcia G, Humbert M, Capel F, Rimaniol AC, Escourrou P, Emilie D, et al. Chemokine receptor expression on allergen-specific T cells in asthma and allergic bronchopulmonary aspergillosis. Allergy. 2007 Feb; 62(2):170–177. PubMed PMID: 17298426. [PubMed: 17298426]
57. Gonzalo JA, Qiu Y, Lora JM, Al-Garawi A, Villeval JL, Boyce JA, et al. Coordinated involvement of mast cells and T cells in allergic mucosal inflammation: critical role of the CC chemokine ligand 1:CCR8 axis. Journal of immunology. 2007 Aug 1; 179(3):1740–1750. PubMed PMID: 17641040.
58. Lloyd CM, Rankin SM. Chemokines in allergic airway disease. Current opinion in pharmacology. 2003 Aug; 3(4):443–448. PubMed PMID: 12901955. Pubmed Central PMCID: 3428843. [PubMed: 12901955]
59. Ahern D, Lloyd CM, Robinson DS. Chemokine responsiveness of CD4+ CD25+ regulatory and CD4+ CD25- T cells from atopic and nonatopic donors. Allergy. 2009 Aug; 64(8):1121–1129. PubMed PMID: 19208087. [PubMed: 19208087]
60. Ozdemir C, Kucuksezer UC, Akdis M, Akdis CA. Specific immunotherapy and turning off the T cell: how does it work? Ann Allergy Asthma Immunol. 2011 Nov; 107(5):381–392. PubMed PMID: 22018608. [PubMed: 22018608]
61. Karlsson MR, Rugtveit J, Brandtzaeg P. Allergen-responsive CD4+CD25+ regulatory T cells in children who have outgrown cow's milk allergy. The Journal of experimental medicine. 2004 Jun 21; 199(12):1679–1688. PubMed PMID: 15197226. Pubmed Central PMCID: 2212808. [PubMed: 15197226]
62. Bedoret D, Singh AK, Shaw V, Hoyte EG, Hamilton R, DeKruyff RH, et al. Changes in antigen-specific T-cell number and function during oral desensitization in cow's milk allergy enabled with omalizumab. Mucosal Immunol. 2012 May; 5(3):267–276. PubMed PMID: 22318492. Pubmed Central PMCID: 3328586. [PubMed: 22318492]
63. Nadeau KC, Schneider LC, Hoyte L, Borras I, Umetsu DT. Rapid oral desensitization in combination with omalizumab therapy in patients with cow's milk allergy. The Journal of Allergy and Clinical Immunology. 2011 Jun; 127(6):1622–1624. PubMed PMID: 21546071. Pubmed Central PMCID: 3396422. [PubMed: 21546071]
64. Varshney P, Jones SM, Scurlock AM, Perry TT, Kemper A, Steele P, et al. A randomized controlled study of peanut oral immunotherapy: clinical desensitization and modulation of the allergic response. The Journal of Allergy and Clinical Immunology. 2011 Mar; 127(3):654–660. PubMed PMID: 21377034. Pubmed Central PMCID: 3060783. [PubMed: 21377034]

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65. Blumchen K, Ulbricht H, Staden U, Dobberstein K, Beschorner J, de Oliveira LC, et al. Oral peanut immunotherapy in children with peanut anaphylaxis. The Journal of Allergy and Clinical Immunology. 2010 Jul; 126(1):83–91. e1. PubMed PMID: 20542324. [PubMed: 20542324]
66. Skripak JM, Nash SD, Rowley H, Brereton NH, Oh S, Hamilton RG, et al. A randomized, double-blind, placebo-controlled study of milk oral immunotherapy for cow's milk allergy. The Journal of Allergy and Clinical Immunology. 2008 Dec; 122(6):1154–1160. PubMed PMID: 18951617. [PubMed: 18951617]
67. Ohkura N, Hamaguchi M, Morikawa H, Sugimura K, Tanaka A, Ito Y, et al. T Cell Receptor Stimulation-Induced Epigenetic Changes and Foxp3 Expression Are Independent and Complementary Events Required for Treg Cell Development. Immunity. 2012 Nov 16; 37(5):785–799. PubMed PMID: 23123060. [PubMed: 23123060]
68. Sakaguchi S, Vignali DA, Rudensky AY, Niec RE, Waldmann H. The plasticity and stability of regulatory T cells. Nature Reviews Immunology. 2013 Jun; 13(6):461–467. PubMed PMID: 23681097.
69. Janson PC, Winerdal ME, Marits P, Thörn M, Ohlsson R, Winqvist O. FOXP3 promoter demethylation reveals the committed Treg population in humans. PLoS One. 2008 Feb 20.3(2):e1612. PubMed PMID: 18286169. [PubMed: 18286169]
70. Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. Nature reviews Immunology. 2004 Oct; 4(10):762–774. PubMed PMID: 15459668.
71. Hwang SL, Chung NP, Chan JK, Lin CL. Indoleamine 2, 3-dioxygenase (IDO) is essential for dendritic cell activation and chemotactic responsiveness to chemokines. Cell research. 2005 Mar; 15(3):167–175. PubMed PMID: 15780178. [PubMed: 15780178]
72. Burks AW, Jones SM, Wood RA, Fleischer DM, Sicherer SH, Lindblad RW, et al. Oral immunotherapy for treatment of egg allergy in children. N Engl J Med. 2012 Jul 19; 367(3):233–243. PubMed PMID: 22808958. Pubmed Central PMCID: 3424505. [PubMed: 22808958]
73. Sampson HA. Peanut Oral Immunotherapy: Is It Ready for Clinical Practice? Journal of Allergy and Clinical Immunology: In Practice. 2013; 1(1):15–21.
74. Keet C, Seopaul S, Knorr SD, Narisety S, Skripak JM, Wood RA. Long-Term Outcomes of Milk Oral Immunotherapy in Children. The Journal of Allergy and Clinical Immunology. 2013; 131(2):AB130.
75. Palomares O, Ruckert B, Jartti T, Kucuksezer UC, Puhakka T, Gomez E, et al. Induction and maintenance of allergen-specific FOXP3+ Treg cells in human tonsils as potential first-line organs of oral tolerance. The Journal of Allergy and Clinical Immunology. 2012 Feb; 129(2):510–520. 20 e1–9. PubMed PMID: 22051696. [PubMed: 22051696]
76. Jutel M, Akdis M, Budak F, Aebischer-Casaulta C, Wrzyszcz M, Blaser K, et al. IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. European journal of immunology. 2003 May; 33(5):1205–1214. PubMed PMID: 12731045. [PubMed: 12731045]
77. Akdis M, Blaser K, Akdis CA. T regulatory cells in allergy: novel concepts in the pathogenesis, prevention, and treatment of allergic diseases. The Journal of Allergy and Clinical Immunology. 2005 Nov; 116(5):961–968. quiz 9. PubMed PMID: 16275361. [PubMed: 16275361]
78. Custovic A, Simpson BM, Simpson A, Hallam CL, Marolia H, Walsh D, et al. Current mite, cat, and dog allergen exposure, pet ownership, and sensitization to inhalant allergens in adults. The Journal of Allergy and Clinical Immunology. 2003 Feb; 111(2):402–407. PubMed PMID: 12589363. [PubMed: 12589363]
79. Samoilova EB, Horton JL, Zhang H, Khoury SJ, Weiner HL, Chen Y. CTLA-4 is required for the induction of high dose oral tolerance. International immunology. 1998 Apr; 10(4):491–498. PubMed PMID: 9620605. [PubMed: 9620605]
80. Sampson HA, Leung DY, Burks AW, Lack G, Bahna SL, Jones SM, et al. A phase II, randomized, doubleblind, parallelgroup, placebocontrolled oral food challenge trial of Xolair (omalizumab) in peanut allergy. The Journal of Allergy and Clinical Immunology. 2011 May; 127(5):1309–1310. e1. PubMed PMID: 21397314. [PubMed: 21397314]
81. Kim EH, Burks W. Managing food allergy in childhood. Current opinion in pediatrics. 2012 Oct; 24(5):615–620. PubMed PMID: 22935752. [PubMed: 22935752]

82. Nguyen KD, Vanichsarn C, Fohner A, Nadeau KC. Selective deregulation in chemokine signaling pathways of CD4+CD25(hi)CD127(lo)/(−) regulatory T cells in human allergic asthma. The Journal of Allergy and Clinical Immunology. 2009 Apr; 123(4):933–939. e10. PubMed PMID: 19152963. [PubMed: 19152963]

83. Soler D, Chapman TR, Poisson LR, Wang L, Cote-Sierra J, Ryan M, McDonald A, Badola S, Fedyk E, Coyle AJ, Hodge MR, Kolbeck R. CCR8 expression identifies CD4 memory T cells enriched for FOXP3+ regulatory and Th2 effector lymphocytes. J. of Immunology. 2007; 177:6940–6951. PubMed PMID: 17082609. [PubMed: 17082609]

84. Garcia, M.; Kohli, A.; Nadeau, K. Tolerogenic Dendritic Cells in Food Allergy and Oral Immunotherapy; Proceedings of the 29th Meeting of the Collegium Internationale Allergologicum.; In Press

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KEY MESSAGES

- Mechanisms of “immune tolerance” during OIT potentially involve induction of antigen-induced Treg.
- Epigenetic changes in the Foxp3 locus may enhance Treg function in this setting.
- Modifications at the DNA level in specific T-cell subsets may be predictive of unresponsiveness during OIT.

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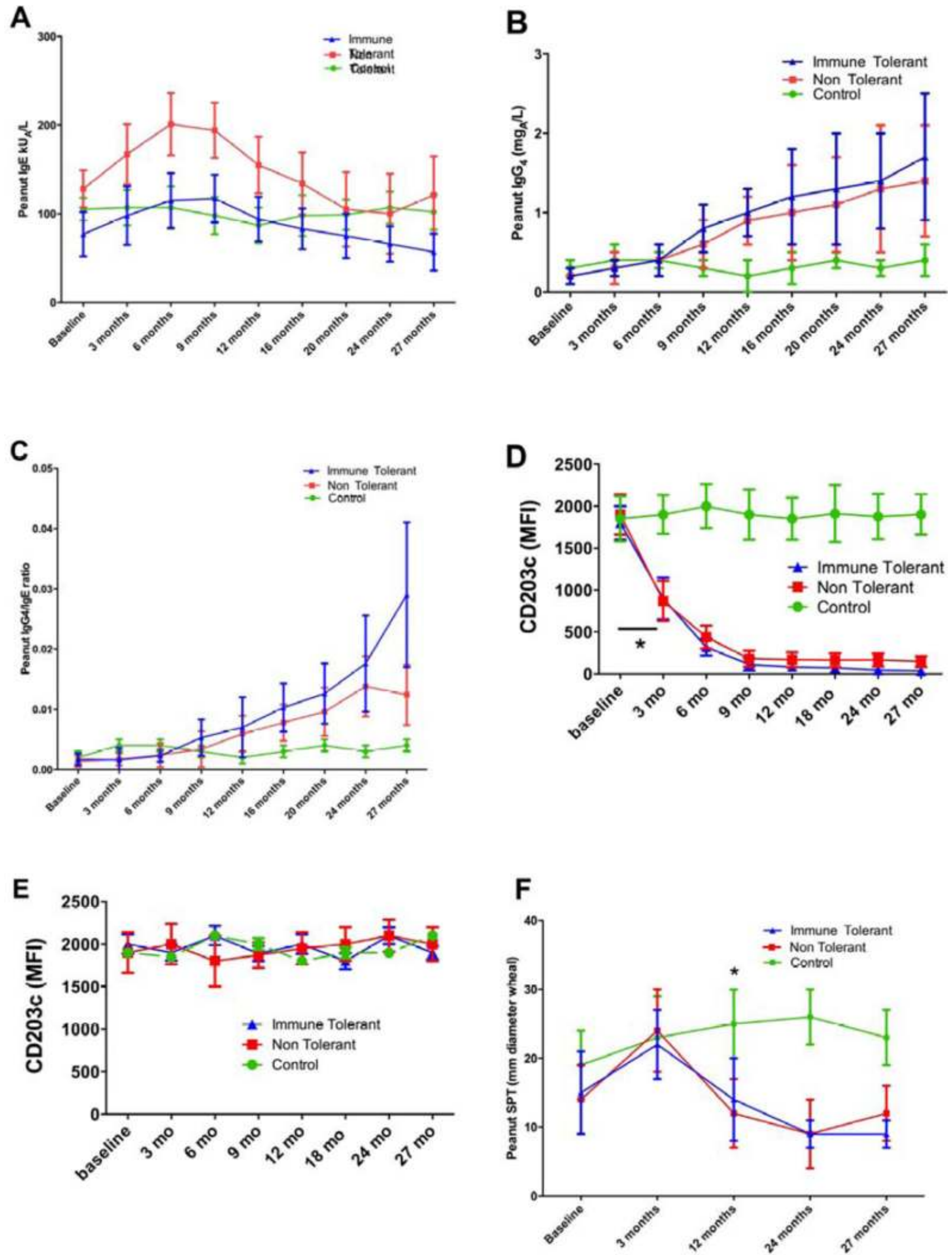

Fig. 1.

OIT-treated participants (squares, n=13, NT or triangles, n=7, IT) or peanut-allergic control participants (circles, n=20). A) Changes in mean peanut-specific IgE levels (kU_A/L +/−SD) among IT (N=7), NT (N=13) and Control (N=20) subjects (NS, P=0.17) B) Change from baseline in mean antibody levels (+/−SD) for peanut-specific IgG4 (mg_A/L). Slight increase in IT and NT compared to Control (NS, P=0.24). C) Mean (+/−SD) of peanut-specific IgG4:IgE ratios. Slight increase in IT and NT compared to Control (NS, P=0.27) D) Expression of CD203c levels on basophils stimulated with peanut allergen (lug/ml). Data are

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presented as mean ± SEM (*P < 0.001, CI: −1222 to −777.8) IT or NT vs. Control. IT vs. NT (NS, P> 0.99) Mg = milligrams of antigen-specific antibody; MFI=mean fluorescence intensity. E) Expression of CD203c levels on basophils stimulated with anti-IgE (NS, P >0.999). F) Significant decrease in SPT diameter starting at 12mo for IT or NT compared to Control (*P<0.001, CI: −16.06 to −5.942). No difference in NT vs. IT (NS, P=0.656).

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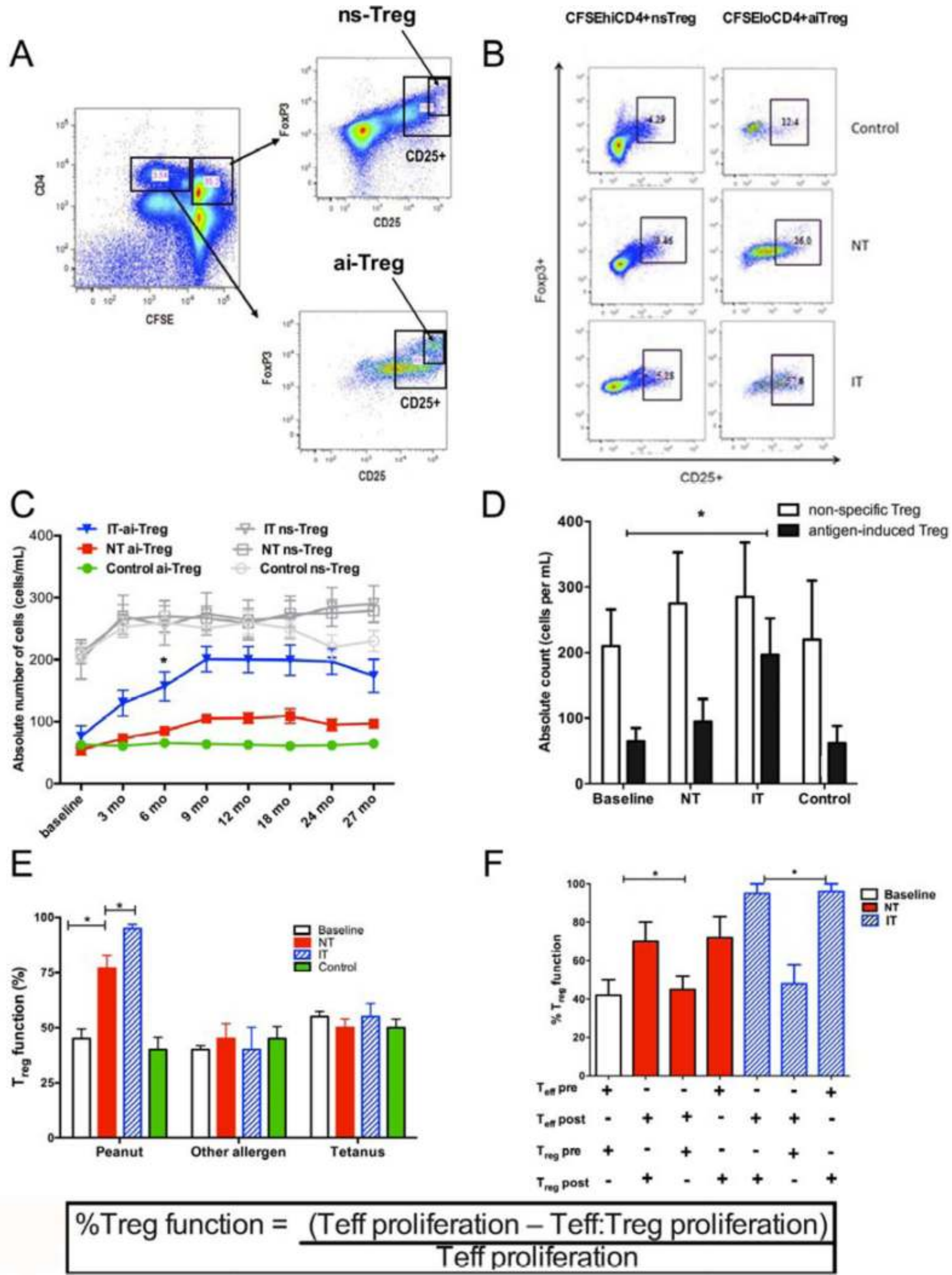

Fig. 2.

A) Representative staining of ai-Treg and ns-Treg. B) Representative staining for ns-Treg and ai-Treg in Control, NT and IT participants at 30mo C) ns-Treg (open shapes) and ai-Treg (closed shapes) absolute counts (*P< 0.002, CI: 2.636 to 141.1,NTN=7, ITN=13, ControlN=20). D) ns-Treg (white bars) and ai-Treg (black bars) at baseline and 24mo of treatment (*P< 0.0001, CI: 41.1 to 162.6, NT N=7, ITN=13, Control N=20). Changes in ns-Treg not significant (P=0.84). E) Treg suppressive activity on conventional responder CD4+ T cells (Teff) measured pre-therapy (baseline) and at 27mo—i.e., after 3mo off treatment

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(filled bars). Suppressed proliferation of Teff in response to peanut stimulation in NT and IT compared to baseline (*P=0.0001, CI: 30.18 to 69.82) but not other allergens or tetanus (P>0.999). F) Suppressive function of Treg from pre and post-OIT. Suppressive function of Treg collected pre-therapy (pre) and at month 27 (post) was assessed towards ai-Teff collected pre- and post-therapy (data represent mean + SEM; *P < 0.001, CI: −32.03 to −17.97).

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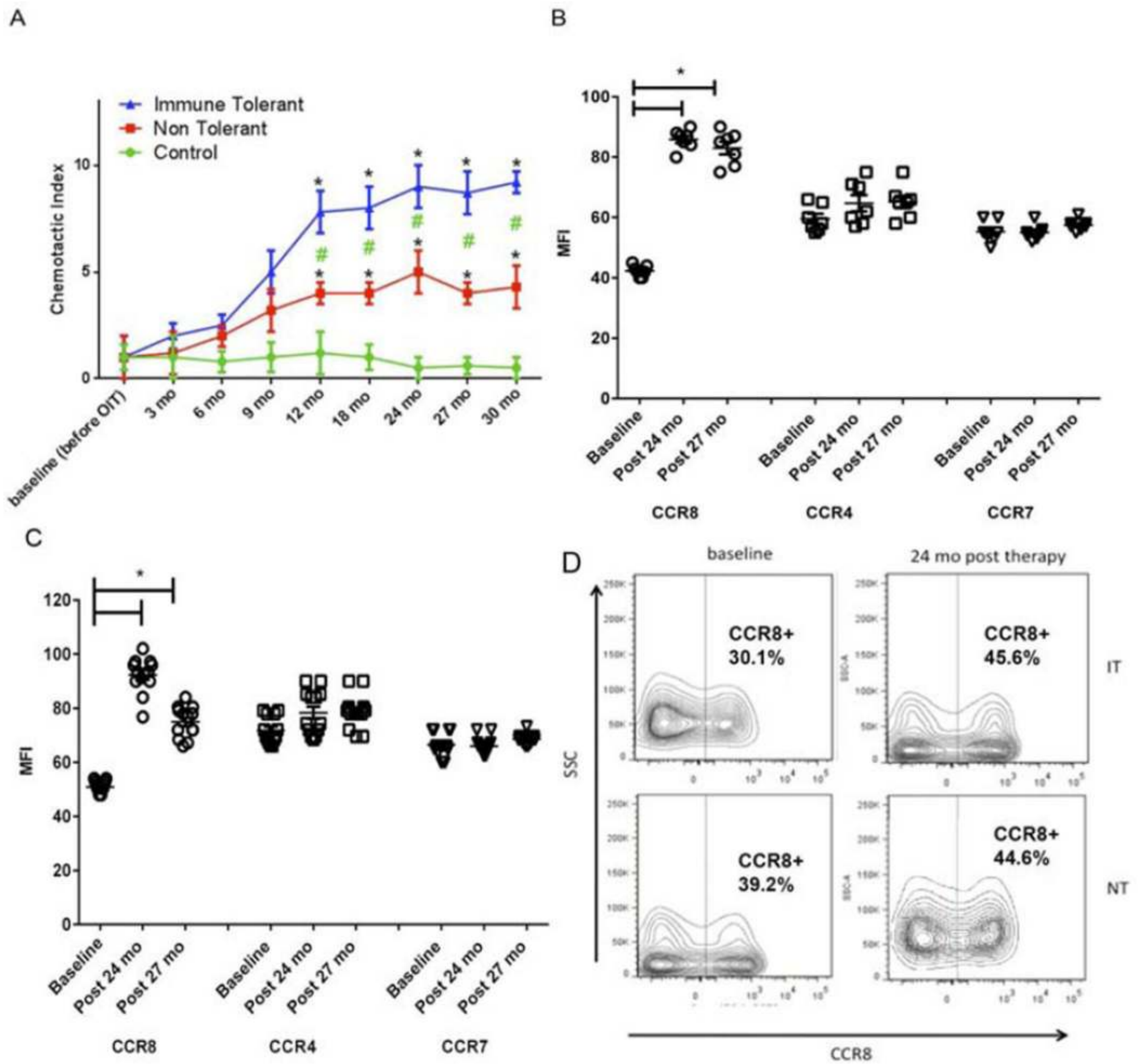

Fig. 3.

A) Chemotactic Indices of ai-Treg toward normal IECs. Indices for cells from immune tolerant (n=7) or non-tolerant (n=13) subjects were significantly higher than those for control (n=20) participants starting at 12mo (*P< 0.001, CI: 5.854 to 7.346). Values for IT subjects were significantly higher than those for NT subjects starting at 12mo (#P<0.0001, CI: 3.004 to 4.596). B and C) Expression levels of chemokine receptors (circles, CCR8; squares, CCR4; triangles CCR7) on ai-Treg populations were identified by flow cytometry and are presented as MFI (xlO) at baseline and at 24mo of treatment and at 27mo (i.e., 3mo post cessation of treatment) in IT patients (Fig. 3B) (*P < 0.001, CI: 38.64 to 48.50) and NT patients (Fig. 3C) (*P<0.001, CI: 34.69 to 48.01). An example of chemokine receptor staining is shown in Fig. 3D.

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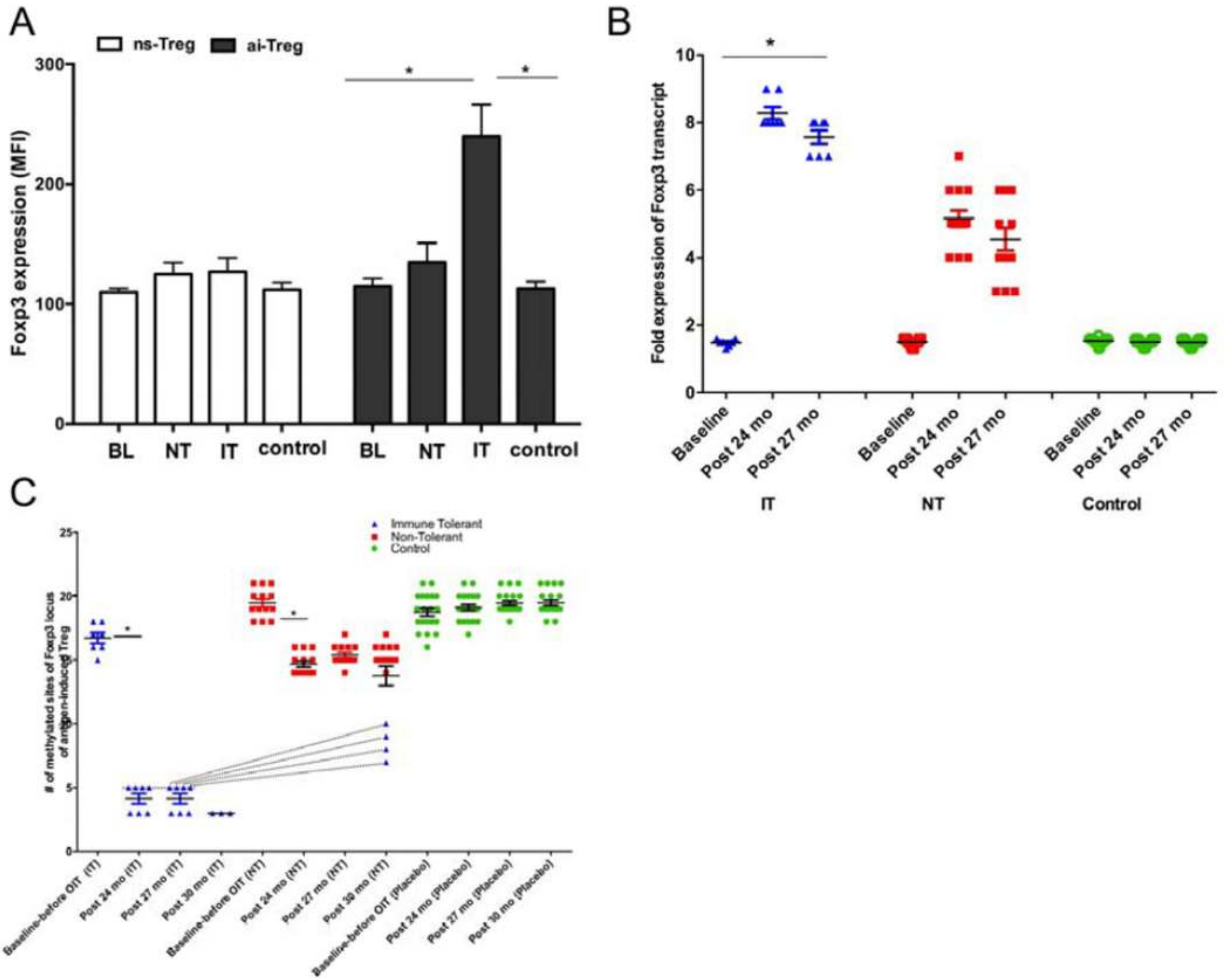

Fig. 4.

(A) Intracellular Foxp3 protein expression levels in ns-Treg (white bars) and ai-Treg (black bars) Values are mean fluorescence intensity (MFI). Significant differences in ai-Treg for IT vs. NT or control (*P <0.001, CI: 90.34 to 159.7), not significant for ns-Treg (P =0.1698). B) *FOXP3* mRNA in Treg isolated from IT OIT participants (triangles), NT OIT participants (squares) and peanut-allergic control participants (circles). Significant differences in IT participants at 24 or 27mo compared to baseline (*P<0.001, CI: 2.871 to 4.437). Difference for NT participants non-significant (P =0.18). C) ai-Treg from participants undergoing OIT (IT, n=7, blue triangles; NT, n=13, red squares) or untreated controls (n=20, green circles). 4/7 IT participants (connected via broken lines) were no longer “tolerant” at 30mo. Data represent mean number of methylated sites + SEM; (IT: * P < 0.001 vs. baseline CI: 12.13 to 14.16, NT: *P < 0.001 vs. baseline CI: 3.919 to 5.620). Not significant for control (P= 0.15). MFI= mean fluorescence intensity.

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Table I

Enhancement of allergen-specific T cell populations during OIT.

Allergen-specific T cell populations[A]		% Treg Mean ±SD	%IL-10+	%Teff	%Th2	%Th1
Peanut+ memory	Pre	3.1 ± 1.3**	18.9 ± 4.0 **	24.6 ± 3.5	57.0 ± 6.8 **	12.3 ± 5.7*
	Post	16.4 ± 3.5	52.9 ± 7.8	23.7 ± 3.9	19.4 ± 7.9	22.6 ± 1.7
Peanut+ naïve	Pre	0.2 ± 0.4**	11.7 ± 3.1	2.6 ± 1.9	17.0 ± 3.7*	8.2 ± 1.8 *
	Post	1.9 ± 0.9	12.3 ± 4.1	3.9 ± 1.3	10.9 ± 2.8	12.6 ± 2.8
OOA+ memory	Pre	4.3 ±1.5	16.6 ± 3.2	27.0 ± 6.3	60.6 ± 3.5	10.8 ± 2.1
	Post	4.6 ± 1.3	17.9 ± 4.8	30.0 ± 5.4	60.7 ± 4.7	14.9 ± 3.4
OOA+ naïve	Pre	0.0	18.3 ± 2.9	3.9 ± 1.6	13.0 ± 4.0	7.9 ± 2.0
	Post	1.4 ± 0.5	18.7 ± 1.8	3.6 ± 1.5	11.7 ± 3.6	8.6 ± 1.3
Peanut+ memory (control)	Pre	3.1 ± 1.6	14.7 ± 1.7	27.4 ± 4.7	60.3 ± 5.2	11.3 ± 2.6
	Post	5.0 ± 1.3	19.4 ± 2.9	26.1 ± 3.8	61.1 ± 3.9	11.4 ± 2.6

[A]Percentages shown are % of CD4+ T cells, mean ±SD.

Significant differences (*P < 0.025, CI: 4.993 to 15.61, ** P < 0.0001 CI: 23.72 to 30.28) between the cell populations pre (n=7) and post (n=7) therapy.

Abbreviations: SD; standard deviation, OOA; other offending allergen, Pre; baseline values prior to starting OIT, Post; 12 months after starting OIT.