IL-10-modulated dendritic cells from birch pollen- and hazelnut-allergic patients facilitate Treg-mediated allergen-specific and cross-reactive tolerance

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Abstract

Background Approximately 70 % of individuals allergic to birch pollen (Bet v 1.01 [Bet]) develop a secondary food allergy (e.g. hazelnut: Cor a 1.04 [Cor]), due to allergen cross-reactivity. However, standard immunotherapy for type I allergies often does not improve the food allergy sufficiently. We analyzed the allergen-specific and cross-reactive suppressive capacity of primary human regulatory T cells (Treg) induced by autologous IL-10-modulated dendritic cells (IL-10 DC) *in vitro* and *in vivo*. **Methods** CD4 $^{+}$ T cells of patients with birch pollen and associated hazelnut allergies were differentiated into Bet-specific or non-specific induced Treg (iTreg). After Bet- or Cor- specific restimulation the phenotype, proliferation and suppressive capacity of iTreg subsets were analyzed. iTreg function was further investigated in humanized mouse models of airway and intestinal allergy, generated by engraftment of peripheral blood mononuclear cells from allergic donors into immunodeficient animals. **Results** After IL-10 DC priming and allergen-specific restimulation (Bet or Cor) non-specific control iTreg remained anergic, whereas Bet-specific iTreg proliferated extensively and exhibited a regulatory phenotype (enhanced expression of CTLA-4, PD-1, TNFR2, IL-10). Accordingly, activated Bet-specific iTreg displayed a high capacity to suppress Bet- and Cor-induced responder T_H2 cell responses *in vitro*, indicating induction of both allergen-specific (birch) and cross-reactive tolerance (hazelnut). *In vivo*, the beneficial effect of Bet-specific iTreg was verified in humanized mouse models of allergic airway and intestinal inflammation, resulting in reduced allergen-induced clinical symptoms and immune responses. **Conclusion** Human IL-10 DC-induced iTreg facilitate allergen-specific and cross-reactive tolerance. Therefore, they are potential candidates for regulatory cell therapy in allergic and autoimmune diseases.

Title Page

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Short title: Treg induced allergen specific- and cross-tolerance

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Abstract

Background

Approximately 70 % of individuals allergic to birch pollen (Bet v 1.01 [Bet]) develop a secondary food allergy (e.g. hazelnut: Cor a 1.04 [Cor]), due to allergen cross-reactivity. However, standard immunotherapy for type I allergies often does not improve the food allergy sufficiently. We analyzed the allergen-specific and cross-reactive suppressive capacity of primary human regulatory T cells (Treg) induced by autologous IL-10-modulated dendritic cells (IL-10 DC)*in vitro* and *in vivo* .

Methods

$CD4^+$ T cells of patients with birch pollen and associated hazelnut allergies were differentiated into Bet-specific or non-specific induced Treg (iTreg). After Bet- or Cor- specific restimulation the phenotype, proliferation and suppressive capacity of iTreg subsets were analyzed. iTreg function was further investigated in humanized mouse models of airway and intestinal allergy, generated by engraftment of peripheral blood mononuclear cells from allergic donors into immunodeficient animals.

Results

After IL-10 DC priming and allergen-specific restimulation (Bet or Cor) non-specific control iTreg remained anergic, whereas Bet-specific iTreg proliferated extensively and exhibited a regulatory phenotype (enhanced expression of CTLA-4, PD-1, TNFR2, IL-10). Accordingly, activated Bet-specific iTreg displayed a high capacity to suppress Bet- and Cor-induced responder T_H2 cell responses *in vitro* , indicating induction of both allergen-specific (birch) and cross-reactive tolerance (hazelnut). *In vivo* , the beneficial effect of Bet-specific iTreg was verified in humanized mouse models of allergic airway and intestinal inflammation, resulting in reduced allergen-induced clinical symptoms and immune responses.

Conclusion

Human IL-10 DC-induced iTreg facilitate allergen-specific and cross-reactive tolerance. Therefore, they are potential candidates for regulatory cell therapy in allergic and autoimmune diseases.

Key words

- type I allergy
- Bet v 1.01
- tolerogenic IL-10 DC
- regulatory T cells
- cross-reactive tolerance

Abbreviations

[^{3}H]TdR: tritiated thymidine, AIT: allergen-specific immunotherapy, Bet: Bet v 1.01, CFSE: Vybrant® CFDA SE Cell Tracer Kit, Cor: Cor a 1.04, ctrl: positive control, eFluor670: Cell Proliferation Dye eFluor 670, IL-10 DC: IL-10-modulated dendritic cells, IL-10 DC_0: unloaded IL-10 DC, IL-10 DC_{Bet}: Bet-loaded IL-10 DC, iTreg: induced Treg, $iTreg_0$: IL-10 DC_0 induced iTreg, $iTreg_{Bet}$: IL-10 DC_{Bet}-induced iTreg, MCh: methacholine, mDC: mature dendritic cells, mDC_0: unloaded mDC, mDC_{Bet}: Bet-loaded mDC, PBMC: peripheral blood mononuclear cells, PFA: pollen-associated food allergy, r: recombinant, SA: suppressor assay, SD: standard deviation, SI: stimulation index, Teff: effector T cells, $Teff_0$: mDC_0 induced Teff, $Teff_{Bet}$: mDC_{Bet}-induced Teff, T_H2: T helper type 2, tolDC: tolerogenic dendritic cells, Treg: regulatory T cells, Tresp: responder T cells.

Text

Introduction

Allergic diseases are becoming the focus of attention, as their incidences as well as severity are increasing worldwide.[1–3] Among the responsible allergens, birch pollen were identified as the third most-diagnosed allergen for respiratory allergy,[4] and around 70% of patients allergic to birch also develop a secondary food allergy.[5–7] The symptoms of this so-called pollen-associated food allergy (PFA) range from nasal, ocular and oral pruritus and angioedema to severe allergic asthma and anaphylaxis,[6,8–10] which has a comprehensive impact on personal quality of life, work or school performance and the socio-economic burden.[4,11]

In the pathogenesis of PFA, patients are first sensitized towards a pollen allergen, such as Bet v 1 (Bet), a birch pollen allergen belonging to the pathogenesis-related (PR)-10 protein family.[12,13] PR-10 proteins share a high degree of amino acid sequence identity as well as structural homology and facilitate cross-reactivity of pollen-specific IgE antibodies and T cells with food allergens, which initiates the development of secondary food allergies.[14–17] PR-10 proteins are found in different plant pollen and in edible plant parts like hazelnut (Cor a 1, Cor), apple (Mal d 1) and carrot (Dau c 1).[7,18]Interestingly, the hazelnut allergen isoform Cor a 1.04 is more similiar to the birch pollen isoallergen Bet v 1.0101 than to the hazel pollen isoallergen Cor a 1.01.[19]

In contrast to the ongoing spread of allergic diseases due to cross-reactivities, the selection of efficient and long-lasting therapies that improve pollen and cross-reactive food allergies together are sparse. The only disease-modifying therapy available is allergen-specific immunotherapy (AIT), which might achieve a 20-50% reduction of combined symptom and medication scores.[20–22] But several years of therapy are needed to maintain the therapeutic success[23,24] and placebo-controlled studies showed that pollen AIT have little to no clinical effect on the associated food allergy.[25–28]Therefore, the current state of the art regarding treatment of PFA remains unsatisfying and novel therapeutic strategies have to be explored.

Autologous tolerogenic dendritic cells (tolDC) are promising candidates for cell-based immunomodulatory therapies, as they can be differentiated from peripheral blood cells of patients *ex vivo* based on standardized protocols.[29–31] After loading with the desired antigen, they are reintroduced into the patients, where they are generally well tolerated without any severe side effects.[32–34] In our group, we found that Interleukin (IL) 10-modulated DC (IL-10 DC) are exceptionally suited for tolerance-inducing therapies, as they displayed a stable, tolerogenic phenotype, with strong migratory and suppressive functions.[30,35–37] Induced Treg (iTreg) primed by these IL-10 DC exhibited a suppressive phenotype resulting in highly efficient antigen-specific regulatory functions.[38,39]In this study, we combined our expertise in IL-10 DC and iTreg biology demonstrating the capacity of IL-10 DC as inducers of iTreg which facilitated allergen-specific and cross-reactive tolerance in birch pollen allergic patients with associated hazelnut allergy *in vitro* and in a humanized mouse model of allergy *in vivo* .

Methods

All experiments with human samples were conducted according to approval of the local ethics committee of Rhineland Palatinate (authorization no. 837.303.13 and 837.054.17 (10888)) and written informed consent was obtained from all participants. Murine experiments were in accordance with current federal, state, and

institutional guidelines (authorisation no. 23 177-07/G 18-1-015).

Methods are described in brief, for further details see Supplementary Methods.

Induction of iTreg and effector T cells (Teff)

Immature dendritic cells (DC) from donors with birch pollen and hazelnut allergies were generated by culture of plastic-adherent peripheral blood mononuclear cells (PBMC) with IL-4 and GM-CSF for 6 days and were then differentiated into mature DC (mDC) by addition of a maturation cocktail or into IL-10 DC by addition of the maturation cocktail and IL-10 (Supplementary Figure 1). Bet-loaded mDC (mDCBet) or IL-10 DC (IL-10 DCBet) were generated by addition of rBet v 1.0101 during DC differentiation. Autologous $CD4^+$ T cells were cocultured with mDC or IL-10 DC to prime effector T cells (Teff) or iTreg, respectively.

Suppressor assay (SA)

$CD4^+CD25^{low}$ responder T cells (Tresp) from donors with birch pollen and associated hazelnut allergy were stimulated with autologous Bet- or Cor- loaded mDC (control, ctrl). iTreg primed by IL-10 DC_{Bet} ($iTreg_{Bet}$) or IL-10 DC0 ($iTreg_0$) were added in 1:1 or 1:2 Tresp:iTreg ratios (SA 1:1 / SA 1:2), respectively. Tresp, mDC and iTreg were stained with different cell proliferation dyes for identification and assessment of T cell proliferation.

Humanized mouse model of type I allergy

As described previously[40,41], NOD.CB17-Prkdc^{scid}/Jγc$^{-/-}$ mice were engrafted with PBMC from donors with birch pollen and associated hazelnut allergy and were simultaneously injected with birch pollen extract (=PBMC + birch, allergic positive control) or without (=PBMC, negative control). $iTreg_{Bet}$ were co-injected at day 0 into animals treated like the allergic positive control. After three weeks, blood samples for human IgE analysis were taken, the mice were challenged rectally with birch pollen or hazelnut extract and the allergen-induced intestinal inflammation was scored endoscopically. On the next day the mice were challenged intranasally with birch pollen extract and allergen-induced airway inflammation was evaluated by measurement of methacholine (MCh)-provoked airway resistance.

Results

Induction of Bet-specific iTreg from birch-pollen allergic patients with associated hazelnut allergy by autologous IL-10 DC

As previously demonstrated by us, human IL-10 DC induce iTreg with a high capacity to suppress T cell responses in an antigen-specific manner.[35,38,42,43] In this study, we wanted to investigate whether these tolerogenic IL-10 DC are able to induce an allergen-specific as well as cross-reactive tolerance in patients suffering from a pollen (birch, Bet v 1) and associated food (hazelnut, Cor a 1) allergy (for patients´ details see Supplementary Table 1). For this purpose, we used our previously established *in vitro* model with human DC to analyze allergen-specific and cross-reactive T cell responses.[12]

IL-10 DC obtained from birch pollen allergic patients with associated hazelnut allergy were loaded with Bet or left unloaded (IL-10 DC_{Bet}/IL-10 DC_0) and cocultured with autologous $CD4^+$ T cells to induce Bet-specific or control iTreg, respectively ($iTreg_{Bet}$/$iTreg_0$). In addition, mDC (mDC_{Bet}/mDC_0) were used to generate Bet-specific and non-specific Teff ($Teff_{Bet}$/$Teff_0$) as controls (see Methods, Supplementary Methods and Supplementary Figure 1).

After primary culture, $Teff_{Bet}$ exhibited a significantly increased proliferative capacity compared to $Teff_0$, demonstrating an allergen (Bet)-specific T cell response (Figure 1A). In contrast, stimulation with IL-10 DC_0 or IL-10DC_{Bet} resulted in a significantly reduced T cell proliferation of both $iTreg_0$ and $iTreg_{Bet}$.

In line with the data of allergen-specific T cell proliferation, analysis of cytokine production after primary culture revealed significantly increased levels of T_H2 cytokines (IL-5, IL-9 and IL-13) and of IL-2 (T cell activation) in supernatants of mDC_{Bet} activated $Teff_{Bet}$ compared to control $Teff_0$, demonstrating highly stimulated Bet-specific Teff (Figure 1B-E). In contrast, supernatants of iTreg primed by IL-10 DC_0 or IL-10

DC_{Bet}, respectively, exhibited reduced amounts of T_H2 cytokines in comparison to Bet-specific control Teff, confirming the diminished activity of iTreg after primary culture. However, we observed very high IL-10 concentrations produced by $iTreg_{Bet}$ compared to control $Teff_{Bet}/Teff_0$ as well as to $iTreg_0$, suggesting the immunosuppressive cytokine IL-10 as mediator of allergen-specific iTreg suppressor function (Figure 1F).

Bet-specific iTreg induced by IL-10 DC exhibited an activated phenotype after Bet-and Cor-specific restimulation

Several studies revealed an anergic phenotype of $CD4^+CD25^+FOXP3^+$natural Treg and iTreg, in particular of iTreg induced by IL-10 DC.[35,38,42,44] In order to prove the allergen-specific and cross-reactive induction of T cell anergy in our system, we have performed restimulation experiments with autologous mDC_0, mDC_{Bet} or mDC_{Cor}, respectively (see Supplementary Methods). Compared to control T cells ($Teff_0$), restimulation of $Teff_{Bet}$ with mDC_{Bet} led to a significantly increased and in the case of mDC_{Cor} slightly enhanced Teff proliferation, demonstrating the induction of both a Bet-specific and cross-reactive, Cor-specific T cell response (Figure 2A). However, also restimulation of $iTreg_{Bet}$ with mDC_{Bet} or mDC_{Cor} resulted in a very pronounced and significantly enhanced T cell proliferation, compared to $Teff_0$. $iTreg_{Bet}$ proliferated even stronger compared to $Teff_{Bet,}$ indicating that allergen-specific iTreg did not display an anergic but activated phenotype after specific (Bet) or cross (Cor)-specific restimulation (Figure 2A). In contrast to $iTreg_{Bet}$, $iTreg_0$ exhibited a significantly lower proliferation and remained anergic regardless of the allergen-specific or cross-specific restimulation with mDC_{Bet} or mDC_{Cor} (Figure 2B).

Profound capacity of Bet-specific iTreg to suppress allergen-specific and cross-reactive T cell responses *in vitro*

In order to analyze the function of iTreg with regard to their suppressive capacity on allergen-specific and cross-reactive responses, we investigated the suppressive capacity of $iTreg_{Bet}$ and $iTreg_0$ on Bet- or Cor-stimulated responder T cells (Tresp) obtained from birch-pollen allergic patients with associated hazelnut allergy in a flow cytometry-based suppressor assay.[35] (see Methods and Supplementary Methods; gating strategy in Supplementary Figure 2).

Coculture with $iTreg_{Bet}$ resulted in a significantly reduced proliferation of allergen (Bet) -specific, and cross-reactive, Cor- specific Tresp, respectively, indicating the induction of allergen-specific (birch) and cross-reactive (hazelnut) tolerance (Figure 3A). Further analysis revealed that higher numbers of iTreg (ratio 1:2) led to a significantly more impaired Tresp proliferation regardless of the allergen-specificity, demonstrating a dose-dependency of the iTreg suppressive capacity (Figure 3A). We also listed the results from each individual allergic donor (Supplementary Figure 3A).

We further compared the suppressive activity of $iTreg_{Bet}$with non-specific $iTreg_0$ on antigen-specific and cross-reactive T cell immune responses (Figure 3B). The experiments revealed a moderately impaired Tresp proliferation after coculture with $iTreg_0$. However, $iTreg_{Bet}$ exhibit a significantly higher suppressive potential for both Bet- and Cor-specific T cell responses, indicating the strong potential of antigen-specific stimulated $iTreg_{Bet}$ as inducers of allergen-specific and cross-reactive tolerance in birch pollen- and hazelnut-allergic patients. Further evidence is depicted in Supplementary Figure 3B, which presents the corresponding data of each individual donor. In 8 out of 10 (80%) experiments with Bet-stimulation, and 6 out of 8 (75%) with Cor-stimulation, $iTreg_{Bet}$ showed a stronger capacity to reduce Tresp proliferation compared to $iTreg_{0,}$ suggesting their specific and cross-reactive suppressive capacity as superior to non-specific iTreg. Thus, the data demonstrated the high, specific and dose-dependent suppressive capacity of $iTreg_{Bet}$ to suppress allergen-specific (birch) as well as cross-reactive (hazelnut) T cell responses, highlighting the importance of allergen-specific iTreg induction.

To support our findings, we harvested the supernatants of suppressor assay samples after Bet- or Cor-specific mDC-stimulation and analyzed the T cell cytokine profile (Figure 3C,D). The presence of $iTreg_{Bet}$ – but not non-specific $iTreg_0$- resulted in significantly reduced levels of the T_H2 cytokine IL-13 (Figure 3C) compared to pronounced cytokine levels produced by control allergen-specific (both, Bet or Cor-stimulated) responder T cells. These results strongly confirmed our T cell proliferation data (Figure 3A,B) and indicated

an allergen-specific and cross\sout-reactive downregulation of T_H2 immunity mediated by Bet-specific iTreg ($iTreg_{Bet}$).

We also found a profound and significant upregulation of the immunosuppressive cytokine IL-10 in suppressor assays with cocultured Bet-specific, but not with non-specific iTreg, when compared to control (Figure 3D). Production of additional T_H2 (IL-5, IL-9) and T_H1 (IFN-y, TNF-α) cytokines were mostly unchanged (see Supplementary Figure 4).

In addition to T cell proliferation and cytokine production, we analyzed the phenotype of Tresp after presence or absence of $iTreg_{Bet}$ in suppressor assays (Supplementary Figure 5A). Coculture of Bet-stimulated Tresp with $iTreg_{Bet}$ in suppressor assays resulted in a significantly impaired activation (reduction of CD25, HLA-DR) and differentiation (reduced CD45RO / increased CD45RA expression) of Bet-stimulated Tresp. Similar results were observed for Cor-stimulated Tresp (Supplementary Figure 5B), confirming the data of the allergen-specific as well as cross-reactive suppressive capacity of $iTreg_{Bet}$ on Tresp proliferation and IL-13 production.

$iTreg_{Bet}$ displayed an activated and suppressive phenotype after allergen-specific and cross-reactive stimulation

In order to identify the phenotype of allergen-specific iTreg in more detail, we performed a flow cytometric analysis of $iTreg_{Bet}$, thereby gating on proliferating (activated) and non-proliferating $iTreg_{Bet}$ in the setting of suppressor assays after Bet- and Cor-specific stimulation (by allergen-loaded mDC) (Figure 4, gating strategy see Supplementary Figure 6). Allergen (Bet or Cor)-specifically restimulated proliferating $iTreg_{Bet}$ populations exhibited significantly higher percentages of $CD45RO^+$ (enhanced differentiation into a memory phenotype) and activated $CD25^+$ and $HLA\text{-}DR^+$ cells compared to Bet- or Cor-specifically stimulated non-proliferating $iTreg_{Bet}$ or Tresp, respectively (Figure 4A). Compared to non-proliferating iTreg and to Tresp, the proliferating $iTreg_{Bet}$ population was characterized by a significantly enhanced expression of CTLA-4, TNFR2, PD-1, IL-10 and ICOS, molecules known to be involved in the immunosuppressive capacity of regulatory T cells, confirming the regulatory phenotype of allergen-stimulated iTreg (Figure 4B). These results emphasized the activation state induced by allergen-specific (mDC_{Bet}) or cross-reactive (mDC_{Cor}) stimulation as prerequisite for iTreg-mediated suppressive activity. For further characterization, the expression of CD49b and LAG3 as parameters for Tr1 differentiation and Treg function were investigated (Figure 4C).[45–48] Compared to Tresp, $iTreg_{Bet}$showed an increase in $CD49b^+LAG3^+$cells after mDC_{Bet} stimulation, although this was not significant under mDC_{Cor} stimulation.

Bet-specific iTreg ameliorated allergic symptoms in humanized mouse models of allergen-induced airway and intestinal inflammation

Humanized mice are a remarkable investigative tool and preclinical study system, which close the gap between exclusively murine and human studies.[49] Here, we used well-established and standardized humanized mouse models of type I allergy to analyze the function of $iTreg_{Bet}$ on allergic symptoms in allergen-induced intestinal and airway inflammation.[41,50,51] An overview of the reconstitution and challenge protocol is visualized in Figure 5A (see also Methods and Supplementary Methods). Briefly, immunodeficient mice (NOD.CB17-$Prkdc^{scid}/J\gamma c^{-/-}$) were engrafted with human PBMC from birch pollen allergic donors with associated hazelnut allergy and injected with birch pollen extract +/- $iTreg_{Bet}$ as indicated.[41,52] After allergen-specific (birch) rectal challenge the intestinal inflammation was evaluated by a clinical score, that revealed a significantly increased inflammatory reaction of animals serving as allergic positive controls (PBMC + birch) compared to negative controls (PBMC), thereby demonstrating the validity of the humanized model and the development of an allergic immune reaction (Figure 5B, C). Intriguingly, injection of $iTreg_{Bet}$ resulted in a significant abrogation of the allergen-specific immune reaction as shown by a reduced intestinal inflammation (Figure 5B, C).

For induction of allergic asthma, the animals were engrafted and boosted as described above and were challenged intranasally with birch pollen extract as published previously.[40,51] Subsequently, the airway resistance was assessed as outcome of the allergic immune reaction.[40,51] In these experiments, allergic positive controls

(PBMC + birch) showed a significantly enhanced airway hyperreactivity in contrast to negative control groups (PBMC) (Figure 5D). Importantly, co-injection of $iTreg_{Bet}$ curtailed the development of allergic asthma symptoms, as the airway resistance was significantly decreased compared to allergic asthma positive control animals (Figure 5D).

We also analyzed the serum concentrations of human birch-specific IgE (Figure 5E) as typical immunological parameter of type I allergic reactions prior to allergen challenge. Compared to enhanced amounts of birch-specific IgE in allergic positive controls (PMBC + birch), treatment with $iTreg_{Bet}$ significantly reduced birch-specific IgE concentrations *in vivo* , confirming our data of significantly reduced clinical symptoms of allergen-induced intestinal and airway inflammation after $iTreg_{Bet}$ application.

In order to investigate the induction of cross-reactive tolerance*in vivo* , the immunodeficient mice were engrafted and boosted as described above, but were challenged rectally with hazelnut extract prior to assessment of the intestinal inflammation (Figure 6). In these experiments, we found a less severe allergic immune response in the hazelnut-challenged compared to the birch-challenged control group (Figure 5C), likely due to lack of *in vivo* booster with hazelnut extract and/or to the donors' less severe sensitization towards the food allergen. However, even after hazelnut challenge co-injection of $iTreg_{Bet}$ resulted in a pronounced inhibition of intestinal allergic symptoms compared to control animals, which was shown with data of individual experiments (Figure 6A). In addition, we observed reduced human hazelnut-specific IgE levels after $iTreg_{Bet}$ application and hazelnut challenge compared to the allergic positive control (Figure 6B). These data indicate the induction of allergen-specific (birch) as well as cross-reactive tolerance (hazelnut) *in vivo* through IL-10 DC-induced $iTreg_{Bet}$.

Discussion

Although PFA is a widespread allergic disorder, the only available disease-modifying therapy for pollen allergy has a rather limited effect on the associated food allergy.[25–27] Therefore in this study, human monocyte-derived IL-10 DC were investigated with regard to their potential to induce allergen-specific (birch) and cross-reactive (hazelnut) tolerance in birch pollen allergic patients with associated hazelnut allergy *in vitro* and *in vivo* . We found that IL-10 DC induce Bet-specific iTreg which show a regulatory phenotype and strong suppressive capacities to inhibit allergen-specific and cross-reactive immune responses *in vitro* . In addition, Bet-specific iTreg were able to ameliorate allergic symptoms *in vivo* in a humanized mouse model of allergic intestinal and airway inflammation.

PFA results from highly conserved protein structures of pollen (e.g. Bet) and food allergens (e.g. Cor), which was shown to facilitate cross-reactions on IgE level and in T cell clones.[12,53,54] In a previous study, we confirmed the data of cross-reactivity between pollen and food allergens in primary T cells directly obtained from patients with allergies to birch pollen and associated food allergens.[12] Here, we focused on the induction of allergen-specific and cross-reactive iTreg to modulate the primary and secondary allergic immune response in patients suffering from PFA.

Bet-specifically stimulated iTreg but not non-specific iTreg underwent vigorous proliferation towards Bet- and Cor-induced restimulation, suggesting activation as prerequisite for suppressive activity. In this context, *Pellerin et al.* investigated peanut-specific Tr1 cells induced *in vitro* by IL-10 DC from allergic subjects.[55] They found a highly proliferative phenotype with T_H2-cytokine profile upon peanut-specific restimulation and, in contrast to our data, suggested a functional impairment of the peanut-specific Tr1 subset.[55] Our experiments revealed that IL-10 DC-induced Bet-specific iTreg did have the ability to suppress allergen-specific responder T cell proliferation, displayed an activated and suppressive phenotype, even though they were highly proliferative. This discrepancy might be due to (1) different protocols for IL-10 DC culture and Treg generation, (2) different allergen-specific immune responses and/or (3) lack of functional assays in the study by *Pellerin et al* .[55] Anergy has been initially described as a fundamental characteristic of functional Treg, but this idea has hence been revised: although breaking Treg anergy can be accompanied by loss of suppressive function, this is not always the case.[56] In fact, it was shown that proliferating Treg can suppress T cell responses *in vivo* [56,57] and Treg that have been stimulated to proliferate can even display an enhanced

suppressive capacity.[57–59]

One very crucial aspect of therapeutic tolerance induction is the allergen-specificity. We are therefore thrilled to report that Bet-specific iTreg showed significantly greater abilities to suppress allergen-specific responder T cell proliferation than non-specific iTreg, as was seen in *in vitro* suppressor assays from up to 80% of allergic donors. These results were strongly supported by the T cell cytokine profile in suppressor assays. Here, we found that Bet-specific iTreg significantly decreased levels of the T_H2 cytokine IL-13, which was not achieved with non-specific iTreg. IL-13 is an IgE-promoting T_H2 cytokine, which contributes to airway inflammation and food-induced anaphylaxis in asthma and type 1 allergies.[60,61] In line with these data, amounts of the immunosuppressive cytokine IL-10 were significantly increased in the presence of Bet-specifically primed iTreg, which was not the case for non-specific iTreg. Accordingly, IL-10 is well known for its suppressive function in regulatory T cell activity, and particularly in control of T_H2-driven allergic diseases.[62] As the described cytokine shift was observed for both, Bet- and Cor-specific responder T cells after coculture with Bet-stimulated iTreg - but not with non-specific $iTreg_0$- these data underlined the induction of an allergen-specific (birch) and cross-reactive tolerance (hazelnut) through IL-10 DC-induced Bet-specific iTreg priming.

In addition, Bet-specific iTreg were able to ameliorate asthmatic and intestinal allergic symptoms provoked by challenge with birch extract and reduced birch-specific IgE in allergic mice *in vivo* . These combined pieces of evidence strongly suggest an allergen-specific tolerance induction *in vitro* and *in vivo* by iTreg which have been stimulated by allergen-loaded tolerogenic IL-10 DC. Intriguingly, Bet-specific iTreg also reduced the allergic gut inflammation and hazelnut-specific IgE levels *in vivo* after challenge with the hazelnut extract in mice engrafted with PBMC from birch pollen allergic patients with associated hazelnut allergy, facilitating cross-reactive tolerance.

Aiming to replace general immunosuppressive therapies, tolerogenic DC (tolDC) have been applied as antigen-specific immune-suppressors in numerous phase 1 clinical trials for multiple sclerosis, type I diabetes, rheumatoid arthritis and organ transplantation.[32,34,63–65] In all studies, tolDC had negligible adverse effects and did not worsen disease symptoms. Clinical outcomes were only investigated in a few trials so far but preliminary evidence for antigen-specific tolerance induction was found.

In a comparative study by *Boks et al.* IL-10 modulation for human tolDC generation was identified as the protocol most suited for tolDC vaccination.[66] We developed a protocol for IL-10 DC that resulted in a subpopulation of tolerogenic $CD83^{high}CCR7^{+}$ IL-10 DC that exhibit a high migratory activity, stability to pro-inflammatory stimuli and profound capacity to induce iTreg with a strong suppressive function.[35] In our current study, we did show that human IL-10 DC through priming of allergen-stimulated iTreg are able to induce specific- and cross-reactive tolerance *in vitro* and*in vivo* and, therefore are promising candidates to modulate pollen as well as associated food allergies.

Combined with previous findings by us and other groups,[35,51,66–68] our study results might further support the development of DC-based tolerance-inducing therapies for allergic and autoimmune diseases.

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Figure Legends

Figure 1: iTreg induced by unloaded or Bet-loaded IL-10 DC displayed an anergic phenotype after primary culture. PBMC for DC generation and T cells were obtained from birch-pollen allergic patients with associated hazelnut allergy. $CD4^+$ T cells were primed with autologous unloaded IL-10 DC (IL-10 DC0) and Bet-loaded IL-10 DC (IL-10 DCBet) or mDC (mDC0/mDCBet), respectively, to induce non-specific and Bet-specific iTreg ($iTreg_0$/$iTreg_{Bet}$) and Teff ($Teff_0$/$Teff_{Bet}$, as controls).**(A)** After 3 days of iTreg induction, the coculture was pulsed with $[^3H]$TdR for 16-18 h to assess T cell proliferation which is shown as stimulation index (SI, mean ± SD) normalized to T cells stimulated with mDC_0 (SI = 1). The data are pooled from 34 independent experiments. **(B-F)** Cytokine concentrations (**B** IL-5, **C** IL-9, **D** IL-13,**E** IL-2 and **F** IL-10) in the supernatants of primary cultures are depicted as mean ± SD normalized to ctrl (=1). P values calculated with paired student's t-test are depicted as asterisks: **** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$, ns = not significant ($p > 0.05$). PC: primary culture (induction)

Figure 2: Bet-specific iTreg lost their anergic phenotype after Bet- and Cor-induced restimulation. In primary culture (PC), $Teff_0$, $Teff_{Bet}$, $iTreg_0$and $iTreg_{Bet}$ were primed for five days by coculture of $CD4^+$ T cells with autologous unloaded and Bet-loaded mDC or IL-10 DC, respectively. After a subsequent resting phase of 3 days, T cells were restimulated (RS) with unloaded, Bet-loaded or Cor-loaded mDC and were pulsed with $[^3H]$TdR for 16-18 h on day 3. T cell proliferation is presented as SI (mean ± SD) normalized to $Teff_0$ stimulated with mDC_0(SI = 1). **(A)** Bet- and Cor-stimulated T cell proliferation of $Teff_0$, $Teff_{Bet}$ and $iTreg_{Bet}$ was pooled from 8 independent experiments.**(B)** Data of 9 independent experiments were pooled demonstrating T cell proliferation of $iTreg_{Bet}$ and $iTreg_0$ restimulated with mDC_{Bet} or $mDC_{Cor,}$ respectively. P values calculated with paired student's t-test are depicted as asterisks: ** $p < 0.01$, * $p < 0.05$, ns = not significant ($p > 0.05$). PC: primary culture (induction), RS: restimulation

Figure 3: Bet-specific iTreg inhibited allergen-specific and cross-reactive responder T cell responses *in vitro*. Bet- and Cor-specific proliferation of autologous $CD4^+CD25^{low}$ responder T cells (Tresp) obtained from birch-pollen allergic donors with associated hazelnut allergy was induced by stimulation with mDC_{Bet}(green) or mDC_{Cor} (brown), respectively. For analysis of the suppressive activity iTreg ($iTreg_{Bet}$ or $iTreg_0$) were added in a Tresp:iTreg ratio of 1:1 or 1:2, respectively. DC, Tresp and iTreg were stained with different proliferation dyes for cell identification and assessment of proliferation by flow cytometry (see Supplementary Figure 2 for gating strategy). **(A, B)** Percentage of proliferating Tresp are shown from one representative experiment (top) and as pooled data (mean ± SD) relative to control (= 100%) from independent experiments (bottom, number of independent experiments indicated below). **(A)**Percentage (mean ± SD) of proliferating Bet- (upper panel, green) and Cor-stimulated (lower panel, brown) Tresp, cocultured with $iTreg_{Bet}$ in the ratios 1: 1 and 1:2 , respectively, are demonstrated (Pooled data: Bet-stimulation: n= 15; Cor-stimulation: n=14). **(B)** Function of $iTreg_{Bet}$ was compared to $iTreg_0$ in suppressor assays with Tresp:iTreg = 1:2 and percentages (mean ± SD) of proliferating Bet- and Cor-stimulated Tresp are depicted as pooled data (Bet-stimulation: n= 10; Cor-stimulation: n=8). **(C)** IL-13 (n=5-9) and **(D)** IL-10 concentrations (n=5-10) in the supernatants of suppressor assay samples are depicted as mean ± SD normalized to ctrl (=1). P values calculated with paired student's t-test are depicted as asterisks: **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns = not significant ($p > 0.05$).

Figure 4: Bet- and Cor-specifically stimulated iTreg showed an activated and suppressive phenotype. Prior to flow cytometry analysis of suppressor assays, T cell populations were stained for expression of extra- and intracellular markers and with different cell proliferation dyes (see supplementary Figure 5 for gating strategy) to distinguish between proliferating and non-proliferating T cells. **(A)**Activation and **(B)** immunosuppressive parameters are pooled from independent experiments as indicated (CD45RO n=5, CD25 n=9, HLA-DR n=10, CTLA-4 n=10, TNFR2 n=9, PD-1 n=10, IL-10 n=5, ICOS n=5) and expression is shown as percentage (mean ± SD), except for ICOS which is presented as the mean fluorescence intensity (mean ± SD). **(C)** Five independent experiments were pooled to show the percentage (mean ± SD) of $CD49b^+LAG3^+$ T cells. P values calculated with paired student's t-test are depicted as asterisks: **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns = not significant ($p > 0.05$).

Figure 5: Bet-specific iTreg abrogated birch-specific allergic symptoms in humanized mouse

models of allergen-induced intestinal and airway inflammation. (A) Immunodeficient mice were engrafted with human PBMC from allergic donors suffering from birch pollen and associated hazelnut allergy and were boosted twice (d0, d8) with birch pollen extract to induce allergic immune responses. iTreg were co-injected to analyze their impact on allergy development. After three weeks, blood samples for IgE analysis were collected and subsequently the mice were challenged with birch pollen extract either rectally for induction of allergic intestinal inflammation or intranasally for allergic asthma induction. **(B)** The allergic intestinal inflammation was monitored by mini-endoscopy and scoring of colitis activity. One representative set of pictures is shown. **(C)**Quantitative endoscopic assessment of colitis activity in all groups is shown as mean ± SD from 8 independent experiments. **(D)** Results of airway resistance as parameter of allergic asthma were pooled from four independent experiments and are depicted as relative changes to baseline in % (mean ± SD). **(E)** The concentration of birch-specific IgE was obtained from 7 independent experiments as indicated and are presented in kU/L. P values calculated with paired student's t-test are depicted as asterisks: **** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$. Figure 5A was created using Servier Medical Art (http://smart.servier.com/).

Figure 6: Bet-specific iTreg facilitated a cross-tolerance to hazelnut in a humanized mouse model of allergic gut inflammation. Immunodeficient mice were treated as described in Figure 5A. After three weeks, the mice were challenged rectally with either birch pollen or hazelnut extract, respectively, 2 h prior to scoring of the intestinal inflammation by mini-endoscopy. **(A)** The endoscopic score of the allergic intestinal inflammation of four independent experiments is depicted as pooled data (mean ± SD). **(B)** Blood samples were collected and human hazelnut-specific IgE was analyzed and is shown as mean value ± SD. Lines connect values from individual experiments. P values calculated with paired student's t-test are shown as asterisks: *** $p < 0.001$, ** $p < 0.01$, ns = not significant ($p > 0.05$)

References

1. Gilles, S, Akdis, C, Lauener, R, Schmid-Grendelmeier, P, Bieber, T, Schappi, G, et al. The role of environmental factors in allergy: a critical reappraisal. Exp Dermatol 2018;27:1193–200.

2. Ose, R, Tu, J, Schink, A, Maxeiner, J, Schuster, P, Lucas, K, et al. Cinnamon extract inhibits allergen-specific immune responses in human and murine allergy models. Clin Exp Allergy 2020;50:41–50.

3. Warren, C, Nimmagadda, SR, Gupta, R, Levin, M. The epidemiology of food allergy in adults. Ann Allergy Asthma Immunol 2023;130:276–87.

4. Wahn, U, Calderon, MA, Demoly, P. Real-life clinical practice and management of polysensitized patients with respiratory allergies: a large, global survey of clinicians prescribing allergen immunotherapy. Expert Rev Clin Immunol 2017;13:283–89.

5. Geroldinger-Simic, M, Zelniker, T, Aberer, W, Ebner, C, Egger, C, Greiderer, A, et al. Birch pollen-related food allergy: clinical aspects and the role of allergen-specific IgE and IgG4 antibodies. J Allergy Clin Immunol 2011;127:616–22.

6. Mastrorilli, C, Cardinale, F, Giannetti, A, Caffarelli, C. Pollen-food allergy syndrome: a not so rare disease in childhood. Medicina (Kaunas) 2019;55:641–52.

7. Vieths, S, Scheurer, S, Ballmer-Weber, B. Current understanding of cross-reactivity of food allergens and pollen. Ann N Y Acad Sci 2002;964:47–68.

8. Eifan, AO, Durham, SR. Pathogenesis of rhinitis. Clin Exp Allergy 2016;46:1139–51.

9. Pfaar, O, Bachert, C, Kuna, P, Panzner, P, Dzupinova, M, Klimek, L, et al. Sublingual allergen immunotherapy with a liquid birch pollen product in seasonal allergic rhinoconjunctivitis with/without asthma. J Allergy Clin Immunol 2019;143:970–77.

10. Papi, A, Brightling, C, Pedersen, SE, Reddel, HK. Asthma. Lancet 2018;391:783–800.

11. Bernstein, DI, Schwartz, G, Bernstein, JA. Allergic rhinitis: mechanisms and treatment. Immunol Allergy Clin North Am 2016;36:261–78.

12. Hofmann, C, Scheurer, S, Rost, K, Graulich, E, Jamin, A, Foetisch, K, et al. Cor a 1-reactive T cells and IgE are predominantly cross-reactive to Bet v 1 in patients with birch pollen-associated food allergy to hazelnut. J Allergy Clin Immunol 2013;131:1384-92.e6.

13. Guryanova, SV, Finkina, EI, Melnikova, DN, Bogdanov, IV, Bohle, B, Ovchinnikova, TV. How do pollen allergens sensitize? Front mol biosci 2022;9:900533.

14. Bohle, B. The impact of pollen-related food allergens on pollen allergy. Allergy 2007;62:3–10.

15. Jahn-Schmid, B, Radakovics, A, Lüttkopf, D, Scheurer, S, Vieths, S, Ebner, C, et al. Bet v 1142-156 is the dominant T-cell epitope of the major birch pollen allergen and important for cross-reactivity with Bet v 1-related food allergens. J Allergy Clin Immunol 2005;116:213–19.

16. Sanchez Acosta, G, Kinaciyan, T, Kitzmuller, C, Mobs, C, Pfutzner, W, Bohle, B. IgE-blocking antibodies following SLIT with recombinant Mal d 1 accord with improved apple allergy. J Allergy Clin Immunol 2020;146:894–900.

17. Asero, R, Ariano, R, Aruanno, A, Barzaghi, C, Borrelli, P, Busa, M, et al. Systemic allergic reactions induced by labile plant-food allergens: Seeking potential cofactors. A multicenter study. Allergy 2021;76:1473–79.

18. Olcese, R, Silvestri, M, Del Barba, P, Brolatti, N, Barberi, S, Tosca, MA, et al. Mal d 1 and Bet v 1 sensitization pattern in children with pollen food syndrome. Allergol Int 2019;68:122–24.

19. Lüttkopf, D, Müller, U, Skov, PS, Ballmer-Weber, BK, Wüthrich, B, Skamstrup Hansen, K, et al. Comparison of four variants of a major allergen in hazelnut (Corylus avellana) Cor a 1.04 with the major hazel pollen allergen Cor a 1.01. Mol Immunol 2002;38:515–25.

20. Durham, SR, Emminger, W, Kapp, A, Colombo, G, Monchy, JG de, Rak, S, et al. Long-term clinical efficacy in grass pollen-induced rhinoconjunctivitis after treatment with SQ-standardized grass allergy immunotherapy tablet. J Allergy Clin Immunol 2010;125:131-8.e1-7.

21. Biedermann, T, Kuna, P, Panzner, P, Valovirta, E, Andersson, M, Blay, F de, et al. The SQ tree SLIT-tablet is highly effective and well tolerated: rsults from a randomized, double-blind, placebo-controlled phase III trial. J Allergy Clin Immunol 2019;143:1058-1066.e6.

22. Bozek, A, Cudak, A, Walter Canonica, G. Long-term efficacy of injected allergen immunotherapy for treatment of grass pollen allergy in elderly patients with allergic rhinitis. Allergy Asthma Proc 2020;41:271–77.

23. Marogna, M, Spadolini, I, Massolo, A, Canonica, GW, Passalacqua, G. Long-lasting effects of sublingual immunotherapy according to its duration: a 15-year prospective study. J Allergy Clin Immunol 2010;126:969–75.

24. Hesse, L, Oude Elberink, JNG, van Oosterhout, AJM, Nawijn, MC. Allergen immunotherapy for allergic airway diseases: use lessons from the past to design a brighter future. Pharmacol Ther 2022;237:108115.

25. Kinaciyan, T, Jahn-Schmid, B, Radakovics, A, Zwolfer, B, Schreiber, C, Francis, JN, et al. Successful sublingual immunotherapy with birch pollen has limited effects on concomitant food allergy to apple and the immune response to the Bet v 1 homolog Mal d 1. J Allergy Clin Immunol 2007;119:937–43.

26. Möller, C. Effect of pollen immunotherapy on food hypersensitivity in children with birch pollinosis. Ann Allergy 1989;62:343–45.

27. Hansen, KS, Khinchi, MS, Skov, PS, Bindslev-Jensen, C, Poulsen, LK, Malling, HJ. Food allergy to apple and specific immunotherapy with birch pollen. Mol Nutr Food Res 2004;48:441–48.

28. Nothegger, B, Reider, N, Covaciu, CE, Cova, V, Ahammer, L, Eidelpes, R, et al. Oral birch pollen immunotherapy with apples: results of a phase II clinical pilot study. Immun Inflamm Dis 2021;9:503–11.

29. Pacciani, V, Gregori, S, Chini, L, Corrente, S, Chianca, M, Moschese, V, et al. Induction of anergic allergen-specific suppressor T cells using tolerogenic dendritic cells derived from children with allergies to house dust mites. J Allergy Clin Immunol 2010;125:727–36.

30. Steinbrink, K, Wolfl, M, Jonuleit, H, Knop, J, Enk, AH. Induction of tolerance by IL-10-treated dendritic cells. J immunol 1997;159:4772–80.

31. Raker, VK, Domogalla, MP, Steinbrink, K. Tolerogenic dendritic cells for regulatory T cell induction in man. Front Immunol 2015;6:569.

32. Zubizarreta, I, Florez-Grau, G, Vila, G, Cabezon, R, Espana, C, Andorra, M, et al. Immune tolerance in multiple sclerosis and neuromyelitis optica with peptide-loaded tolerogenic dendritic cells in a phase 1b trial. Proc Natl Acad Sci U S A 2019;116:8463–70.

33. Domogalla, MP, Rostan, PV, Raker, VK, Steinbrink, K. Tolerance through education: how tolerogenic dendritic cells shape immunity. Front Immunol 2017;8:1764.

34. Nikolic, T, Zwaginga, JJ, Uitbeijerse, BS, Woittiez, NJ, Koning, EJ de, Aanstoot, HJ, et al. Safety and feasibility of intradermal injection with tolerogenic dendritic cells pulsed with proinsulin peptide-for type 1 diabetes. Lancet Diabetes Endocrinol 2020;8:470–72.

35. Kryczanowsky, F, Raker, V, Graulich, E, Domogalla, MP, Steinbrink, K. IL-10-modulated human dendritic cells for clinical use: identification of a stable and migratory subset with improved tolerogenic activity. J immunol 2016;197:3607–17.

36. Kubsch, S, Graulich, E, Knop, J, Steinbrink, K. Suppressor activity of anergic T cells induced by IL-10-treated human dendritic cells: association with IL-2- and CTLA-4-dependent G1 arrest of the cell cycle regulated by p27Kip1. Eur J Immunol 2003;33:1988–97.

37. Hubo, M, Trinschek, B, Kryczanowsky, F, Tuettenberg, A, Steinbrink, K, Jonuleit, H. Costimulatory molecules on immunogenic versus tolerogenic human dendritic cells. Front Immunol 2013;4:82.

38. Steinbrink, K, Graulich, E, Kubsch, S, Knop, J, Enk, AH. CD4(+) and CD8(+) anergic T cells induced by interleukin-10-treated human dendritic cells display antigen-specific suppressor activity. Blood 2002;99:2468–76.

39. Li, X, Yang, A, Huang, H, Zhang, X, Town, J, Davis, B, et al. Induction of type 2 T helper cell allergen tolerance by IL-10-differentiated regulatory dendritic cells. Am J Respir Cell Mol Biol 2010;42:190–99.

40. Bellinghausen, I, Weigmann, B, Zevallos, V, Maxeiner, J, Reissig, S, Waisman, A, et al. Wheat amylase-trypsin inhibitors exacerbate intestinal and airway allergic immune responses in humanized mice. J Allergy Clin Immunol 2019;143:201–12.

41. Eschborn, M, Weigmann, B, Reissig, S, Waisman, A, Saloga, J, Bellinghausen, I. Activated glycoprotein A repetitions predominant (GARP)-expressing regulatory T cells inhibit allergen-induced intestinal inflammation in humanized mice. J Allergy Clin Immunol 2015;136:159–68.

42. Steinbrink, K, Jonuleit, H, Muller, G, Schuler, G, Knop, J, Enk, AH. Interleukin-10-treated human dendritic cells induce a melanoma-antigen-specific anergy in CD8(+) T cells resulting in a failure to lyse tumor cells. Blood 1999;93:1634–42.

43. Muller, G, Muller, A, Tuting, T, Steinbrink, K, Saloga, J, Szalma, C, et al. Interleukin-10-treated dendritic cells modulate immune responses of naive and sensitized T cells in vivo. J Invest Dermatol 2002;119:836–41.

44. Pandiyan, P, Zheng, L, Ishihara, S, Reed, J, Lenardo, MJ. CD4+CD25+Foxp3+ regulatory T cells induce cytokine deprivation-mediated apoptosis of effector CD4+ T cells. Nat Immunol 2007;8:1353–62.

45. 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. Nat Med 2013;19:739–46.

46. Hanazawa, A, Hayashizaki, K, Shinoda, K, Yagita, H, Okumura, K, Löhning, M, et al. CD49b-dependent establishment of T helper cell memory. Immunol Cell Biol 2013;91:524–31.

47. Huang, CT, Workman, CJ, Flies, D, Pan, X, Marson, AL, Zhou, G, et al. Role of LAG-3 in regulatory T cells. Immunity 2004;21:503–13.

48. Maruhashi, T, Sugiura, D, Okazaki, IM, Okazaki, T. LAG-3: from molecular functions to clinical applications. J Immunother Cancer 2020;8.

49. Bellinghausen, I, Saloga, J. Analysis of allergic immune responses in humanized mice. Cell Immunol 2016;308:7–12.

50. Meyer-Martin, H, Hahn, SA, Beckert, H, Belz, C, Heinz, A, Jonuleit, H, et al. GARP inhibits allergic airway inflammation in a humanized mouse model. Allergy 2016;71:1274–83.

51. Martin, H, Reuter, S, Dehzad, N, Heinz, A, Bellinghausen, I, Saloga, J, et al. CD4-mediated regulatory T-cell activation inhibits the development of disease in a humanized mouse model of allergic airway disease. J Allergy Clin Immunol 2012;129:521-8, 528.e1-7.

52. Vizzardelli, C, Gindl, M, Roos, S, Mobs, C, Nagl, B, Zimmann, F, et al. Blocking antibodies induced by allergen-specific immunotherapy ameliorate allergic airway disease in a human/mouse chimeric model. Allergy 2018;73:851–61.

53. Zulehner, N, Nagl, B, Briza, P, Roulias, A, Ballmer-Weber, B, Zlabinger, GJ, et al. Characterization of the T-cell response to Dau c 1, the Bet v 1-homolog in carrot. Allergy 2017;72:244–51.

54. Fritsch, R, Bohle, B, Vollmann, U, Wiedermann, U, Jahn-Schmid, B, Krebitz, M, et al. Bet v 1, the major birch pollen allergen, and Mal d 1, the major apple allergen, cross-react at the level of allergen-specific T helper cells. J Allergy Clin Immunol 1998;102:679–86.

55. Pellerin, L, Jenks, JA, Chinthrajah, S, Dominguez, T, Block, W, Zhou, X, et al. Peanut-specific Tr1 cells induced in vitro from allergic individuals are functionally impaired. J Allergy Clin Immunol 2017;141:202–13.

56. Walker, LS, Chodos, A, Eggena, M, Dooms, H, Abbas, AK. Antigen-dependent proliferation of CD4+ CD25+ regulatory T cells in vivo. J Exp Med 2003;198:249–58.

57. Klein, L, Khazaie, K, Boehmer, H von. In vivo dynamics of antigen-specific regulatory T cells not predicted from behavior in vitro. Proc Natl Acad Sci U S A 2003;100:8886–91.

58. Yamazaki, S, Iyoda, T, Tarbell, K, Olson, K, Velinzon, K, Inaba, K, et al. Direct expansion of functional CD25+ CD4+ regulatory T cells by antigen-processing dendritic cells. J Exp Med 2003;198:235–47.

59. Caramalho, I, Lopes-Carvalho, T, Ostler, D, Zelenay, S, Haury, M, Demengeot, J. Regulatory T cells selectively express toll-like receptors and are activated by lipopolysaccharide. J Exp Med 2003;197:403–11.

60. Noah, TK, Knoop, KA, McDonald, KG, Gustafsson, JK, Waggoner, L, Vanoni, S, et al. IL-13-induced intestinal secretory epithelial cell antigen passages are required for IgE-mediated food-induced anaphylaxis. J Allergy Clin Immunol 2019;144:1058-1073.e3.

61. Burgess, G, Boyce, M, Jones, M, Larsson, L, Main, MJ, Morgan, F, et al. Randomized study of the safety and pharmacodynamics of inhaled interleukin-13 monoclonal antibody fragment VR942. EBioMedicine 2018;35:67–75.

62. Schulke, S. Induction of interleukin-10 producing dendritic cells as a tool to suppress allergen-specific T helper 2 responses. Front Immunol 2018;9:455.

63. Bell, GM, Anderson, AE, Diboll, J, Reece, R, Eltherington, O, Harry, RA, et al. Autologous tolerogenic dendritic cells for rheumatoid and inflammatory arthritis. Ann Rheum Dis 2017;76:227–34.

64. Benham, H, Nel, HJ, Cheng Law, S, Mehdi, AM, Street, S, Ramnoruth, N, et al. Citrullinated peptide dendritic cell immunotherapy in HLA risk genotype–positive rheumatoid arthritis patients. Sci Transl Med 2015;7.

65. Sawitzki, B, Harden, PN, Reinke, P, Moreau, A, Hutchinson, JA, Game, DS, et al. Regulatory cell therapy in kidney transplantation (The ONE Study): a harmonised design and analysis of seven non-randomised, single-arm, phase 1/2A trials. Lancet 2020;395:1627–39.

66. Boks, MA, Kager-Groenland, JR, Haasjes, MS, Zwaginga, JJ, van Ham, SM, Brinke, A ten. IL-10-generated tolerogenic dendritic cells are optimal for functional regulatory T cell induction - a comparative study of human clinical-applicable DC. Clin Immunol 2012;142:332–42.

67. Bellinghausen, I, König, B, Böttcher, I, Knop, J, Saloga, J. Inhibition of human allergic T-helper type 2 immune responses by induced regulatory T cells requires the combination of interleukin-10-treated dendritic cells and transforming growth factor-beta for their induction. Clin Exp Allergy 2006;36:1546–55.

68. Amodio, G, Gregori, S. Human tolerogenic DC-10: perspectives for clinical applications. Transplant Res 2012;1:14.

Fig. 1

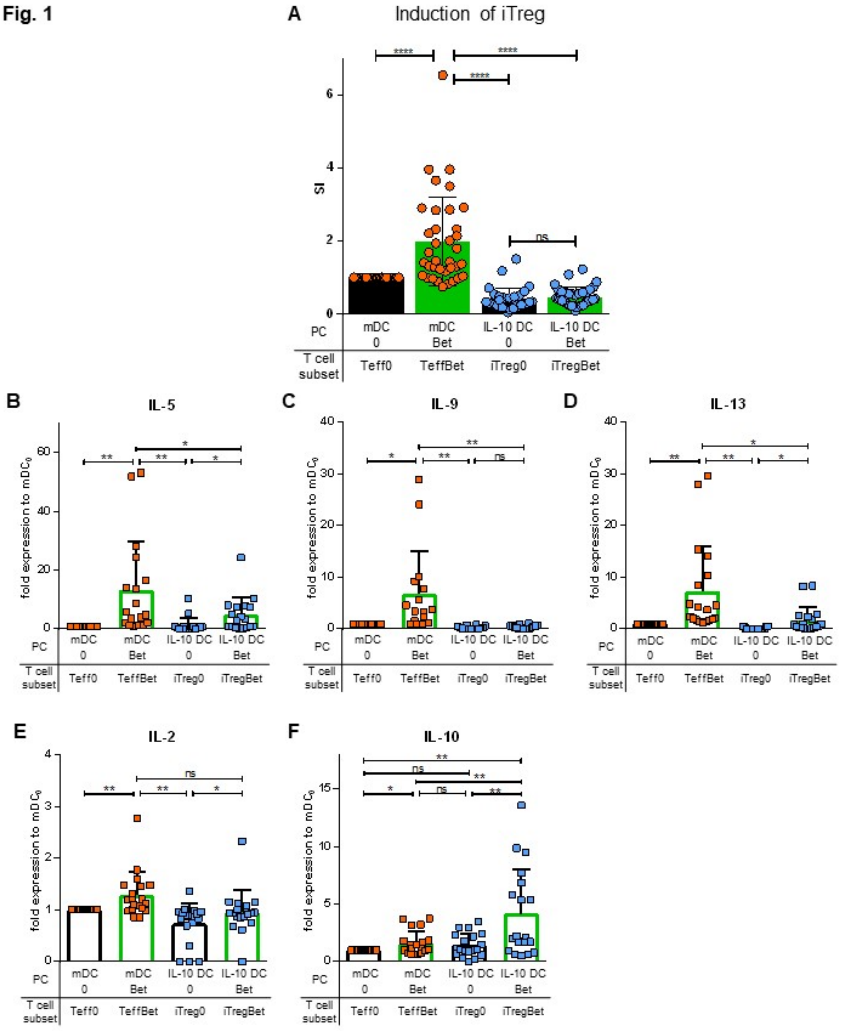

Fig. 2 A Restimulation of iTregBet **B** Restimulation of iTreg0

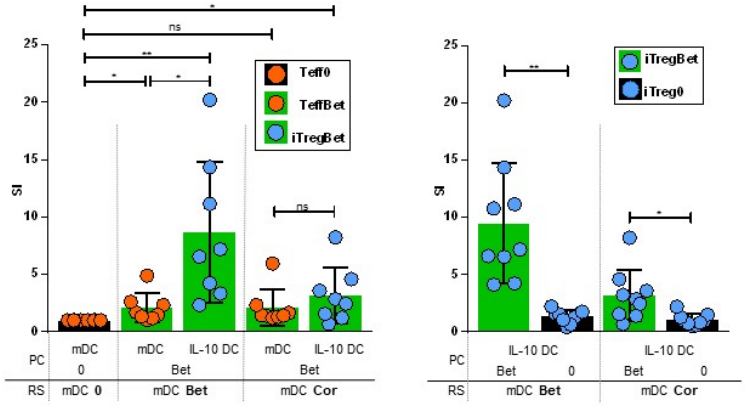

Fig. 3

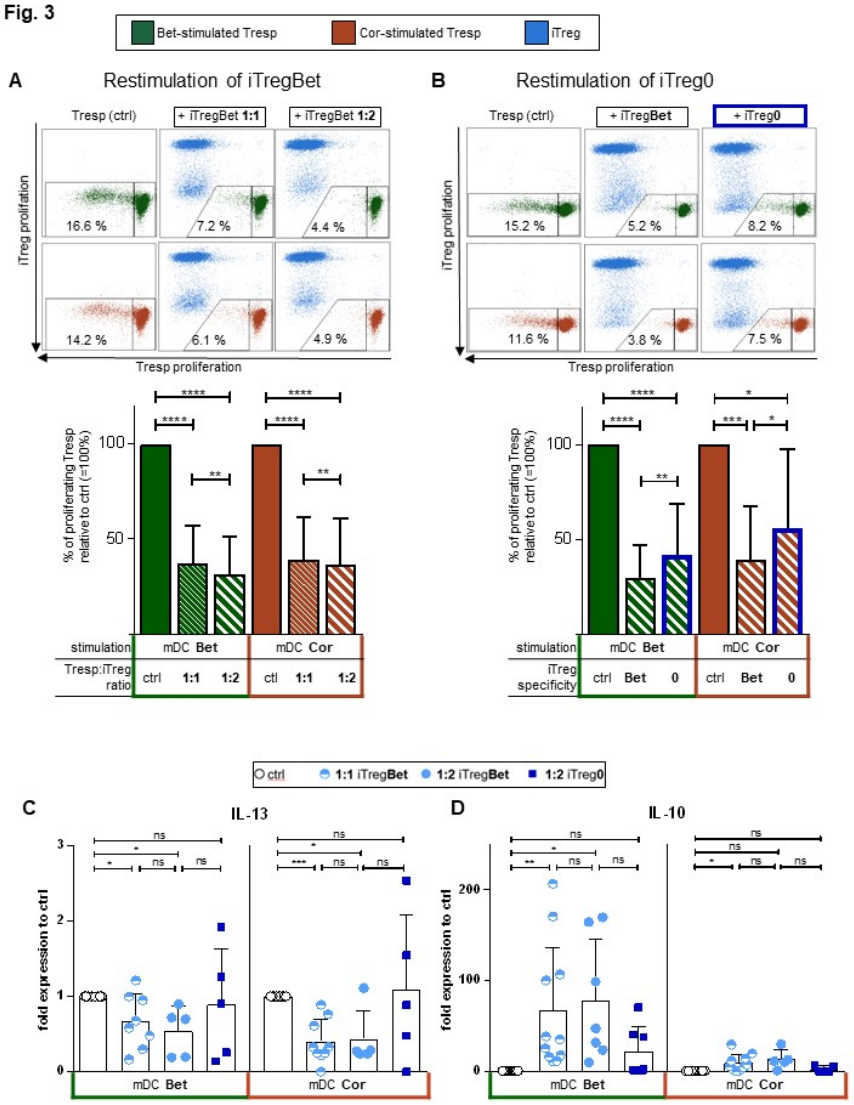

Fig. 4

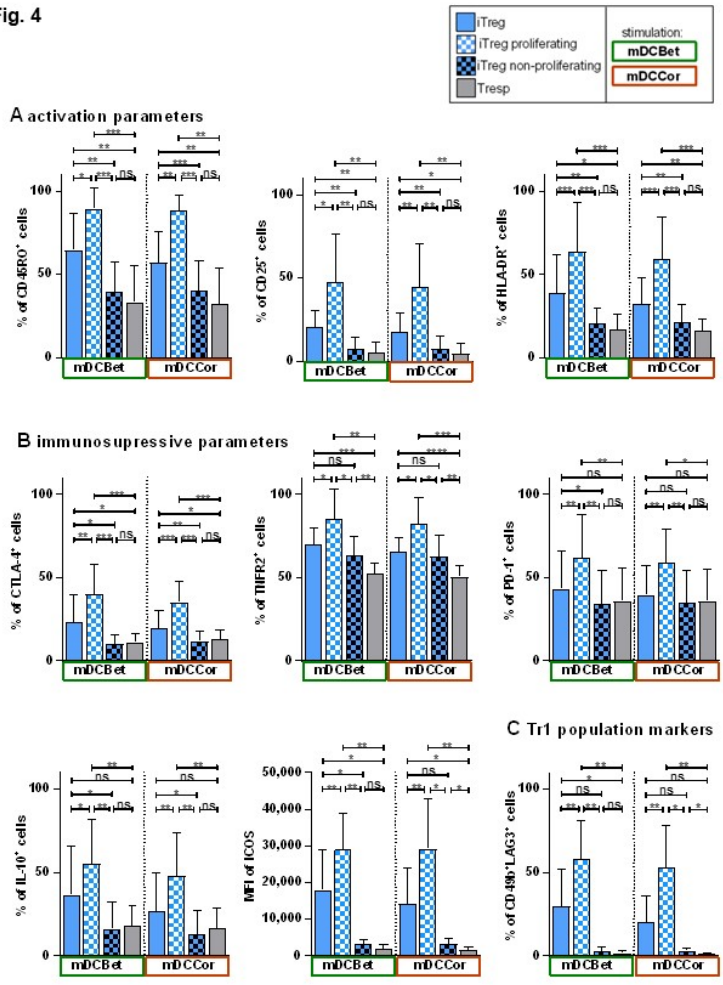

Fig. 5

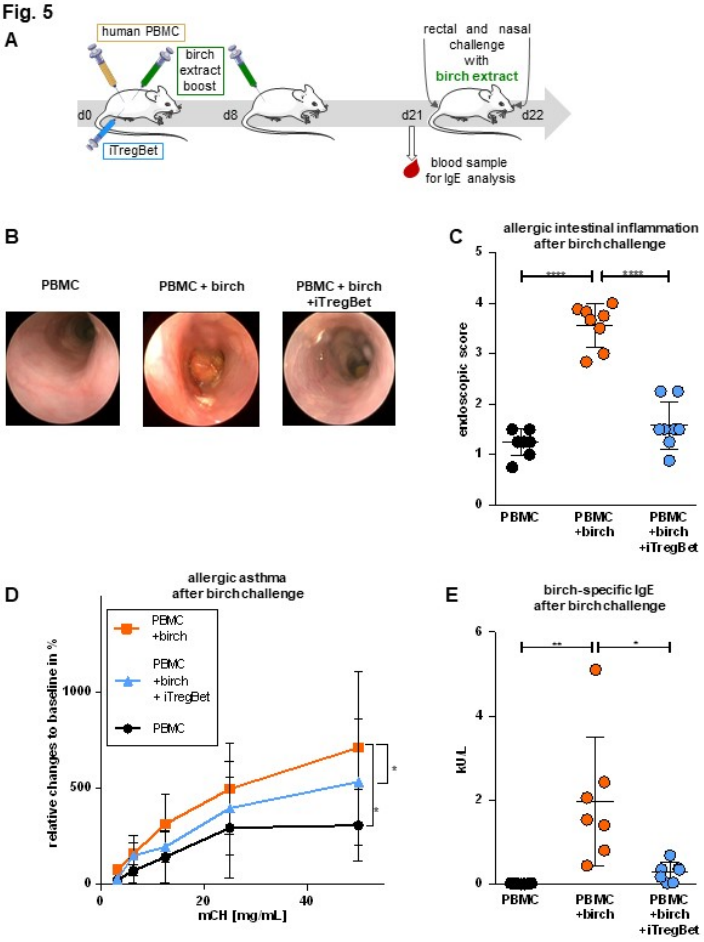

Fig. 6