

Impact of systemic immuno-suppression after solid organ transplantation on allergen-specific responses

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Abstract

Introduction: The immunosuppressive therapy in solid organ transplantation targets mainly the T- and B-cell-mediated immune response. However, there is evidence that it neither suppresses sensitization nor clinical manifestation of allergic diseases in organ-transplanted patients.

Objective: This study addresses the question whether allergen-specific responses are altered by systemic immunosuppression via negative effects on the T-regulatory cell compartment and a more pronounced suppression on Th1-type T-cell responses.

Material and methods: Peripheral blood mononuclear cells from 65 solid organ-transplanted (kidney, liver, lung) children, adolescents, and young adults and 18 healthy, matched controls were included, and their clinical and sensitization status assessed. Allergen-specific proliferation, intracellular cytokine production, frequency of forkhead box P3 (FOXP3)⁺CD3⁺CD4⁺CD25^{high} cells, mRNA expression of IL-10, transforming growth factor (TGF)- β and FOXP3 (real-time RT-PCR) of peripheral blood mononuclear cells or bronchoalveolar lavage fluid (BAL)-derived cells, and the inhibitory capacity of T-reg cells were investigated.

Results: Immunosuppression led to a significantly altered regulatory marker profile expressed by enhanced TGF- β mRNA production and a reduced frequency of FOXP3⁺CD4⁺CD3⁺ cells in solid organ transplanted individuals. FOXP3 expression in BAL cells of lung-transplanted patients was significantly decreased. Allergen-specific proliferation was not significantly altered despite long-term immunosuppression. However, suppression of allergen-specific responses via the T-regulatory cell fraction was deficient in immunosuppressed individuals.

Conclusion: The results suggest an insufficient control of allergen-specific responses via the Treg-cell compartment under systemic immunosuppression.

The prevalence of allergies is constantly rising, and causal treatment, with exception of immunotherapy, is still missing. Based on current knowledge, the triangle of Th1, Th2 and T-regulatory cells forms the basis of the disease (1, 2). If the balance between these different T-cell subsets is altered, disease manifestation occurs and allergen-specific tolerance is broken.

Comparable rates of allergic sensitization and allergic symptoms in transplanted individuals, in particular to food allergens, but also to inhalant allergens have been published (3, 4). Despite the potent immunosuppressive properties of a posttransplantation protocol sensitization to allergens on the one hand and allergy-related clinical symptoms have been reported on the other hand (3–8). This is astonishing because

a sufficient control of allogeneic responses can be achieved. Therefore, investigations of allergen-specific responses in organ-transplanted individuals may also provide new insights into the pathogenesis of allergic diseases.

To date, the insufficient control of allergic diseases under systemic immunosuppression is explained via four main theories. First, systemic immunosuppression in organ-transplanted individuals is thought to be more efficient in the suppression of Th1- than Th2-type responses, which consequently leads to a relative shift toward Th2. This may result in an insufficient suppression of Th2-type responses (7, 9–11). Second, calcineurin inhibitors (CNI) downregulate Treg-cell frequency in general (12–14) which may result in an insufficient control of allergen-specific effector cells. Third, transfer

of pathogenic cell subsets and/or IgE via the transplantation may occur (6, 15, 16). Fourth, in context with tacrolimus and food allergy development, an increase in the gut permeability and therefore a facilitated uptake of potentially allergenic molecules resulting in sensitization and allergy development is assumed (17).

Although there are numerous reports about allograft responses, little information is available about the impact of systemic immunosuppression on allergen-specific responses and Treg function in the context of allergy. Literature available so far mainly relates to case reports or selected subgroups and is based on clinical results (5–7, 16, 18, 19). This study characterizes allergen-specific responses after systemic immunosuppression *in vitro* in a cross-sectional design in organ-transplanted children, adolescents, and young adults, based on clinical characteristics. The results suggest an insufficient control of allergen-specific responses via the Treg-cell compartment under systemic immunosuppression.

Materials and methods

Study population

Sixty-five solid-organ recipients of kidney, liver, or lung allografts at the Medical University of Vienna were enrolled from 2004 to 2005 and evaluated in this cross-sectional study. A detailed characterization of the study population in terms of sensitization patterns and clinical symptoms related to type-I allergy has been published by Dehlink et al. (3).

In brief, information on the history of atopic diseases, i.e. allergic rhinoconjunctivitis, allergic bronchial asthma, atopic dermatitis, and food allergy, was collected by a detailed questionnaire based on the International Study of Asthma and Allergies in Childhood (ISAAC) (20). The underlying disease leading to organ transplantation, personal and family history of atopic disease, immunosuppressive medication since transplantation and blood levels of the respective immunosuppressive agent were assessed. Skin prick tests with eight common allergen extracts (birch pollen, 6-grass pollen mix, *Dermatophagoides pteronyssinus*, *Alternaria alternata*, peanut, cat and dog dander, and hen's egg; ALK-Scherax, Hamburg, Germany) as well as specific IgE measurement enquiring eight common inhalant and ten common nutritional allergens were conducted (timothy grass, rye, birch, and mugwort pollen, house dust mite, cat and dog dander, *Cladosporium herbarum*, hen's egg white, cow's milk, codfish, wheat flour, soy bean, peanut, hazelnut, Brazil nut, almond, and coconut) with all serum samples. Additional allergens were included if indicated by the patient's history. Specific and total IgE levels in peripheral blood were determined using the CAP-fluoroenzyme immunoassay (Phadia AB, Uppsala, Sweden). Sensitization was defined as SPT and/or CAP-fluoroenzyme immunoassay positivity. Peripheral blood was taken from 65 patients. Peripheral-blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation and stored in liquid nitrogen. All investigations were performed in patients that had no signs of infection or acute rejection. In addition an age-matched, nontransplanted group served as control.

At the time point, *in vitro* experiments were performed, frozen cells were thawed, counted, and functional assays were performed. Whenever the cell count allowed, mRNA isolation of 1 Mio PBMCs was undertaken.

T-cell assays

To assess the cytokine predominance of PBMCs from transplanted individuals, intracellular cytokines were stained as explained previously (21, 22). In brief, cells were stimulated with 10 ng/ml phorbol myristateacetate, 1.25 mM ionomycin, in the presence of 5 mg/ml Brefeldin A (all from Sigma Chemical Co. BioSciences, St Louis, MO, USA) for 4 h. Thereafter, cells were washed, fixed, permeabilized, and stained for CD3 (PerCP), CD4 (FITC) and the cytokines (INF- γ APC), IL-13 (PE), IL-4 (PE) or the respective isotype controls (all BD Biosciences, Franklin Lakes, NJ, USA). After pre-gating on CD3⁺CD4⁺-cells within the lymphocyte gate, the percentage of cytokine-producing cells was measured. A minimum of 10⁵ cells were analyzed by flow cytometry (FACSCalibur; BD Biosciences).

To determine the frequency of Treg cells in peripheral blood of included subjects, unstimulated cells were fixed with formaldehyde, surface marker (CD3, CD4, CD25), and intracellular forkhead box P3 (FOXP3) staining was performed according to the manufacturer's instructions (eBioscience, San Diego, CA, USA). All CD4⁺ FOXP3⁺ were CD25^{high} upon back-gating.

Allergen-specific proliferation assays

Peripheral blood mononuclear cells (10⁵/well) were cultured in serum-free medium (Ultra culture complete; BioWhittaker, Walkersville, MD, USA) supplemented with 2 mM L-Glutamin and 170 mg/l gentamycinsulphate (both from Sigma Chemical Co. BioSciences, St Louis, MO, USA) and stimulated with the respective allergens (Bet v 1, Phl p 1 (Biomay, Vienna, Austria), Fel d 1 (kind gift from Hans Grönlund, Department of Medicine, Clinical Immunology and Allergy Unit, Karolinska Institute, Stockholm, Sweden; all applied at a concentration of 10 μ g/ml), Der p 1 (kind gift from Wayne R. Thomas, Centre for Child Health Research, Telethon Institute for Child Health Research, University of Western Australia, Perth, Australia); OVA (Profos AG Regensburg, Germany); Ara h 1 (kind gift from Clare Mills, Institute of Food Research, Norwich, UK; all applied at a concentration of 50 μ g/ml) for 6 days. At day 6, antigen-specific proliferation was assessed by [³H]-thymidine incorporation assay (Amersham-Pharmacia, Freiburg, Germany). Proliferation was expressed as stimulation index (SI) reflected as fold expression of the unstimulated control. A stimulation index of >2 was considered as positive.

Real-time RT-PCR

To assess the presence of the two regulatory cytokines IL-10 and transforming growth factor (TGF)- β and the Treg cell-associated transcription factor FOXP3 in peripheral

blood of transplanted individuals and controls at certain mRNA level, real-time RT-PCR measurements were taken.

After thawing, cells were counted, and total RNA extraction from 1 Mio PBMCs was performed (Gene Elute Total RNA Miniprep kit; Sigma Chemical Co. BioSciences) and reverse-transcribed into cDNA (SuperScript III First-Strand Synthesis SuperMix; Invitrogen, Carlsbad, CA, USA). Expression levels were assessed by real-time RT-PCR with QuantiTect Primer Assay for IL-10, TGF- β , and FOXP3 (Qiagen, Hilden, Germany) using a Mastercycler[®] ep realplex. Cycle threshold values for genes of interest were normalized to 18S.

The sample with the lowest amount of the respective gene of interest was set as internal standard to one, and relative expression was calculated as follows:
 $2^{-(\text{dCt gene of interest} - \text{dCt lowest sample})}$.

Treg inhibition and depletion assays

To define the inhibitory potency of the putative Treg-cell-subset, CD4⁺CD25⁺ regulatory T cells were isolated using the CD4⁺CD25⁺ T-cell isolation-kit (Miltenyi Biotech, Bergisch Gladbach, Germany) according to the manufacturer's instruction. In brief, CD4⁺ T cells were negatively selected, and thereafter, the CD25⁺ fraction was isolated via positive and the CD25⁻ via negative selection. Fractions had a purity of >95% (CD4⁺CD25⁺) or >92% (CD4⁺CD25⁻).

The regulatory potential of Tregs was assessed by adding CD4⁺CD25⁺ to effector CD4⁺CD25⁻ T cells. Cell subsets alone (either only Treg or Teff) or the combination thereof (1 : 2) were stimulated with 250 ng/ml plate-bound anti-CD3 (clone UCHT1, Dako, Glostrup, Denmark) or the respective antigen in the presence of autologous, irradiated PBMCs (3000 rad; 5×10^4 cells per well), respectively. After 6 days of culture, cells were pulsed with [³H]-thymidine for the last 16 h of the culture. The proliferation is expressed as percent proliferation of the CD4⁺CD25⁻ cells.

Because of limited cell numbers, the net impact of the regulatory compartment on allergen-specific proliferation in PBMCs was measured indirectly via CD25 depletion of PBMCs with CD25⁺ MicroBeads (Purity >95%; Miltenyi Biotech). Proliferation of the respective allergen was measured via [³H]-thymidine incorporation at day 6 as described earlier.

Statistics

Statistical comparison between the groups was performed by nonparametric Mann-Whitney *U*-test or Wilcoxon signed rank test. Correlation was measured via Spearman test. *P*-values were declared significant at levels <0.05.

Results

Patient characteristics

Sixty-five transplanted (Tx) and 18 nontransplanted (Co) children, adolescents, and young adults have been enrolled from 2004 to 2005. Within the transplanted group, 72.3% underwent kidney, 24.6% liver, and 3.1% lung transplantation. The

Table 1 Patients characteristics

	Transplant group	Control group
Number, <i>n</i>	65	18
Gender, m/f (%)	29/36 (44.6%/55.4%)	11/7 (61.1%/38.9%)
Age (years)	13.77 $\pm$ 5.48	24.0 $\pm$ 12.2
mean $\pm$ SD (range)	(1.42–24.25)	(8.8–45.2)
Transplanted organ, <i>n</i> (%)		
Kidney	47 (72.3%)	–
Liver	16 (24.6%)	–
Lung	2 (3.1)	–
Immunosuppression, <i>n</i> (%)		
CS	59 (90.8%)	–
Aza	17 (26.2%)	–
MMF	37 (56.9%)	–
CyA	28 (43.1%)	–
Tac	29 (44.6%)	–
Rap	10 (15.4%)	–
Time on tx (months)	63.36 $\pm$ 51.72	–
mean $\pm$ SD (range)	(2–252)	
Sensitization, % (<i>n</i>)	24.6% (16)	33.6% (6)
Inhalant allergens	18.5% (12)	27.8% (6)
Nutritive allergens	9.2% (6)	27.8% (6)
Allergy, % (<i>n</i>)	12.3% (8)	22.2% (4)
Rhinitis/rhinoconjunctivitis	10.8% (7)	11.1% (2)
Bronchial asthma	4.6% (3)	11.1% (2)
Atopic dermatitis Urticaria	–	5.5% (1)
Food allergy	1.5% (1)	5.5% (1)
Total IgE, % (<i>n</i>)	26.2% (17)	16.7% (3)
Mean $\pm$ SD (range)	56.2 $\pm$ 109.4 (1.9–532)	51.5 $\pm$ 80.1 (1.9–298)

Sixty-five solid organ recipients of kidney, liver, or lung allografts at the Medical University of Vienna were included from 2004 to 2005 and evaluated. Sensitization was defined as SPT and/or CAP-fluoro-enzyme immunoassay positivity.

M, male; f, female; CS, corticosteroids; aza, azathioprine; MMF, mycophenolate mofetil; CyA, cyclosporin A; Tac, tacrolimus; rap, rapamycin.

mean duration of immunosuppression was 65 months. Eight of 65 transplanted individuals displayed clinical symptoms in form of rhinitis/rhinoconjunctivitis, bronchial asthma, or food allergy (Table 1). One-fourth of the population was sensitized to at least one of the tested allergens. Sensitization to allergens was mainly to inhalant allergens. The percentage of sensitization and clinically relevant allergy was comparable between the control group and the transplanted group.

Immunosuppressive medication *in vivo* does not attenuate allergen-specific lymphoproliferation *in vitro*

Antigen-specific responses of PBMCs were compared to a nonimmunosuppressed control population. Allergen-specific proliferation was not compromised in transplanted individuals despite systemic immunosuppression for several years (Fig. 1 A). The time of immunosuppression did not impact the rate of positive responses. Moreover, the two groups did not differ with regard to proliferation in response to IL-2 (Fig. 1B).

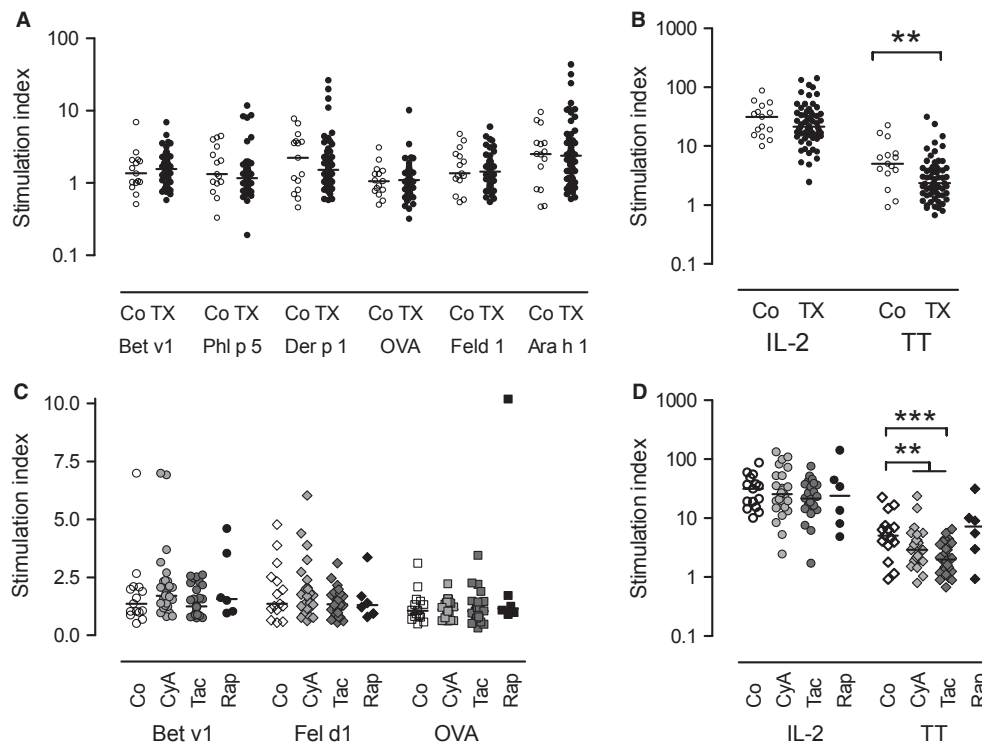


Figure 1 Allergen-specific responses of organ-transplanted individuals are not significantly altered compared to a matched nontransplanted control population. Peripheral blood mononuclear cells from organ-transplanted individuals ($n = 62$) and a matched control population ($n = 15$) were subjected to a panel of relevant

inhalant and food allergens [Bet v1, Phl p5, Der p1, OVA, Fel d1, and Ara h1; (A)] and the recall antigen Tetanus Toxoid (TT; (B)]. Sub-analysis, according to the immunosuppressive regimen taken, was performed [(C, D); $**P < 0.01$, $***P < 0.001$; Mann-Whitney U -test].

It has been reported that mainly Th1-type responses are deprived in transplanted patients. In contrast, it has been hypothesized that an insufficient control of Th2-type allergen-specific responses was related to a more efficient suppression of Th1-type responses (10, 11). In this study proliferation of tetanus toxoid (TT) was significantly reduced in transplanted individuals (Fig. 2B).

Calcineurin inhibitors, in particular tacrolimus, have been linked to allergy development after transplantation (7, 9). Therefore, a subgroup analysis was performed with three important allergens (Bet v1, Fel d1, OVA) and to the recall antigen TT. Again, allergen-specific proliferation was not influenced by any of the immunosuppressive drugs regimens applied (Fig. 1C). However, TT-specific responses were significantly reduced by CNI, and this effect was more pronounced in the tacrolimus (Tac)-treated group when compared to the second CNI cyclosporin A (CyA)-treated group. The mTOR inhibitor rapamycin (Rap), on the contrary, did not impact the TT-specific recall response (Fig. 1D).

CD4⁺ T-cell subsets of immunosuppressed individuals are not significantly altered by calcineurin inhibitor treatment

To investigate whether CNI treatment may alter the general ability of CD4⁺ T-cells to produce Th1-type cytokines after immunosuppression, intracellular cytokine staining was

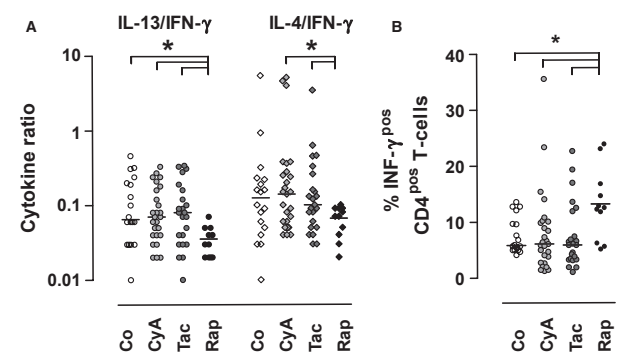


Figure 2 The T-helper cell polarization and calcineurin inhibitors. Peripheral blood mononuclear cells from organ-transplanted ($n = 61$) and a matched control population ($n = 18$) were extensively washed and stimulated for 4 h with PMA and Ionomycin in the presence of Brefeldin A, and intracellular cytokine production was assessed via flow cytometry. The percentage of cytokine (IFN- γ , IL4, and IL13)-producing CD3⁺CD4⁺ cells was measured (Mann-Whitney U -test). Cytokine ratios were calculated from the percentages of positive CD4⁺ T cells.

performed. Interestingly, no general differences were observed, neither with regard to INF- γ , IL-5, and IL-4 producing CD4⁺ T-cells nor with regard to Th1/Th2 ratios calculated

(Fig. 2A). However, rapamycin treatment was associated with an elevated frequency of INF- γ -producing CD4^{pos} T-cells (Fig. 2B) and a more Th1-type response.

Calcineurin inhibitor treatment is associated with a reduced frequency of Tregs in the periphery and reduced FOXP3 expression in the allograft

Suppression of allergen-specific proliferation despite effective T-cell cytokine deprivation by immunosuppression may be because of insufficient control by Treg cells. Therefore, mRNA expression in PBMCs of the Treg-cell marker and transcription factor FOXP3 and the two regulatory cytokines for induced Treg cells IL-10 and TGF- β were measured. Calcineurin inhibitors did neither affect FOXP3 expression nor IL-10 mRNA levels in general. TGF- β was significantly upregulated by either of the immunosuppressive protocols (Fig. 3A). However, upon sub-analysis of the sensitized

individuals, a significant correlation between FOXP3 expression and the number of allergens with a positive proliferative response was observed (Fig. 3B).

To investigate whether mRNA data is reflecting the frequency of Tregs in the periphery at a protein level, FOXP3 protein levels were assessed via intracellular FOXP3 staining of CD4⁺ cells. Both, Tac and CyA, were associated with a significant reduction in the Treg-cell subset (Fig. 3C). Rapamycin-treated patients had a frequency of FOXP3⁺CD4⁺ cells in the periphery corresponding to the nonimmunosuppressed control group (Fig. 3C).

To investigate the impact of immunosuppression in an organ that is both, the allograft and a potential side of sensitization, FOXP3 mRNA expression was assessed in the cellular fraction of bronchoalveolar lavage fluid (BAL) from lung-transplanted individuals that underwent surveillance bronchoscopy more than 6 months post transplantation. Cells from nontransplanted control individuals had signi-

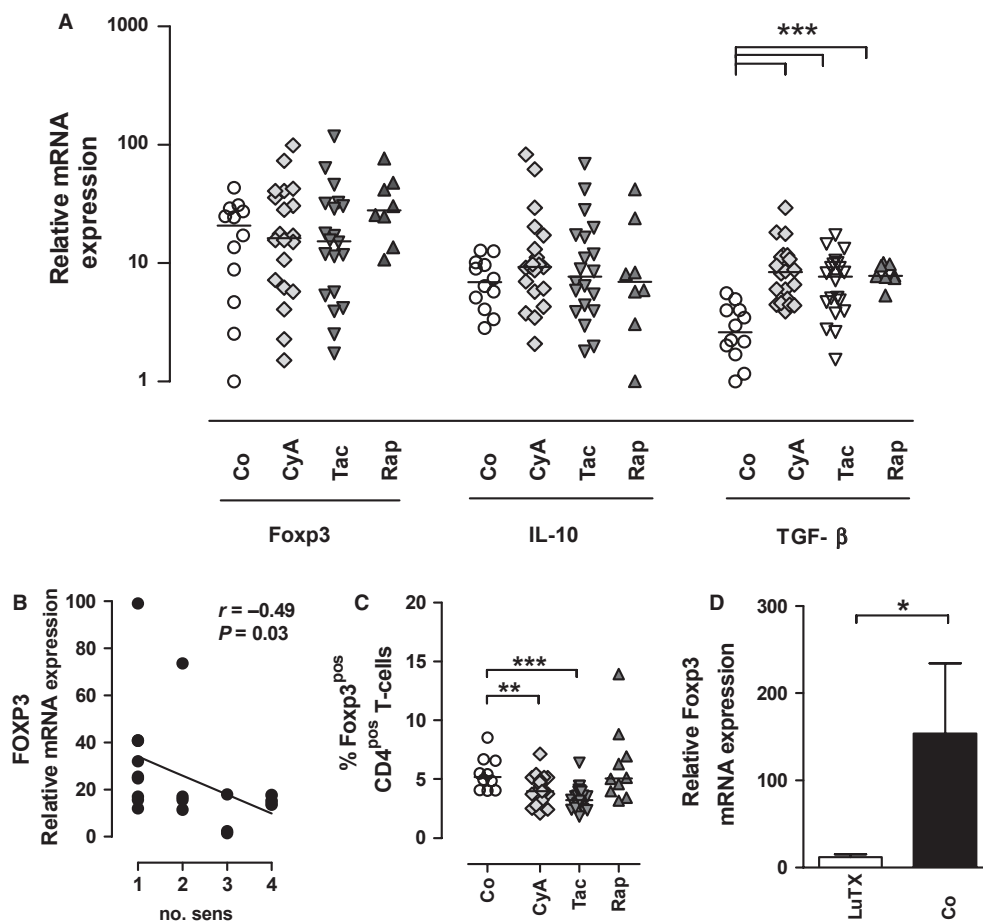


Figure 3 Treg-cell frequency and transforming growth factor (TGF)- β production is modulated by the immunosuppressive medication. Immunosuppression with calcineurin inhibitors (CNI) (Tac $n = 20$, cyclosporin A $n = 20$, Rap $n = 8$) is associated with an upregulation of TGF- β mRNA in peripheral blood mononuclear cells (PBMCs) (A), with a significantly reduced frequency of Tregs (C).

Forkhead box P3 (FOXP3) mRNA expression correlated with the number of allergens PBMCs responded to *in vitro* (B, spearman test). FOXP3 mRNA expression in the cellular fraction of bronchoalveolar lavage from lung-transplanted individuals was significantly reduced (D; LuTX $n = 6$ all CNI treatment; vs Co $n = 11$); $P < 0.05$, Mann-Whitney U -test).

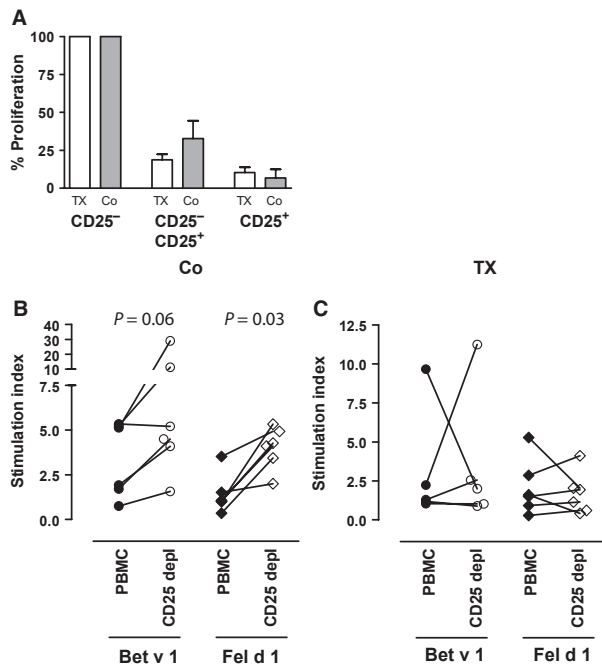


Figure 4 Insufficient control of allergen-specific proliferation by T-regulatory cells from transplanted individuals. CD4⁺CD25⁺ cells from PBMC of transplanted (TX; $n = 6$) and nontransplanted individuals (Co; $n = 3$) were purified, and their suppression of anti-CD3 induced proliferation of CD4⁺CD25⁻ cells measured at a ratio of 1 : 2. (A). Suppressive potential of the CD25⁺ fraction on allergen-specific proliferation was monitored by the removal of the respective fraction from peripheral blood mononuclear cells (B and C; Co $n = 6$; TX $n = 6$).

ificantly higher FOXP3 mRNA levels when compared to those from lung-transplanted individuals (Fig. 3D).

The control of allergen-specific responses by Treg-cells is altered in immunosuppressed individuals

To evaluate possible effects on the potency of the Treg compartment, CD4⁺CD25⁺ T-cells were isolated from PBMCs, and inhibition assays were performed. Tregs from controls and transplanted individuals displayed equal suppressive capacities on TCR cross-linked CD4⁺CD25⁻ T cells if applied at the same number (Fig. 4A).

To measure the impact of the Treg compartment on allergen-specific T-cell responses, CD25⁺ cells were removed. While allergen-specific responses in the control group were consistently upregulated, this was not the case in the transplanted group (Fig. 4B).

Discussion

Comparable rates of allergic sensitization and allergic symptoms in transplanted individuals have been published (3, 4). However, there is scant information on the impact of sys-

temic immunosuppression on allergen-specific responses. In this study, we report minor effects of immunosuppression on allergen-specific responses most likely because of an insufficient control of allergen-specific responses via the Treg-cell compartment.

Although there are numerous reports about tolerance related to the allograft, the impact of systemic immunosuppression on allergen-specific responses and Treg-cell function in the context of allergy is not really understood. Conclusions drawn from studies on allo-T-cell responses do not necessarily have to be identical with allergen-specific responses because a more prominent effect of Th1-type responses is hypothesized (7, 9–11).

To date, type I allergy in transplanted individuals has mainly been linked to the CNI tacrolimus but not cyclosporine A and mainly to liver transplanted children that are transplanted at a very early time point in life. We do see minor, but nonsignificant differences between these two CNI with regard to FOXP3⁺ CD4⁺ cells which is in line with our clinical outcomes that suggested a higher but nonsignificantly increased percentage of sensitization in the Tac-treated group (3).

We demonstrate that the potency of Treg-cells to suppress TCR-related stimulation is not significantly altered when compared to nontransplanted individuals. However, there may be a partial loss of allergen-specific tolerance in conjunction with the lower number of Tregs present in total. As there is no doubt about the efficacy of CNI in suppressing T-cell cytokine response and proliferation, a partial break of allergen-specific tolerance via an insufficient control of allergen-specific responses by the Treg-cell compartment may explain a comparable frequency of allergen-specific T-cell responses after long-time immunosuppression (mean time > 5 years).

The approach taken in this study differs from many *in vitro* studies undertaken in the field of transplantation so far. Cellular responses from transplanted individuals were not investigated *in vitro* in the presence of the immunosuppressive drug. This is an important point because the anti-proliferative and general suppressive effects of CNI on Th1- and Th2-cells are described. However, it is not clear what kind of effect long time immunosuppression has on allergen-specific responses. At present, several hypotheses try to explain the insufficient control of allergic diseases under immunosuppression. One of the most prominent theories proceeds from the assumption that a more efficient suppression of Th1-type cytokines and of IL-2 is responsible for a relative shift direction Th2. This is not fully supported by our data as the commitment of PBMCs of transplanted patients did not differ significantly from the control cohort as expressed by the Th1/Th2 ratio. However, there is indirect evidence which favors this hypothesis: proliferative responses to the recall antigen TT which is known to be of a more Th1-type are significantly lower in the immunosuppressed individuals whereas allergen-specific proliferation is not. Interestingly, the diminished responses to TT were not observed in the rapamycin-treated group. Data from mouse experiments also support the inability to treat established allergic response via inhibition of T-cell co-stimulation (23).

Allograft tolerance in lung-transplanted individuals has been linked to local FOXP3 expression in tissue (24). We recruited a small cohort of patients that underwent surveillance bronchoscopy after lung transplantation and under CNI treatment and compared it with a group of nontransplanted individuals with bronchoscopy because of foreign body aspiration, recurrent infections for unknown reasons or malformations and compared the FOXP3 expression of BAL cells. We do believe that lower amount of FOXP3 expression reflects a reduced degree of Treg-cell function as patients included had no apparent clinical signs of infection. However, an upregulation of FOXP3 by a latent subclinical pro-inflammatory state cannot be completely excluded.

Patients treated with the mTOR inhibitor rapamycin displayed a higher Foxp3 mRNA expression and a higher frequency of FOXP3⁺ CD4⁺ cells in the periphery when compared to CNI-treated individuals. In addition, T-cells tended to a more Th1-type response upon stimulation with

PMA/Ionomycin. This is in line with recent data (25, 26). However, the number of individuals included does not allow conclusions about its impact on clinical symptoms and sensitization.

In conclusion, this study systemically defines the expression of Treg-cells and regulatory markers in general and at an allergen-specific level in organ-transplanted children, adolescents, and young adults. Allergen-specific proliferation is not altered after immunosuppressive therapy in organ-transplanted individuals despite potent suppression of T-cell responses. This lack of control of allergen-specific responses may relate to concomitant suppression of the T-reg-cell compartment and the effector T-cell compartment by immunosuppressive drugs.

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