

ORIGINAL ARTICLE

Allergic sensitization in kidney-transplanted patients prevails under tacrolimus treatment

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Summary

Background Type I allergies have repeatedly been reported after solid organ transplantation despite T cell-targeted immunosuppressive therapy. A causal relationship with tacrolimus has been proposed.**Objective** The present study directly compared the occurrence of allergic sensitization and disease under tacrolimus- vs. cyclosporin A-based immunosuppressive therapy.**Methods** The prevalences of IgE-mediated sensitization and allergy were assessed in a cross-sectional study of kidney-transplanted adults receiving tacrolimus ($n = 100$) or cyclosporin A ($n = 100$). Methods included a standardized questionnaire, skin prick test and measurement of total and specific IgE against common nutritive and inhalant allergens.**Results** The prevalence of sensitization was significantly higher in the tacrolimus- than in the cyclosporin A-treated group (34%, $n = 34$, vs. 20%, $n = 20$; $P = 0.026$). The rate of clinically relevant allergy in patients receiving tacrolimus was twice that in patients receiving cyclosporin A (15%, $n = 15$, vs. 8%, $n = 8$; $P = 0.12$). No other factor (age, serum drug level, concomitant immunosuppressive medication, time since transplantation, underlying disease) was found to have an influence on sensitization or allergy prevalence (logistic regression).**Conclusion and Clinical Relevance** Our results suggest that post-transplant immunosuppression with tacrolimus is associated with an increased occurrence of IgE-mediated sensitization and probably manifestation of allergic disease, which has to be treated specifically despite immunosuppressive therapy.**Keywords** calcineurin inhibitor, IgE, immunosuppressive therapy, tacrolimus, transplantation
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Introduction

Lifelong immunosuppressive therapy is a prerequisite for successful organ transplantation. The immunosuppressive drugs applied target mainly the specific immune response, in particular T lymphocytes [1]; consequently, some of the same drugs applied in transplantation medicine are used in severe allergic diseases as well [2, 3]. Therefore, one would expect that transplant recipients do not show clinical signs of type I allergy. However, allergic diseases have repeatedly been observed in patients after solid organ transplantation. The majority of reports describe food allergy in young liver-transplanted children receiving tacrolimus immunosuppression [4–7]. The prevalences observed in these patients range from 6 to 20%

[8, 9]. Fewer publications report other types of post-transplant allergies, such as allergic asthma or atopic dermatitis [8], and other patient collectives, such as heart and intestinal transplant recipients [10, 11].

The factors explaining the failure of immunosuppressive therapy to prevent type I hypersensitivity in transplant recipients are not entirely clear; however, several mechanisms have been proposed. First, by suppression of predominantly T-helper (Th) 1 cytokine production (IL-2, IFN- γ), calcineurin inhibitors (CNI) might lead to an imbalance of T cell response towards a more Th2-shifted cytokine profile, thereby predisposing to allergic reactions [5, 12]. Secondly, immunosuppressants could inhibit the regulation of allergic disease by interference with generation or function of regulatory T cells [13]. Thirdly, some of

the underlying diseases leading to organ transplantation, such as minimal change glomerulonephritis or cystic fibrosis, have been found to be associated with increased allergy prevalence [14, 15]. Finally, a potentially allergen-uptake promoting effect in the gut mucosa by tacrolimus [5, 7] and the transfer of allergy from donor to transplant recipient especially after bone marrow or liver transplantation [16, 17] have been described.

We previously assessed the prevalence of type I sensitization and allergic disease in 78 kidney-, liver- and lung-transplanted children and 42 lung-transplanted adults. Concordant with other publications, we could confirm the existence of type I allergies despite immunosuppressive therapy in these patients. Allergic symptoms were found in comparable numbers as in the general non-immunosuppressed population [18–20] and occurred in all transplant recipients regardless of the type of transplanted organ. Moreover, allergies against inhalant allergens, such as allergic rhinoconjunctivitis and asthma, prevailed. Interestingly, we observed higher rates of sensitization and allergy in tacrolimus-treated patients compared with patients receiving cyclosporin A (CsA) in both study groups. However, this difference was not statistically significant [21, 22].

The present study was therefore planned to directly compare these two drugs regarding the occurrence of allergic sensitization and clinically relevant allergic disease in a larger patient population. Secondary aim was to reassess other potential risk factors for post-transplant allergic sensitization, including patient age, gender, time since transplantation, serum drug level, concomitant immunosuppressive medication and underlying disease.

Methods

The study protocol was approved by the Ethics Committee of the Medical University of Vienna.

Study population

Patients were approached consecutively at the outpatient clinic of the Department of Internal Medicine, Division of Nephrology, Medical University of Vienna. Inclusion criteria were kidney transplantation, tacrolimus- or cyclosporin-based immunosuppression for at least 3 months and age between 18 and 55 years. Sample size was calculated by power analysis using the results of our survey on allergies in lung-transplanted adults (chi-square test) [22]. Patients assessed in our previous studies on the topic of post-transplant allergic disease were not included in the analysis. A total of 228 patients meeting the inclusion criteria were approached; 28 (CsA: 14, tacrolimus, 14) declined to participate. Immunosuppressive therapy, time since transplantation, age at transplantation, year of transplantation, serum drug level of

cyclosporin A or tacrolimus and underlying disease leading to organ transplantation were assessed.

Interview

Data on symptoms of atopic disease, i.e. allergic rhinoconjunctivitis, allergic bronchial asthma, atopic dermatitis and food allergy were collected by a detailed questionnaire. Patients were classified as having actual atopic disease if they reported allergic symptoms in relationship with allergen exposure during the previous 12 months that concurred with the results of skin prick test (SPT) or specific IgE measurement. Personal history of allergic disease before transplantation was not assessed. Family history of type 1 allergy was ascertained for first-degree relatives. Because allergies tend to be overestimated in self-report [23, 24], questionnaires were conducted in form of an interview by a trained physician blinded to the immunosuppressive treatment used.

Skin prick testing

SPTs were performed following established guidelines with commercially available allergen extracts (ALK-Abelló, Hamburg, Germany) [25]. A panel of nine common allergens was tested, including birch pollen, grass pollen mix, *Dermatophagoides pteronyssinus*, *Alternaria alternata*, *Cladosporium herbarum*, peanut, cat dander, dog dander and hen's egg protein. A weal diameter ≥ 3 mm was regarded as a positive test result. All interviews and SPTs were performed by the same investigator, who was blinded to the immunosuppressive treatment used.

Total and specific serum immunoglobulin E measurement

The total and specific serum IgE measurement was conducted using ImmunoCAP (Phadia, Uppsala, Sweden) according to the manufacturer's instructions. Specific IgE levels were determined using screening assays for nine common inhalant (timothy grass, rye, birch and mugwort pollen, house dust mite, cat and dog dander, *Alternaria alternata* and *Cladosporium herbarum*) and six common nutritive (hen's egg, cow's milk, codfish, wheat flour, soy bean and peanut) allergens. Results were assigned to CAP classifications 0–6. Specific IgE levels above the detection limit of 0.35 kU/L (CAP class ≥ 1) were considered as positive. Total serum IgE was measured within a range of 2–5000 kU/L.

Blood differential count

To assess eosinophilia (defined as $> 6\%$ eosinophilic granulocytes of total white blood cells), blood differential

count was measured in all patients at the time of IgE measurement.

Statistical analysis

Statistical analysis was performed using SAS-9.1 and SPSS for Windows, version 17.0. Univariate analysis was performed to detect an association of immunosuppressive therapy and other potential risk factors with sensitization, allergy, eosinophilia or total IgE. The χ^2 test was used to compare categorical variables, while numeric variables were analysed using a *t*-test. Additionally, a stepwise multivariate logistic regression model was applied for each response variable.

Results

Patient collective

The total sample size consisted of 200 patients (117 male, 83 female), including 100 individuals receiving tacrolimus- and 100 individuals receiving CsA-based immunosuppressive regimens. Interview, IgE measurement and blood differential count were performed in all patients; 164/200 (82%) patients additionally underwent SPT. The mean patient age was 40.8 ± 8.2 years (range: 18–54.6). Patients had undergone transplantation 3–353 months before study inclusion (median time post-transplantation: 97 months). The indications for transplantation are listed in Table 1. Serum trough levels of CsA (C0-level) ranged from 29 to 424 ng/mL, serum trough levels of tacrolimus from 1.9 to 13.2 ng/mL. In 22 patients, CNI was switched during the post-transplant period before study inclusion; all of them were on the same CNI for at least 12 months. Concomitant immunosuppressive therapy included corticosteroids ($n = 154$), azathioprine ($n = 26$), mycophenolate mofetil ($n = 143$) and rapamycin ($n = 1$).

Detailed patient characteristics of the two groups (tacrolimus- vs. CsA-based immunosuppression) are shown in Table 2. The samples did not differ significantly in age, gender, family history of atopy and underlying disease. A difference was found regarding total time of immunosuppression, with the median time since first transplantation being higher in patients receiving CsA. Moreover, CsA-treated patients were more likely to receive azathioprine, whereas tacrolimus-treated patients more commonly received mycophenolate mofetil as concomitant immunosuppressive medication.

Sensitization

Patients were classified as sensitized in the presence of a positive SPT and/or positive-specific IgE. On comparing patients according to their immunosuppressive regimen, we found a sensitization rate of 34% (34/100) in the

Table 1. Underlying disease leading to kidney transplantation in patients under immunosuppression with cyclosporin A or tacrolimus

Underlying disease	CsA (<i>n</i> = %)	Tacrolimus (<i>n</i> = %)
Glomerulonephritis, unclassified	14	18
Focal segmental glomerulosclerosis	5	7
IgA nephropathy	3	8
Rapidly progressive glomerulonephritis	3	2
Cystic kidney disease	7	11
Vesicoureteral reflux	9	7
Diabetes mellitus type 1	2	7
Inborn kidney dysplasia/aplasia	4	2
Recurrent pyelonephritis	5	1
Urolithiasis	2	4
Alport syndrome	2	2
Hypertensive nephropathy	2	2
Other: systemic lupus erythematosus (3), amyloidosis (3), Goodpasture's syndrome (3), diabetes mellitus type 2 (3), tumor (2), juvenile nephronophthisis (2), toxic (2), septic (1), transfusion-induced kidney damage (1), hyperuricaemia (1), hereditary renal tubular acidosis (1), interstitial nephritis (1), Henoch-Schönlein purpura (1), leucocytoclastic vasculitis (1)	13	12
Terminal renal insufficiency of unknown origin	29	17

tacrolimus group versus 20% (20/100) in patients receiving CsA (Fig. 1). Univariate statistical analysis demonstrated this difference to be significant ($P = 0.026$, Chi-square test). Thus, patients receiving tacrolimus were significantly more likely to be sensitized against one or more allergens than patients receiving CsA (Fig. 1).

The sensitization profile was comparable in both groups, with sensitization against inhalant allergens being much more common than against nutritive allergens (inhalant/nutritive: CsA 19/4, tacrolimus 34/4). No influence of immunosuppression was observed on the height of specific IgE (categorized into CAP classes) or on the number of sensitizations.

Allergy

Similarly, clinically significant allergy was found considerably more often in tacrolimus- than in CsA-treated patients. Fifteen percent (15/100; 15%) had an allergy in the tacrolimus group, as compared with 8% (8/100; 8%) in the CsA group. However, this difference was not statistically significant ($P = 0.12$; Fig. 1).

In both groups, the most common symptoms were allergic rhinitis or rhinoconjunctivitis. One patient treated with tacrolimus and CsA each had symptoms of bronchial asthma, while the only patient presenting with atopic dermatitis received a CsA-based immunosuppression.

Table 2. Characteristics of tacrolimus- and CsA-treated transplant recipients

	Cyclosporin A (n = 100)	Treated transplant recipients	P-value
Age in years	41.9±8.1	39.8±8.1	0.52
Mean±SD (range)	(21.9–54.2)	(17.6–54.7)	
Age at 1st tx in years	29.9±9.8	32.2±9.4	0.09
Mean±SD (range)	(4.9–52.9)	(8.5–54.1)	
Gender m : f	55 : 45	62 : 38	0.32
Time since tx in months	147.6 (137.9)	60.2 (216.9)	<0.01*
Median (IQR) (range)	(3.7–353)	(3–328)	
<i>Underlying disease (n = 145)</i>			
% imm.-mediated	45.7%	58.5	0.12
(imm.-/non-imm.-mediated; n)	(32/70)	(48/82)	
<i>Concomitant is Medication (n=%)</i>			
Corticosteroids	74	80	0.31
Azathioprine	22	4	<0.01*
Mycophenolate mofetil	60	83	<0.01*
Rapamycin	1	–	0.32
Positive family history for atopy (n = %)	20	20	1.00

m, male; f, female, imm., immunologically; IQR, interquartile range; n, number; SD, standard deviation; tx, transplantation.

Total immunoglobulin E and eosinophilia

The prevalence of elevated total IgE (mean total IgE 37.3±81 kU/L; range <2–834) was rather low in both patient groups, with 10% (20/200) of all patients displaying elevated total IgE. Eosinophilia was present in 30 patients (30/200; 15%). As expected, both total IgE and eosinophilia were more prevalent in sensitized and allergic individuals ($P<0.01$, Wilcoxon rank sum test). No influence of treatment group (CsA or tacrolimus) on the risk of increased IgE (10% in both groups; median total IgE 38.7±94.2 vs. 35.9±65.8 kU/L) or eosinophilia (14% vs. 16%) was found.

Skin prick test vs. specific immunoglobulin E

The results of SPT and specific serum IgE measurement were not consistent in all patients, with a higher rate of positive SPT than positive specific IgE (27% vs. 18.5%). This difference was more pronounced in patients receiving tacrolimus. Forty-one percent (14/34; 41%) of patients in the tacrolimus group and 10% (2/20; 10%) of patients receiving CsA had a positive SPT but no specific IgE. In contrast, only two patients (2/54; 3.8%), both receiving CsA, had elevated specific IgE but a negative SPT. Thirty-six patients (18%, 14 receiving tacrolimus, 22 receiving CsA) declined SPT. Of these, four in the tacrolimus and three in the CsA group had positive specific IgE.

Other tested variables

Sensitized and allergic patients were more likely to have a family history of atopy (see Table 3). This was found equally in the tacrolimus and the CsA group.

No influence of the other variables, including age (at transplantation and at evaluation), year of transplantation, gender, time since transplantation, concomitant immunosuppressive medication, induction therapy, number of transplantations and underlying disease (immunologically mediated vs. non-immunologically mediated), on sensitization, allergy, total IgE or eosinophilia was found in univariate analysis (chi-square, Wilcoxon rank sum and *t*-test; Table 3).

Multivariate analysis

To adjust for potentially confounding factors, a stepwise logistic regression model was performed for the target variables sensitization and allergy accounting for the factors immunosuppression (CsA vs. tacrolimus), underlying disease, age, gender, number of transplantations, concomitant immunosuppressive medication and time since first transplantation. As in univariate analysis, the results showed a significantly higher risk for sensitization under tacrolimus treatment ($P = 0.027$, OR 2.06: L-95%CL: 1.09 U-95%CL: 3.91). None of the other factors had an effect on sensitization or allergy prevalence.

Subgroup analysis was performed to investigate the effect of serum drug level (tacrolimus or cyclosporin A, respectively) on sensitization and allergy again by stepwise logistic regression separately for the two treatment groups. In both subgroup analyses, no significant influence of serum drug level was found.

Discussion

The presence of post-transplant allergic disease was first described by Lacaille et al. [12]. Since then, various case-

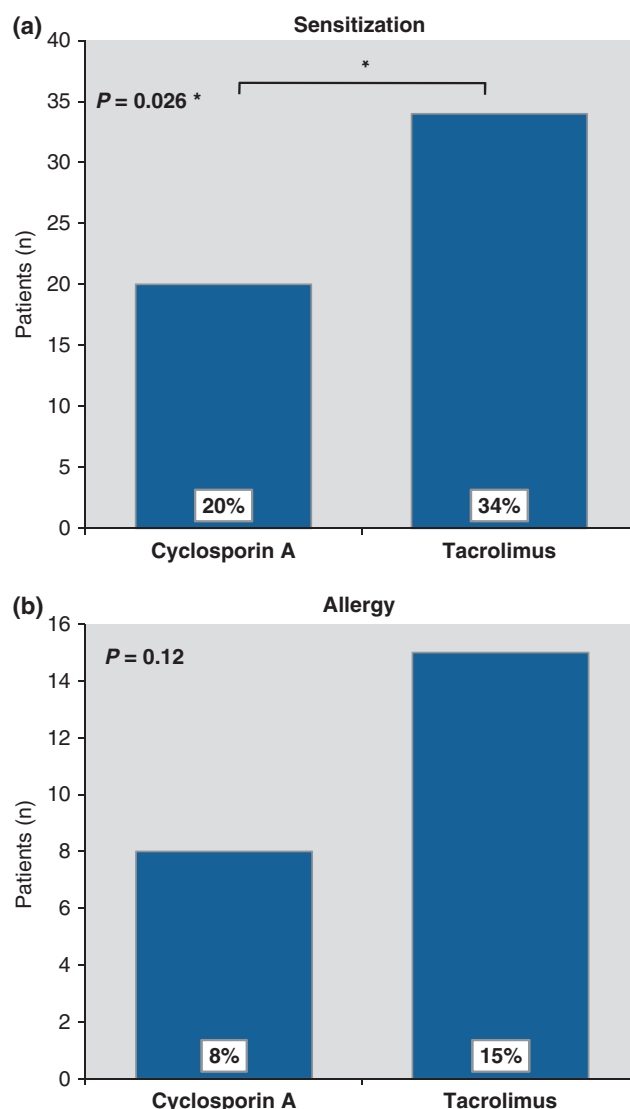


Fig. 1. Sensitization and allergy prevalence according to immunosuppressive therapy (CsA vs. tacrolimus) (A) Patients treated with tacrolimus had a significantly higher risk for atopic sensitization than patients receiving CsA ($P=0.026$). (B) The prevalence of clinically manifested allergic disease was higher in the tacrolimus group compared with the CsA group, although this was not statistically significant ($P=0.12$). $n = \%$.

series have been reported [5, 6, 9, 17]; however, to date, the reason for this phenomenon is unclear. Tacrolimus was repeatedly suspected to be a risk factor or even the cause for post-transplant allergy [5, 12] as only very few reports of IgE-mediated allergic reactions existed in patients receiving CsA [9, 17]. As earlier publications were mainly case reports or not focusing on that particular issue, this theory was not verified.

In the present cross-sectional study, we demonstrate allergy and sensitization rates in immunosuppressed kidney-transplanted adults, which are comparable to those in the general population [20, 26]. This is in line with earlier

results in other subgroups of organ-transplant recipients [5, 9, 21, 22]. New in the present analysis is the evidence of a significantly higher sensitization prevalence in transplant recipients treated with tacrolimus compared with patients receiving CsA. Based on the fact that the prevalence of sensitization varies significantly in these two groups, which differ only by the CNI used in their immunosuppressive protocol, we assume that these immunosuppressants have an effect on Th2-mediated immune responses.

Both tacrolimus and CsA are CNI, which reduce the transcription of growth and activation factors of lymphocytes, most importantly IL-2. Despite the fact that both CNI have the same intracellular target, tacrolimus has been shown to be more effective in preventing acute rejection episodes in kidney transplant recipients and is associated with increased early graft survival [27]. These differences in clinical efficacy as well as the distinct side-effect profiles of the two drugs could be related to certain additional features in their modes of action. Understanding the specific molecular targets is essential in the development of a more specific immunosuppressive therapy. Our results, showing a different effect of CsA and tacrolimus on Th2-mediated immune responses, are thus of interest.

One of the underlying mechanisms for the higher sensitization rate in tacrolimus-treated patients might be a variable effect of CNI on distinct Th cell cytokines. Dysregulation of the Th1/Th2 balance has repeatedly been considered to be the underlying cause of the phenomenon of post-transplant allergy [5, 12]. A more profound Th1/Th2 shift by tacrolimus as compared with CsA therapy has been proposed and is supported by own *in vitro* assays showing a dose-dependent Th1/Th2 shift in patients under tacrolimus but not CsA therapy (see supporting information).

Moreover, tacrolimus seems to have an inhibitory effect on the generation of immune regulatory T cells (T_{regs}), which has not been described under CsA therapy [13]. We recently performed *in vitro* assays on peripheral blood mononuclear cells (PBMCs) of transplanted patients to outline the role of allergic immune response regulation after transplantation. We showed a decreased number of CD4+CD25 T_{regs} in transplanted patients, which was more prominent in patients receiving tacrolimus compared with patients receiving CsA [13].

A third distinct characteristic of tacrolimus is its negative effect on the energy production of intestinal mucosa cells, thereby potentially impairing gut barrier, facilitating allergen uptake and promoting allergic disease [28]. This effect has not yet been demonstrated on other mucosal surfaces, but it is conceivable that the same effect also increases allergen uptake in the mucosa of the respiratory tract system.

The present study was designed to directly compare the prevalence of allergic sensitization and disease in a large collective of transplanted patients receiving two different

Table 3. Comparison of sensitized and non-sensitized kidney transplant recipients

	Non-sensitized (n = 146)	Sensitized (n = 54)	Non-allergic (n = 177)	Allergic (n = 23)
Age in years	41.3±7.9	41.7±9.0	40.9±7.9	40.0±10.3
Mean±SD (range)	(20.5–54.7)	(17.6–54.1)	(20.5–54.7)	(17.6–54.1)
Time since tx in months	94.3	97.0	97.7	60.7
Median (range)	(3.3–353.1)	(3.0–300)	(3.3–353.1)	(3.0–300)
<i>Underlying disease (n = 145)</i>				
% imm.-mediated	56.4	42.8	53.0	50.0
(imm./non-imm.-mediated; n)	(62/48)	(18/24)	(70/62)	(10/10)
<i>Concomitant is medication % (n)</i>				
Corticosteroids	76.0 (111)	79.6 (43)	75.1 (133)	91.3 (21)
Azathioprine	13.7 (20)	11.1 (6)	13.3 (23)	13.0 (3)
Mycophenolate mofetil	72.6 (106)	68.5 (37)	72.3 (128)	65.2 (15)
Rapamycin	0.86 (1)	–	0.56 (1)	–
<i>Serum drug level</i>				
CsA (ng/mL) mean±SD	91.0±37.0	83.5±48.2	85.5±49.8	90.0±25.6
Tacrolimus (ng/mL) mean±SD	6.8±2.4	6.7±2.5	6.8±2.5	6.6±2.2
Positive family history of atopy % (n)	17.8 (26)	25.9 (15)	18.6 (33)	30.4 (7)

CsA, cyclosporin A, imm., immunologically, is, immunosuppressive, n, number, SD, standard deviation, tx, transplantation.

CNI in order to confirm the previously reported trend of an increased risk in tacrolimus-treated patients. The cross-sectional design applied has the drawback that we could not prospectively analyse the development of allergic sensitization. We did not assess pre-transplant allergic disease retrospectively as such self-reported data are highly inconsistent [24]. A prospective comparison of tacrolimus and CsA, however, is difficult because of the limited use of CsA in newly transplanted patients. Thus, we do not have valid data on atopic status before transplantation. We consequently do not know whether the increased risk for sensitization in tacrolimus-treated patients is due to a more allergy-promoting or rather a less allergy-suppressive activity of tacrolimus. The question of how sensitization and allergy rate changed by transplantation or transplantation-associated factors, such as immunosuppression, and the verification of the hypothesis that post-transplant immunosuppressive therapy facilitates new development of allergic disease thus needs to be approached by a prospective study, which is ongoing. Nonetheless, even the persistence of a pre-existing allergy after transplantation is surprising, considering the profound immunosuppressive therapy in these patients.

Because of the non-prospective nature of our study, subjects were allocated to either CsA- or tacrolimus-based immunosuppression according to various criteria, including the date of transplantation, participation in other clinical trials, number of transplantations and individual tolerability or comorbidities. To check for potential confounders, we analysed the two groups for other differences besides the type of CNI. We found a longer transplantation time in the CsA group and different rates of azathioprine and mycophenolate mofetil medication. An effect of these factors on sensitization and allergy prevalence was excluded by multivariate logistic regres-

sion. Both transplantation time and concomitant immunosuppressive medication were consistent between sensitized and non-sensitized as well as allergic and non-allergic patients (Table 3). However, we cannot exclude completely the possibility that other, non-assessed factors, such as distinct comorbidities in the two groups, contribute to the differences in sensitization rate.

In contrast to previous reports describing mainly food allergies after transplantation, we observed predominantly inhalant allergies. We think that this discrepancy is related to the higher age of our recipients compared with the patients presented by other authors. In the general population, food allergy prevails in the early years of life and then decreases significantly in prevalence [29]. It is conceivable that young children are especially vulnerable to react to the effects of immunosuppression with symptoms of food allergy, whereas older transplanted children and adults rather display symptoms of allergic disease more common in that age, like allergic rhinoconjunctivitis or asthma. Moreover, we focused on kidney transplant recipients, which might share some but not all mechanisms relevant for the explanation of tacrolimus-associated food allergy in liver-transplanted children. An effect of liver transplantation, which might not occur via transplantation of other solid organs, is the possible transfer of allergy by sensitized lymphocytes or pluripotent blood stem cells [17, 30]. Also, maintenance immunosuppression after liver transplantation is lower in dosage and number of drugs compared with that after transplantation of other solid organs.

We did expect higher levels of total IgE in our patients, as there have been reports of elevation of total IgE under CNI application [9, 11, 31]. However, contradictory findings on reduced or unchanged IgE expression in mice models and humans under CNI application have also been

reported [32–34]. It seems that the effects of CNI on serum IgE depend on the drug concentration, drug administration methods and target tissue [32, 35]. Another unexpected finding was the high discrepancy between the results of SPT and specific serum IgE measurement, which was much more prominent in patients receiving tacrolimus than in patients under CsA treatment. Differences of up to 30% (mostly positive SPT with negative immunoassay) have been reported in the literature. These discrepancies are thought to be more owing to the restraints of the immunoassay, with limitations in the detection of lower IgE levels [36]. As total IgE was low in both groups, we suggest that soluble serum IgE is reduced by immunosuppressive therapy, while allergic reactions, including the weal-and-flare-reaction of the prick test, can still be mediated by cell-bound specific IgE. The topical application of tacrolimus successfully suppresses the skin lesions of atopic dermatitis; however, this is rather based on its inhibitory effect on proinflammatory cytokines than on its anti-allergic effects [37].

In contrast to some previous case reports of very severe food allergies in liver-transplanted children, the observed allergic symptoms in our patient collective were mostly mild to moderate and did not include life-threatening reactions. A direct effect on the choice of immunosuppressive agent is thus not given by our results. However, physicians involved in the care of transplant recipients should be aware of the possibility of allergic reactions also in adult solid organ transplant recipients to allow early diagnosis and to avoid misinterpretation of symptoms as infections or side-effects of post-transplant medication. Especially in tacrolimus-treated patients, the personal history of allergic disease should be investigated before transplantation, and thorough work-up of symptoms suggestive of type 1 allergy including measurement of specific IgE levels or a SPT should be performed after transplantation. Adequate anti-allergic treatment in

affected individuals is required in addition to immunosuppressive therapy.

Finally, analysis of the effects of CNI on type I hypersensitivity reactions should lead to a better understanding of rejection and tolerance mechanisms. On the one hand, Th2 cells have been shown to play a role in transplant rejection when classical pathways of rejection are inhibited, for example by recruiting eosinophils and by contributing to vasculopathy in chronic rejection [38]. Thus, it is conceivable that distinct effects of CNI on Th2-type immune responses also have an influence on graft survival. On the other hand, the effect of CNI on CD4+CD25 high T_{regs} might have an influence on the development or failure to achieve graft tolerance [39]. Further *in vitro* studies addressing the influence of CsA and tacrolimus on regulatory mechanisms in detail are of interest.

In conclusion, in this first study to directly compare post-transplant allergy and sensitization rates in patients receiving different CNI in their immunosuppressive protocol, we demonstrate a significantly increased prevalence of post-transplant sensitization and, albeit not statistically significant, a higher rate of clinically relevant allergy in patients receiving tacrolimus.

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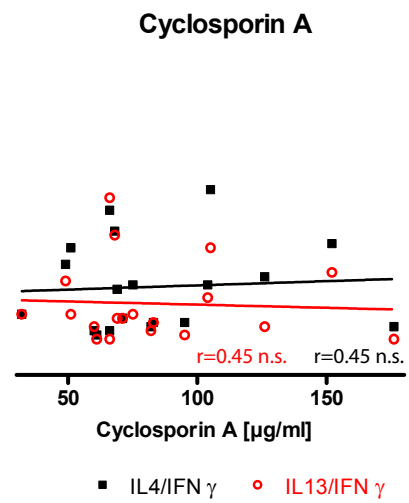
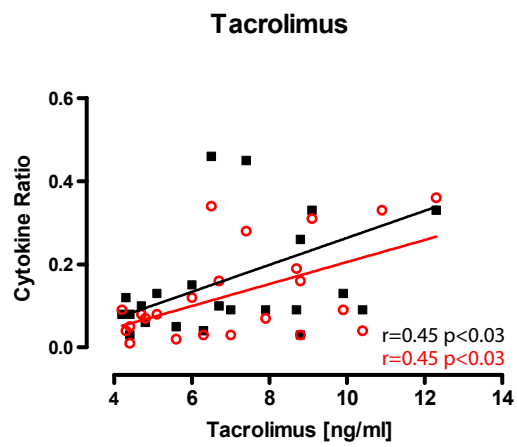
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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Allergic sensitization in kidney-transplanted patients prevails under tacrolimus treatment.

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Allergic sensitization in kidney-transplanted patients prevails under tacrolimus treatment

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METHODS

Peripheral blood obtained from 62 recipients of kidney-, liver- or lung allografts (29 male, 33 female, aged 1.4 to 24.3 years, mean age 14.6 ± 5.5 years) were recruited from 2004 to 2005 as described by Eiwegger et al. Blood concentration of tacrolimus (whole blood trough level) or cyclosporin A (second hour serum level) was measured in all patients at time of PBMC sampling.

Peripheral blood mononuclear cells (PBMCs) were isolated by density-gradient centrifugation over Ficoll-Paque (Pharmacia, Uppsala, Sweden). Prior to cytokine staining, PBMCs were stimulated with 10 ng/mL phorbol myristateacetate and 1.25 mM ionomycin in the presence of 5 mg/mL Brefeldin A (all from Sigma Chemical Co. Bio Sciences, St Louis, MO, USA) for 4 hours. Thereafter, cells were washed, fixed, permeabilized and stained for CD3, CD4, INF- γ , IL-13, IL-4 and the respective isotype controls (all BD Biosciences, NJ, USA). A minimum of 10^5 cells was analyzed by flow cytometry (FACSCalibur, BD Biosciences, NJ, USA).

RESULTS

Ratio of T helper (Th) 1 (INF- γ) and Th2 (IL-4, IL-13) cytokines were calculated and correlated to blood concentration of CsA and tacrolimus. A significant correlation of both INF- γ /IL-4 and INF- γ /IL-13 to tacrolimus concentration was found. In contrast, calcineurin A serum concentration did neither correlate with INF- γ /IL-4 nor with INF- γ /IL-13 ratio (Spearman test).

Figure legends

Figure E1: Correlation of Th1/ Th2 cytokine ratio to serum levels of tacrolimus and CsA

Peripheral blood mononuclear cells from 62 kidney-, liver- and lung-transplanted children and adolescents (mean age 14.6 ± 5) were stimulated for four hours with PMA and ionomycin in the presence of Brefeldin A. The ratio of INF- γ to IL-13 or IL-4 producing cells significantly correlated with blood concentration of tacrolimus (whole blood trough level) but not CsA (second hour serum levels). Spearman test $p < 0.05$