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ORIGINAL ARTICLE

Sirolimus in renal transplant recipients with tuberous sclerosis complex: clinical effectiveness and implications for innate immunity

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Summary

Tuberous sclerosis complex (TSC) is caused by constitutively activated mammalian target of rapamycin (mTOR) resulting in nonmalignant tumours of several organs and consequently renal failure. Recent reports suggest a possible beneficial role of the mTOR-inhibitor (mTOR-I) sirolimus for TSC; however, safety and efficiency of sirolimus in TSC patients after renal transplantation, both as primary immunosuppressant as well as anti-proliferative agent, are still undefined. Moreover, it is currently unknown whether the TSC mutation affects the primary immune response in these patients. In this article, we report on three TSC patients after renal transplantation who have been converted from a calcineurin-inhibitor (CNI)-based immunosuppression to sirolimus. During 2 years of follow-up, renal allograft function was stable or even improved, and no significant sirolimus-associated side-effects were noted. Beneficial effects of sirolimus against TSC were detected in the skin, along with improved spirometric measurements and an arrest of astrocytoma progression. We show that the inflammatory immune response was significantly altered in TSC patients as compared with controls and sirolimus potently affected both inflammatory cytokine production and vascular endothelial growth factor levels in these patients. Larger studies are warranted to further examine the relationship between clinical parameters and the molecular response to mTOR-inhibition in TSC patients after renal transplantation.

Introduction

Tuberous sclerosis complex (TSC) is an autosomal-dominant multisystem disease that affects at least one out of 6000 live-born [1]. TSC is caused by various mutations, affecting at least one allele in the tumour-suppressor genes *TSC1* and *TSC2*. Somatic mutations in the corresponding second allele, which have been demonstrated in TSC-specific tumours, result in hyperactivation of mammalian target of rapamycin (mTOR) and distorted cell growth in various organ compartments [2].

The kidney is affected in the majority of TSC patients, with angiomyolipoma (AML) and cysts being common, while renal carcinomas being rare [3,4]. Importantly, AML can cause haemorrhage or infiltrate the kidney leading to renal failure in up to 60% and end-stage renal

disease (ESRD) in 15% of all affected TSC patients [5–9]. Neurological manifestations of TSC are also common and include cortical tubers, subependymal nodules and giant cell astrocytomas typically leading to epileptic seizures and reduced intelligence in TSC patients [10]. Pulmonary lymphangioleiomyomatosis (LAM), which almost exclusively manifests in women, occurs in about 30% of TSC-affected female patients and is characterized by progressive cystic destruction of the lung and accumulation of smooth muscle-like tissue throughout the bronchioles, alveolar septa, perivascular spaces and lymphatics. Consequently, airway remodelling and loss of lung function may occur, and 10-year survival after diagnosis of LAM is about 70% [11].

In TSC patients, hyperactivated mTOR-signalling is considered the key factor in the disease, and hence inhibition of mTOR-signalling emerges as a rational pharmacological approach to have a negative impact on disease progression and recurrence. Recent data from case reports and small clinical trials indicate that the mTOR-inhibitor (mTOR-I) sirolimus may be beneficial for TSC patients, as it leads to a regression of AML, LAM and astrocytomas [12–14]. Sirolimus however, is primarily employed in solid organ transplantation because of its capacity for blocking lymphocyte proliferation, and may be of advantage in transplanted patients who develop malignancies.

Renal transplantation in TSC patients with ESRD was described as safe and effective [15,16]. Recently, it was argued that mTOR inhibition might be of special advantage in renal transplant patients with TSC [17]. However, solid organ recipients are subjected to a variety of signalling inhibitors as part of their immunosuppression, rendering the benefit of sirolimus questionable in these particular patients. Importantly, although mTOR-signalling has recently become known to exert a fundamental influence on the innate immune system, there are no data concerning the influence of a disturbed mTOR-pathway on immunocompetent cells of TSC patients. Inhibition of mTOR-signalling in TSC patients has also been argued to be of potential danger to the patients, as disturbed signalling feedback loops might actually enhance potentially detrimental TSC-signalling [18].

In this article, we report on three TSC patients after renal transplantation who were converted from a calcineurin-inhibitor- (CNI) to an mTOR-inhibitor-based immunosuppression and discuss the potential implications as well as the immunological peculiarities of our findings for TSC and renal transplantation.

Material and methods

Three TSC patients were converted from a CNI-based regimen to sirolimus at 12 months (patient 3, Fig. 3a), 32 months (patient 1, Fig. 1a) and 128 months after renal transplantation (patient 2, Fig. 2a) respectively. We exhaustively report the medical course before and after the conversion for a follow-up time of up to 24 months, along with several specific findings concerning various organ compartments, including the immune system. Sirolimus serum target levels were between 4 and 8 ng/ml. Mycophenolate mofetil (MMF) doses and steroid use are described for each patient in Figs 1–3 respectively.

Neuroimaging data include magnetic resonance imaging (MRI) and computerized tomography (CT). Pulmonary lesions were analysed by high-resolution CT. Pulmonary function parameters such as forced expiratory volume and vital capacity were obtained, and cardiopulmonary exercise tests (CPET) performed. Skin-associated TSC lesions were monitored over time by photos, and the clinical dermatological status was repeatedly assessed. Renal function and morphology was reported in terms of serum-creatinine values and biopsy results. Vascular endothelial growth factor (VEGF) levels (ELISA from R&D Systems, Minneapolis, MN, USA) from patients 1 and 2 were measured before and after conversion.

For immunological analyses, whole blood samples were diluted, incubated for 90 min with sirolimus, and stimulated with 100 ng/ml lipopolysaccharide (LPS) for 24 h, as described elsewhere [19]. IL-12p40, IL-10, IL-6 and TNF-α were determined by ELISA (Kits from R&D Systems). For comparing the cytokine findings in TSC patients after renal transplantation with the same in other renal transplant patients without TSC, we similarly measured cytokine levels of LPS-stimulated peripheral blood mononuclear cells (PBMCs) from 20 control patients receiving cyclosporine- and 20 control patients receiving sirolimus-based immunsuppression. For these analyses, informed consent was obtained from all patients, and the blood draw was approved by the local ethics committee.

Results

Patient 1

The first patient, a 35-year-old Caucasian male was diagnosed with TSC in childhood and developed ESRD at the age of 23 as a result of deterioration of the concomitant renal cystic disease. After being on haemodialysis for 7 months, the patient received a renal allograft from his mother which failed 39 months later after repeated episodes of acute rejection. The patient remained on haemodialysis for another 25 months before receiving a kidney from a cadaveric donor. The patient received an immunosuppressive regimen with cyclosporine A (CsA), azathioprine and prednisone. Fourteen months after transplantation with an uneventful clinical course including an

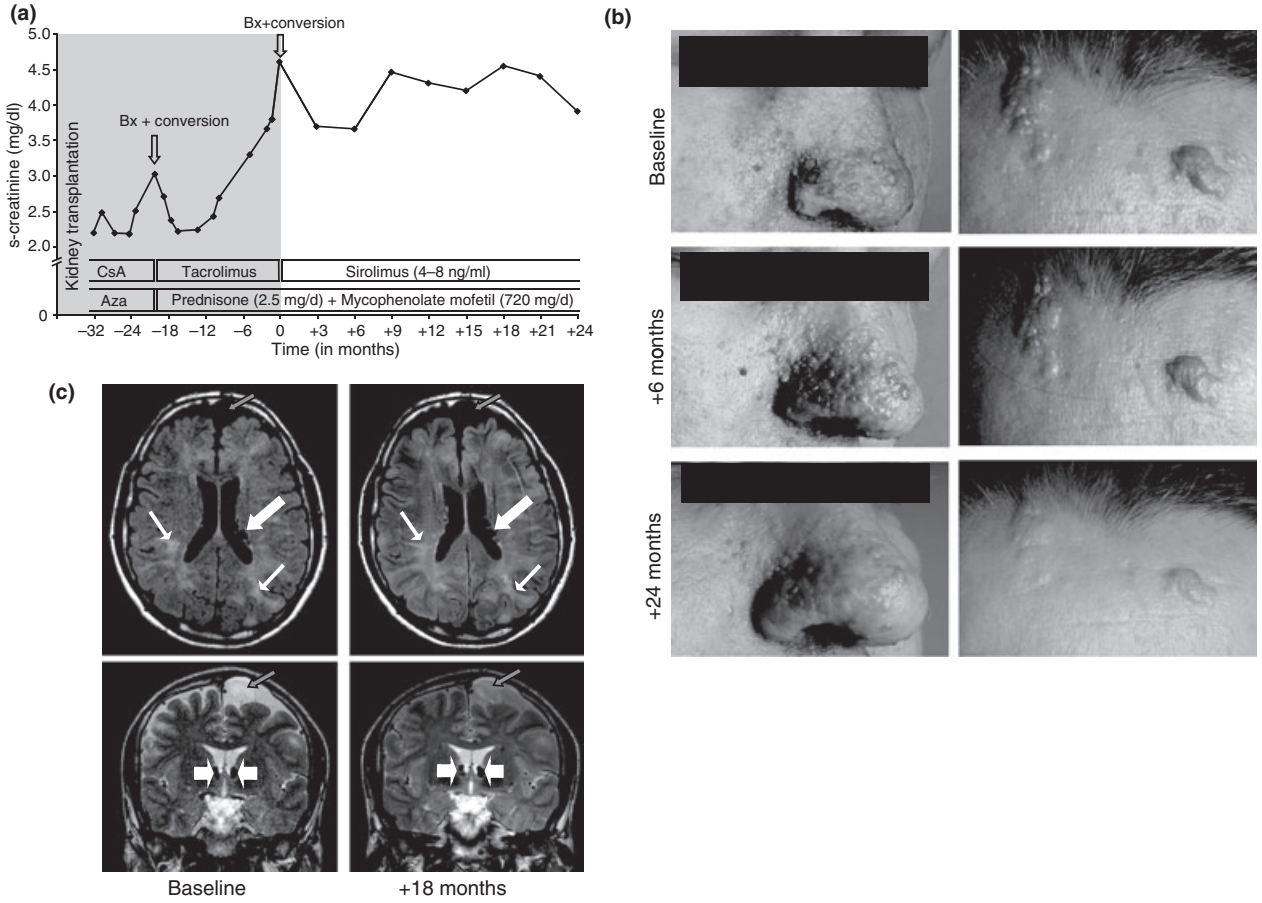
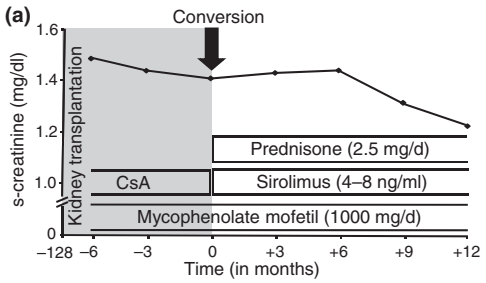
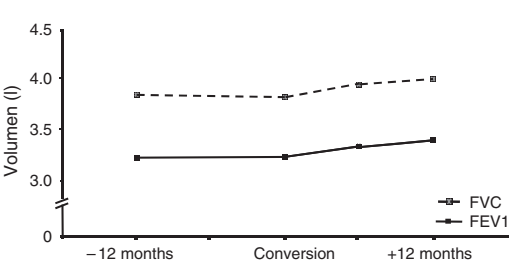


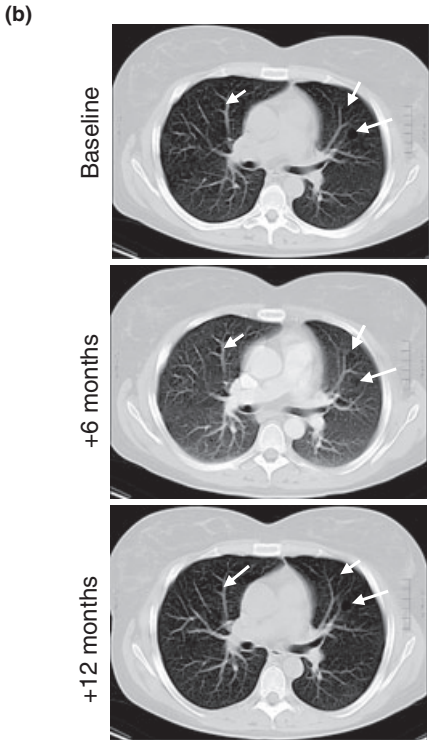
Figure 1 Patient 1 (a) Renal allograft function and immunosuppressive regimen. (b) Magnetic resonance imaging (MRI) scans of the brain before (baseline) and 18 months after conversion to sirolimus. Upper left: axial fluid-attenuated inversion recovery (FLAIR) sequence. Subependymal nodules in both ventricles, on the left side with cystic appearance (thick white arrow). Multiple subcortical tubera, hyperintense compared with adjacent grey/white matter (thin white arrows). Secondary finding: left frontal arachnoidal cyst (thin grey arrow). Lower left: Coronal view, T2-weighted sequence. Calcified (hypointense) giant cell astrocytomas at both foramina of Monro (thick short white arrow). Secondary finding: left frontal arachnoidal cyst (thin grey arrow). Upper right: Axial-FLAIR-sequence, 18 months after conversion to sirolimus: Unchanged in comparison to baseline. Lower right: Coronal view, T2-weighted sequence, 18 months after conversion to sirolimus: unchanged in comparison to baseline. (c) Facial angiofibromas and forehead plaques before, 6 and 24 months after conversion to sirolimus.

episode of urinary tract infection and a revised lymphocele, a renal biopsy revealed chronic transplant nephropathy and an acute interstitial rejection grade 1A (BANFF 97). The patient was treated with high-dose corticosteroids and was converted to tacrolimus and MMF. Ten months later, another acute rejection was diagnosed, along with further progression of chronic interstitial rejection, glomerulitis and severe chronic glomerulopathy. Because of high-grade arteriosclerosis and putative ameliorative effects of sirolimus on TSC-lesions, we replaced tacrolimus by sirolimus. At conversion, the patient had a glomerular filtration rate (GFR) of 16 ml/min (MDRD). Twenty-four months after the conversion, he displayed a GFR of 19 ml/min, the allograft function being stable over the whole observational period (Fig. 1a).

To determine the potential effects of sirolimus on TSC-associated lesions of the skin, the patient was repeatedly presented to the dermatologist. Before conversion, the patient exhibited a rash of reddish papules on the nose, the nasolabial folds and cheeks, in a butterfly distribution, compatible with facial angiofibromas, as well as two flesh-coloured tumours on the forehead ('forehead plaques'). Discrete ungual/subungual fibromas were detected on three toes. Ash-leaf spots or areas of thick leathery skin ('shagreen patches') were not present, but the patient reported about a few hypopigmented lesions on the lower extremities that were visible in earlier years. Six months after conversion to sirolimus, facial angiofibromas remained unchanged, whereas after 24 months, size and degree of erythema of the lesions was decreased (Fig. 1b).







(d)

Lung function, exercise capacity and gas exchange variables during exercise over the period

	Baseline	+6 months	+12 months
FEV1 (l)	3.27	3.37	3.43
FEV1% pred.value	102	105	108
FVC (l)	**3.84**	**3.96**	**4.01**
TLC (l)	5.65	5.9	5.8
TLC % pred. value	110	115	113
RV (l)	1.79	1.77	1.67
RV % pred.value	32	30	29
DLCO (mmol/minkPa)	7.77	6.67	7.29
DLCO % pred value	**89**	**77**	**84**
VO_2 (ml/min)	1700	1667	1472
VO_2 (% predicted)	100	99	90
VO_2 (ml/min/kg)	20.7	20.8	20
$Watt_{max}$ (Watt)	73	80	83
$Watt_{max}$ (% predicted)	52	58	62
Lactat $(mmol/l)_{max}$	**2.3**	**3.6**	**3.9**
PaO_2 $(mmHg)_{max}$	94.5	102.2	94.5
VE/VCO_2	35	39	31
Vd/Vt	22	0.15	11
Vt (l)	1.25	1.82	1.75
VEmax (l)	52.3	71	44.4
P(A-a)O_2 $(mmHg)_{max}$	**19.7**	**22.1**	**20.5**
P(a-ET)CO_2 (mmHg)	1.8	0.4	0.1

FEV1 = forced expiratory volume in 1 second, TLC = total lung capacity, RV = residual volume, DLCO = diffusing capacity of the lung for carbon monoxide, VO_2 = maximum oxygen uptake, W_{max} = maximum working capacity, PaO_2 = partial arterial oxygen pressure at maximum exercise, VE/VCO_2 = ventilatory equivalent for carbon dioxide, Vd/Vt = dead volume % tidal volume at exercise, D Vt= tidal volume, VEmax = maximum minute ventilation, P(A-a)O_2 = alveolar arterial oxygen content difference, P(a-ET)CO_2 = arterial end-tidal pCO_2 difference.

Figure 2 Patient 2 (a) Renal function and immunosuppression. (b) Thin-section CT of the thorax at the time of conversion (baseline), 6 and 12 months after conversion to sirolimus. White arrows show the typical thin-walled lung cysts in patients with LAM. (c) Pulmonary function evaluated by forced vital capacity (FVC) and forced vital capacity at 1 s (FEV_1) 12 months before conversion, at the time of conversion (baseline), 6 and 12 months after conversion to sirolimus. (d) Specific results of the pulmonary function analysis as well as of CPETs at the time of conversion (baseline), 6 and 12 months after therapy.

Neurologically, the patient suffered from several epileptic seizures until the age of 23. An MRI scan of the brain before conversion revealed several bilateral, calcified, subependymal nodules, giant cell astrocytomas, and cortical tubers. Eighteen months after conversion to sirolimus, a second MRI scan did not reveal any further tissue growth (Fig. 1c). Moreover, the patient reported cessation of sensations similar to the epileptic auras that he was experiencing earlier, after conversion to sirolimus.

After conversion, the patient developed three episodes of diarrhoea and two urinary tract infections (*Escherichia coli*). Apart from mild hyperlipidaemia, which was treated with rosuvastatin, no other mTOR-I-specific side-effects were observed. In summary, conversion to sirolimus was safe and beneficial for deteriorating allograft function and despite severe degree of chronic allograft damage, the patient's kidney recovered in terms of a conspicuous improvement of GFR after sirolimus therapy. Moreover, a positive influence of mTOR inhibition on CNS lesions can be deduced from the improvement of neurological symptoms as well as from the unchanged MRI scans, as giant cell astrocytomas tend to steadily grow in TSC patients [20]. Impressively, sirolimus significantly affected

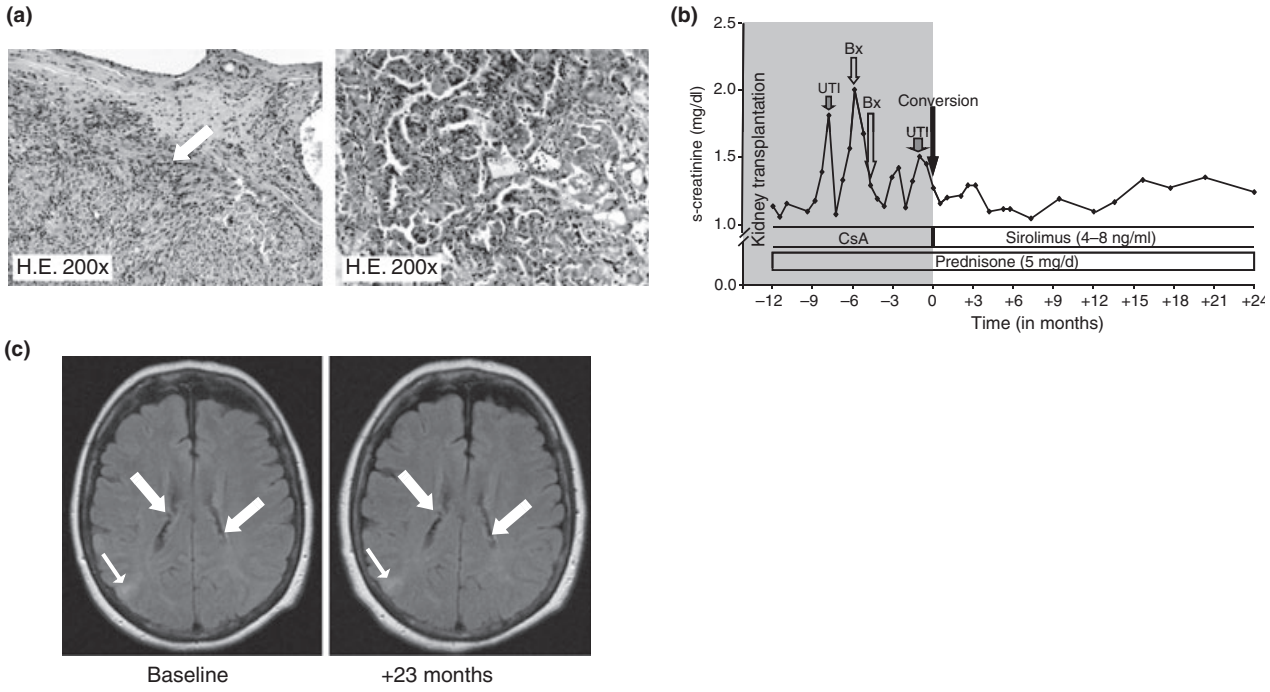


Figure 3 Patient 3 (a) Histological findings in the removed kidney: Fibrotic renal tissue consistent with ESRD; embedded multiple myolipoma nodules (one of them highlighted by the arrow) with spindle-shaped, partially vacuolized cells in dense trabeculae (left) and foci of papillary renal cell carcinoma with papillary structure, nuclear polymorphism, bleeding and foam cells (right). Haematoxylin & eosin staining, 200× amplification. (b) Renal function and immunosuppression. (c) Magnetic resonance imaging (MRI) scans of the brain before (baseline), and 23 months after conversion to sirolimus. Left: axial-FLAIR-sequence. Subependymal nodules in both ventricles (thick white arrows). Tuber in the right parietooccipital region (thin white arrow). Right: axial-FLAIR-sequence, 23 months after conversion to sirolimus: unchanged in comparison to baseline.

skin lesions including angiofibroma quality in this patient after a prolonged period of treatment.

Patient 2

A 40-year-old Caucasian female patient displayed recurring episodes of various types of epilepsy and concomitant discrete angiofibromas since she was 14 months old. At the age of 24 years, acute bleeding of renal AML necessitated transcatheter tumour embolization, followed by serial renal resections and finally nephrectomy attributable to uncontrollable haemorrhages. After 3 months of haemodialysis, the patient received a kidney from her father and was immunosuppressed with CsA, MMF and prednisone (Fig. 2a). After transplantation, the patient developed one episode of bacteraemia (*Proteus mirabilis*) and one episode of fever of unknown origin.

Approximately 7 years after transplantation, the patient noticed recurrent episodes of laboured breathing. Two years thereafter, a CT of the thorax was performed because of several respiratory infections unresponsive to antibiotic treatment. Multiple, thin-walled, cystic, intrapulmonary lesions were detected (Fig. 2b), and a lung biopsy revealed the diagnosis of LAM. Treatment consisted of inhalative corticosteroids and later anti-oestrogens, however, the severe dyspnoea did not resolve.

Immunosuppression was changed from CsA to sirolimus because of further clinical progression of LAM. High-resolution CT scans, pulmonary function analyses and CPET were performed at baseline, 6, and 12 months after conversion to sirolimus. Morphologically, no alteration of the cystic lesions was observed. However, forced expiratory volume at 1 s (FEV_1) increased by 100 ml at 6 months and by 160 ml at 12 months after conversion to sirolimus. Forced vital capacity (FVC) at 12 months increased by 170 ml (Fig. 2c,d). The residual lung volume fell by 120 ml after 1 year of sirolimus therapy. In the CPET, an increase of the maximum exercise capacity from 52% to 62% (Wmax in percentage predicted) was observed, without differences of the maximum oxygen uptake. The pulmonary gas exchange (paO_2, $AadO_2$) was normal without diffusion limitation at baseline and after 6 and 12 months (Fig. 2d). Clinically, the patient experienced significant relief of dyspnoea and also cessation of recurrence of respiratory infections.

Dermatological examinations revealed various hypomelanic, white macules (ash leaf spots) randomly distributed on the entire integument. Additionally, facial

angiofibromas were present from early childhood on and had been successfully treated with ablative laser therapy. Neurologically, the patient experienced recurring episodes of epilepsy throughout childhood that finally disappeared after treatment with levetiracetam. A CT scan of the brain before conversion was compared with an MRI scan 12 months after sirolimus therapy and showed no further growth of subependymal nodules, giant cell astrocytomas and tubers (not shown).

Sirolimus-specific side-effects were not observed and graft function was excellent. The lack of astrocytoma growth, even after an extended observation period, may indicate an effect of the mTOR inhibitor. Clinically, pulmonary function parameters improved, and the patient reported a subjective improvement of dyspnoea along with resolution of respiratory infections.

Patient 3

A 1955-born Caucasian female was haemodialysed since January 1997 because of chronic interstitial nephritis and simultaneously occurring renal cysts. In May 2003, the enormous renal size necessitated bilateral nephrectomy prior to a potential transplantation. The histology revealed AML and a high-grade papillary renal cell carcinoma in both kidneys (Fig. 3a). An MRI scan of the brain was performed in May 2005 when the patient was hospitalized because of an episode of haemodialysis-associated hypotonia, and showed various atypical calcifications in the lateral ventricles. Upon further investigation, the patient reported episodes of epilepsy before the age of 20 that were symptomatically treated. Dermatological examination confirmed the diagnosis of TSC with the presence of discrete shagreen patches on the right shoulder and a number of Koenen tumours.

In September 2005, the patient received a cadaveric renal allograft. The initial immunosuppressive regimen consisted of cyclosporine A, steroids and MMF. An increase in serum-creatinine necessitated a renal biopsy revealing acute transplant rejection classified as grade 2B (BANFF 97), and moderate chronic transplant rejection. The patient was successfully treated with corticosteroids and a control biopsy showed no more signs of rejection, but mild interstitial fibrosis and tubular atrophy. Additionally, the patient experienced persistent abdominal discomfort and diarrhoea as well as two episodes of urinary tract infection necessitating antibiotic treatment (Fig. 3a). Upon MMF withdrawal, the clinical symptoms completely resolved. Six months thereafter, the patient was converted to sirolimus to potentially affect the course of TSC (Fig. 3b).

An MRI scan was performed and displayed TSC-typical tubers in both occipital lobes and subependymal nodules in both ventricles. Twenty-three months later a control MRI demonstrated stable central nervous system morphology and no evidence of tumour growth.

Renal function remained excellent over a period of 24 months after conversion to sirolimus, and no sirolimus-associated side-effects apart from one episode of diarrhoea were observed. The discrete TSC-associated skin lesions, however, remained unchanged.

These data indicate that conversion to sirolimus was safe and beneficial for allograft function. With regard to the discrete TSC-specific skin lesions, no obvious effects of mTOR inhibition were observed even after a treatment period of 24 months. Nevertheless, we also did not observe a progression of any TSC-specific manifestations especially within the CNS.

Immunological features

To assess, whether sirolimus therapy is effective in the TSC patients, we measured serum levels of VEGF-A. VEGF-A, which has been advocated to be increased in LAM [21] and which is sensitive to mTOR inhibition was significantly suppressed in the serum of both patients after they were converted to sirolimus (Fig. 4a).

Recent reports indicate a pivotal role of the TSC/mTOR-pathway in regulating innate immunity, and importantly, mTOR-activation has been shown to induce IL-10 and to suppress IL-12 production from innate immune cells [19]. Previously, it was demonstrated that IL-6 and TNF-α serum levels were significantly higher after conversion from a CNI-based immunosuppression to sirolimus, while IL-10 secretion was suppressed in renal transplant patients [22]. Despite the long-standing notion that the TSC/mTOR signalling pathway is important for lymphocyte activation, the immune system of TSC patients has never been analysed. Therefore, we stimulated whole blood of patient 1 and patient 2 at baseline and 3 months after the conversion to sirolimus with LPS and compared it with transplanted non-TSC patients treated with CsA or sirolimus (Sir) therapy. Interestingly, IL-10-levels were clearly elevated (Fig. 4b), whereas IL-12 levels were decreased (Fig. 4c) in the two TSC patients as compared with non-TSC patients indicating constitutive activation of mTOR at the level of the innate immune system. After conversion to sirolimus, we observed a clear, at least threefold decrease in IL-10 (Fig. 4b) and an approximately twofold increase in IL-12p40 (Fig. 4c) in the supernatants of LPS-stimulated blood samples as compared with baseline. The proinflammatory cytokine TNF-α (Fig. 4d) was deviated in a similar way as IL-12, namely increased approximately threefold in patient 1 and 2 during immunosuppressive therapy with sirolimus, while IL-6 production was increased in patients 1 and decreased in patient 2 after conversion (Fig. 4e).

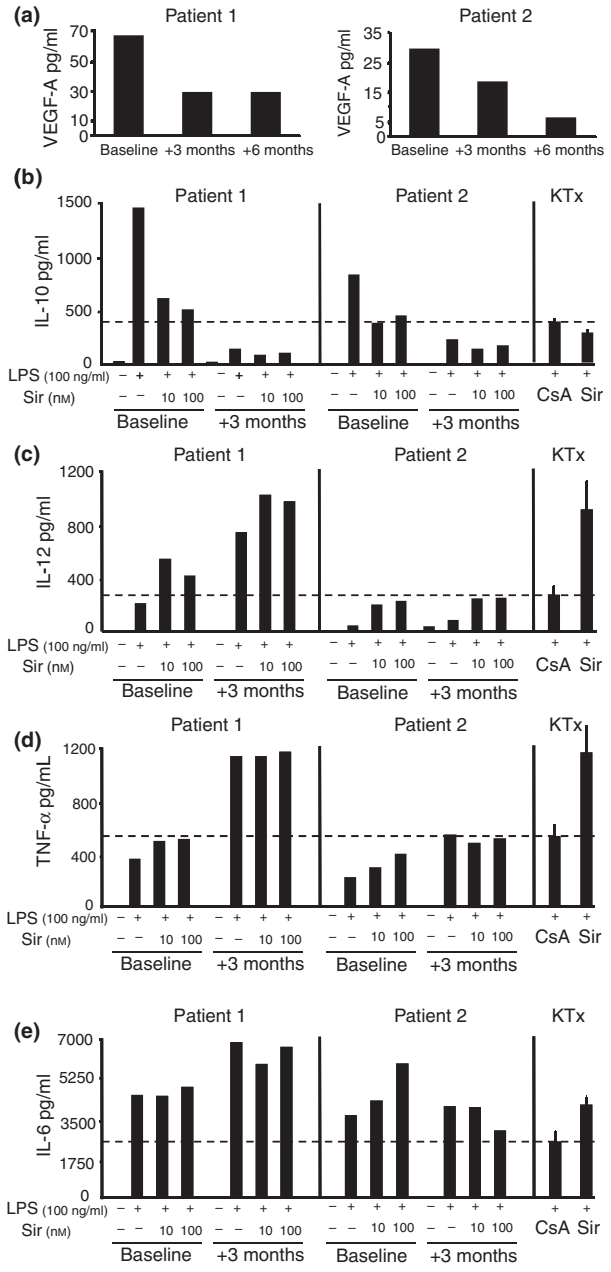


Figure 4 Immunological data (a) VEGF-A serum levels from patients 1 and 2 at baseline, 3 and 6 months after conversion to sirolimus. Whole blood samples of patients 1 and 2 were stimulated with 100 ng/ml lipopolysaccharide (LPS), and partially pretreated with 10 and 100 nM sirolimus. To compare cytokine responses in renal transplant patients with tuberous sclerosis complex (TSC) to renal transplant patients without TSC (KTx = kidney transplantation), we collected blood samples from 40 kidney transplant recipients receiving either CsA- (n = 20) or sirolimus-based (n = 20) immunosuppression and stimulated the samples with LPS. Cytokine responses after 24 h of LPS stimulation are shown for IL-10 (b), IL-12p40 (c), TNF-α (d), IL-6 (e).

In blood-samples pretreated with rapamycin for 90 min before stimulation with LPS, the production of IL-12p40 was strongly increased in PBMCs from patients immunosuppressed with CNI as described previously ([19], Fig. 4c). Similarly, TNF-α was increased, whereas IL-10 was strongly decreased (Fig. 4b,d). In PBMCs from TSC patients converted to sirolimus, exogenous rapamycin further increased IL-12p40 production even beyond the values observed without rapamycin (Fig. 4c). IL-10, however, was not decreased upon addition of rapamycin, and TNF-α remained similarly high (Fig. 4b,d).

Together with all the above findings, the immunological analyses indicate that blocking mTOR in TSC patients leads to a prominent deviation of cytokine production in peripheral leucocytes. Moreover, we were able to show that the innate immune response is profoundly altered in TSC patients and is equilibrated after sirolimus therapy.

Discussion

Inhibition of mTOR holds promise as a molecularly targeted therapy against TSC, and several recent studies have found beneficial effects of sirolimus against TSC-associated lesions. Concerning renal AML, Bissler *et al.* [13] reported beneficial effects of sirolimus in TSC or sporadic LAM patients over a period of 18 months. These results were confirmed by Davies *et al.* [14] in their interim analysis of an ongoing study with 13 patients.

In our analysis, the effect of sirolimus against renal AML could not be assessed, as patient 1 did not have AML, whereas patients 2 and 3 underwent bilateral nephrectomy. Concerning LAM, a pulmonary involvement that specifically affects up to 30% of women with TSC, patient 2 revealed consistent improvements in FEV1 and FVC and also in the maximum exercise capacity. Moreover, recurrent respiratory infections completely resolved and LAM also did not continue to progress after conversion.

The effects of sirolimus on TSC-associated cerebral lesions have been reported controversially [13,23]. While all three patients in our series showed a cerebral involvement of TSC, sirolimus might not have been effective against tubers *per se*, as these lesions are dysplastic rather than neoplastic. Patient 1 still reported sensations similar to the epileptic auras that the individual experienced prior to sirolimus treatment, indicating a residual neurological impairment. This patient reported that the neurological sensations had completely stopped after treatment with sirolimus. While pre-existing astrocytomas are well-known to be continuing to grow [20], we observed a stabilization of astrocytoma size potentially indicating effectiveness of mTOR inhibition.

One recent report demonstrated dramatically reduced TSC-specific facial angiofibromas as early as 3 months after sirolimus treatment in a female transplant recipient [24]. All patients described in our case series displayed TSC-specific lesions on the skin. Only in the first patient, sufficient numbers of facial angiofibromas were present at the time of conversion for the purpose of analysing any sirolimus-associated benefit. The change of the distinct erythemas towards a more flesh-coloured appearance of the facial angiofibromas is impressive and can be attributed to sirolimus therapy.

In this article, we demonstrate that peripheral blood lymphocytes (PBL) from sirolimus-treated TSC patients display a distorted cytokine profile, including up-regulation of the proinflammatory cytokine IL-12 and suppression of the anti-inflammatory cytokine IL-10. While a conspicuous role of mTOR in regulating innate immunity has recently been demonstrated [25], our *ex vivo* data are the first to demonstrate such an effect in humans with TSC. The immunological implications of these findings are not clear at present. Nevertheless, our data suggest that the immune response may be profoundly altered in TSC patients. This effect is most strongly seen in the ability to produce higher levels of the anti-inflammatory cytokine IL-10 when compared with non TSC patients. It will be interesting to investigate whether the activation status of the mTOR pathway in the leucocytes is indeed higher in TSC patients, as has been suggested by the difference in basal IL-10 and IL-12 levels between TSC patients and renal transplant patients treated with CNI (Fig. 4b,c). Thus it is tempting to speculate that the adaptive immune response might be similarly altered in TSC patients. Furthermore, it might be hypothesized that altered cytokine profiles as shown for a variety of autoimmune, infectious and malignant diseases might also be a critical factor for disease pathogenesis and treatment in TSC patients [26]. Hence, future studies analysing the immune system of TSC patients may further contribute to a better understanding of the disease pathology.

In summary, the cases presented in this article demonstrate that converting renal transplant patients with TSC to mTOR-I-based immunosuppression with sirolimus was safe, associated with a stable renal allograft function, and effective with regard to primary disease pathology. In all three patients, the course of TSC did not show signs of any progression of the underlying disease. Moreover, a clear clinical benefit of sirolimus therapy on the skin in patient 1 and on pulmonary function parameters in patient 2, along with subjective improvements in all patients were observed. Furthermore, cerebral lesions, especially astrocytomas, did not continue to grow and may indicate a potential benefit of mTOR inhibition [20]. Finally, the potent immunomodulatory effects of sirolimus in these patients may serve as an ancillary translational method in further prospective analyses aimed to test mTOR-I therapy in TSC patients. Further studies in renal transplant recipients with TSC are warranted to define the risks and putative benefits of mTOR inhibition on the progression of established tumours as well as on tumour prevention in the context of immunosuppression.

Authorship

MHa, MDS, MHe: designed and performed the study. WE, MHa, MDS: followed the patients. MP, MHa, WHH: performed the immunological research. DP: analysed the radiological findings. AS: did the pathology analysis. KV: performed and analysed the spiroergometria. AG: performed the dermatological controls. TW, GJZ, RO: collected and analysed data. TW, MHe, MDS, MHa wrote the paper. MHe, MDS, MHa: final approval of the manuscript.

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