End-stage renal failure and regulatory activities of CD4\(^+\)CD25\(^{bright}\)+FoxP3\(^+\) T-cells


Abstract

Background. The defensive immune system in patients with end-stage renal failure is impaired at multiple levels. This state of immune incompetence is associated with continuous activation of the immune system. An additional explanation for this state of activation may be the disturbed function of CD4\(^+\)CD25\(^{bright}\)+FoxP3\(^+\) regulatory T-cells.

Methods. The phenotype and function of peripheral regulatory T-cells from patients with end-stage renal failure (N = 80) and healthy controls (N = 17) was studied by flow cytometry, RT-PCR and mixed lymphocyte reaction.

Results. The basal IL-2 mRNA level was high in patients-PBMC (P = 0.0002 versus healthy controls). The absolute number of CD4\(^+\)CD25\(^{bright}\)+ FoxP3\(^+\) T-cells was low in patients (P < 0.05 versus healthy controls). Furthermore, proliferation of patient-PBMC upon allogenic stimulation was impaired (P < 0.0001 versus healthy controls). The regulatory function of CD4\(^+\)CD25\(^{bright}\)+ T-cells was determined in the setting of direct allorecognition. First, the effect of

References


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depletion of CD25bright+ cells from patient-PBMC on proliferation was low. Second, co-culture of CD25bright+ cells with CD25reg/dim cells (1:10 ratio) showed impaired regulatory function (P < 0.001 versus healthy controls), which was especially pronounced in patients on dialysis. The FOXP3 mRNA level was also low upon stimulation (P = 0.0002 versus healthy controls).

**Conclusions.** In line with previous studies, we observed an overactivated but functionally compromised immune system in patients with end-stage renal failure. It now appears that in this setting, regulation by CD4+CD25bright+FoxP3+ T-cells is also impaired.

**Keywords:** dialysis; end-stage renal failure; FoxP3; patients; regulatory T-cells

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### Introduction

Patients with end-stage renal failure (ESRF) suffer from inadequate responses upon vaccination, e.g. hepatitis B [1,2], susceptibility to infections and an increased incidence of malignancies [1,3]. Studies have shown that these clinical observations are accompanied with failure of the defensive immune system at multiple levels, despite evidence of activation by markers on immune competent T-cells [4–6]. This state of immune incompetence, associated with continuous activation of the immune system, may result from the high level of uraemic toxins that is caused by the renal failure itself and is further intensified by treatment with dialysis [7–10]. However, an additional explanation for this state of activation may be a disturbed function of regulatory T-cells.

The CD4+CD25bright+ T-cell, which specifically expresses the transcription factor FoxP3+, controls immune responses of effector T-cells to auto and foreign antigens [11–13]. CD4+CD25bright+FoxP3+ T-cells prevent organ-specific auto-immune diseases, control anti-tumour responses, anti-viral responses and immune responses to allo-antigens in the setting of organ transplantation [14–16]. CD4+CD25bright+FoxP3+ T-cells exert their suppressive function in a cell–cell contact-dependent manner by inhibiting the IL-2 and IFN-γ production of effector T-cells [17,18]. IL-2 is a crucial cytokine for the function, homeostasis and survival of CD4+CD25+FoxP3+ T-cells [19,20]. Previous reports on the IL-2 system in ESRF patients showed high spontaneous release and expression of IL-2 and its receptor (R) by circulating T-cells [21,22]. However, after stimulation, the expression of the IL-2R and the production capacity of IL-2 were low compared to those induced in cells from healthy controls [21–24]. Both phenomena may have a significant negative influence on the number and function of CD4+CD25+FoxP3+ T-cells in ESRF patients.

The effect of ESRF on immunoregulatory activities of CD4+CD25+FoxP3+ T-cells has not been extensively investigated. In a T-cell-dependent murine model of anti-glomerular basement membrane glomerulonephritis, treatment with CD4+CD25+ T-cells suggested therapeutically valuable inhibition of these suppressor cells [25]. Furthermore, it has been shown that in these animals CD4+CD25+ T-cells protect against injury of kidney cells [26]. While these animal studies suggest a controlling role for these cells in kidney disease and renal function, it remains unknown whether they influence the function of T effector cells in patients with ESRF. Here we postulate that in ESRF patients the function of CD4+CD25bright+FoxP3+ T-cells is impaired resulting in their characteristic overactivated but functionally compromised immune system.

**Patients and Methods**

**Subjects**

The medical ethical committee (MEC) of Erasmus Medical Centre approved the study protocol and all patients provided informed consent (MEC 2004-264). Inclusion of patients started in February 2004. Peripheral blood samples were obtained from 80 stable patients (Table 1) with ESRF and from 17 HC, consisting of 10 males and 7 females with a mean age of 52 ± 8.6 years. Patients were on haemodialysis (HD, N = 40) or peritoneal dialysis (PD, N = 26) and 14 patients were not yet on dialysis (ND). Patients showed high spontaneous release and expression of IL-2 and its receptor (R) by circulating T-cells [21,22]. However, after stimulation, the expression of the IL-2R and the production capacity of IL-2 were low compared to those induced in cells from healthy controls [21–24]. Both phenomena may have a significant negative influence on the number and function of CD4+CD25+FoxP3+ T-cells in ESRF patients.

**Flow cytometric analysis**

Blood samples were collected in heparinized tubes and analysed for the presence of T-cell subsets by four-colour flow cytometry using mAbs directly conjugated to fluorescein (FITC), phycoerythrin (PE), allophycocyanin (APC) or peridinin chlorophyll protein (PerCP). 100 µl blood was incubated with 10 µl of the dual mAb combination CD45-FITC/CD14-PE; IgG1-FITC/IgG2a-PE as isotype control. Further to this, we used the mAb CD3-FITC, CD4-PerCP, CD8-APC, CD19-APC, CD16/CD56-PE and CD25-PE (epitope B, clone M-A251). The antibodies were purchased.

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from BD Biosciences (San Jose, CA, USA) and R&D Systems (Abingdon, UK). After 30 min of incubation at room temperature, red blood cells were lysed with FACS lysis solution (BD Biosciences) for 10 min. Cells were washed twice and analysed on a flow cytometer (FACSCalibur, BD Biosciences) using SimulSet and CELL Quest Pro software (BD Biosciences). The number of leukocytes was determined by the cell counter CASY® model TT (Schräf System GmbH, Reutlingen, Germany). To establish an analysis gate that included at least 90% of the lymphocytes, the CD45/CD14 reagent was used. At least 20 000 gated lymphocyte events were acquired from each tube.

Expression of FoxP3 and CD127

A specific marker for CD4+CD25bright- Treg is FoxP3 and recently it was shown that the expression of CD127 inversely correlates with FoxP3 expression and the suppressive function of Treg [11,29]. Experiments on our study cohort were performed on fresh materials before the anti-FoxP3 antibody was available for flow cytometric analysis. Therefore, we stained whole blood of an additional cohort of ESRF patients (N = 34) as well as HC (N = 9) with CD4-PerCP, CD25-PE (epitope B), CD127-FITC (eBioscience, San Diego, CA, USA) and FoxP3-APC (clone PCH101, eBioscience). The isotype controls for FoxP3 and CD127 were IgG2A-APC and IgG1-FITC, respectively. Patient characteristics were comparable with our study population.

Isolation of peripheral blood lymphocytes

Peripheral blood mononuclear cells (PBMC) were freshly isolated from the interphase, washed twice in RPMI 1640 (BioWhittaker, Verviers, Belgium) and resuspended in the human culture medium (HCM) consisting of RPMI 1640-Dutch Modification (Gibco, BRL, Scotland, UK) supplemented with 4 mM l-glutamine (Gibco BRL), 100 IU/ml penicillin (Gibco BRL), 100 µg/ml streptomycin (Gibco BRL) and 10% heat inactivated pooled human serum. The latter consists of serum from 20 subjects (males & females), which was purchased from the blood bank (Sanquin, The Netherlands) and pooled in home. Subjects contained all ABO blood groups and were tested for HLA-antibodies. If the test was positive, the serum was not used. We did not use the same batch for all experiments, but each batch was tested for adequate cell growth prior to our experiments. For the analysis of IL-2 and FOXP3 mRNA expression levels 2 × 10^6 PBMC samples were snap-frozen directly after isolation.

Isolation of CD25bright+ cells

After isolation, PBMC were washed once and resuspended in 45 µl MACS-buffer/10 × 10^6 PBMC prepared according to manufacturers protocol (Miltenyi, Bergisch Gladbach, Germany). PBMC were then incubated with anti-CD25 microbeads (Epitope A, Miltenyi Biotec) followed by a positive selection (POSSELD-program) on the autoMACS® (Miltenyi), resulting in a CD25bright enriched fraction, which was referred to as the CD25bright fraction. Cells not selected by the microbeads were referred to as the CD25negdim fraction [16]. To control for the autoMACS procedure on cells, 6 × 10^6 PBMC were treated by the same protocol in the absence of anti-CD25 microbeads. Purity of the fractions was measured by flow cytometry using CD4-PerCP, CD8-APC, CD25-PE (epitope B, BD Bioscience) and FoxP3-APC (clone PCH101).

Mixed lymphocyte reactions

To check for cell viability and proliferative capacity, 5 × 10^6 CD25negdim cells and PBMC were stimulated with 1 µg/ml phytohaemagglutinin (PHA; Murex Biotech Ltd, Kent, UK) for 3 days. At Day 2, 0.5 µCi/well was added to the culture and 16 h later, samples were harvested and radioactivity was measured using a β-counter (PerkinElmer, Oosterhout, The Netherlands).

In the MLR, 5 × 10^5 PBMC and CD25negdim cells were stimulated with 5 × 10^4 HLA-A,-B and DR fully mismatched (2-2-2) irradiated (40 Gy) PBMC (allo-Ag). All cultures were performed in HCM, in triplicate in a 96-well round bottom plate for 7 days. At Day 6, ^3H-thymidine was added to the cultures and 16 h later samples were harvested and radioactivity was counted.

Regulation of allo-Ag stimulated responder cells by CD25bright+ cells

Regulation of proliferation by CD25bright+ cells was quantified both by their depletion from PBMC and by co-culture experiments with CD25negdim responder cells. After depletion the increase in proliferation reflects the regulatory capacity of the CD25bright+ cells [16]. Isolated CD25bright+ cells were added to CD25negdim responder cells at a ratio of 1:5, 1:10, 1:20 and 1:40. The effect was then calculated as the percentage of inhibition (%IH).

FOXp3 and IL-2 mRNA expression

Messenger RNA (mRNA) was extracted from unstimulated PBMC and from allo-Ag stimulated PBMC (7 days) from 16 patients and 17 HC. cDNA transcription and amplification was performed as described before [16]. In brief, total RNA was isolated using the High Pure RNA Isolation Kit (Roche Applied Science, Penzberg, Germany), according to the manufacturer's instructions. cDNA was synthesized from 500 ng RNA with random primers (Promega, Leiden, The Netherlands).

Real-time PCR (RT-PCR) was performed on the Taqman (ABI PRISM™ 7700 Sequence detector, Applied Biosystems, Foster City, CA, USA). IL-2 (Hs00174114_m1) and FOXP3 (Hs00203958_m1) mRNA measurements were performed using Assay on Demand (Applied Biosystems) as described before [16,30]. For the absolute quantification of mRNA expression levels, we used the 2^-ΔΔCt procedure and denoted target expression levels as copy number/500 ng RNA [31].

Statistics

To determine if three or more groups were statistically different for a certain parameter, the one-way ANOVA Dunnett’s multiple comparison test was used. Frequency analysis was performed with the Chi-squared test. Correlation was analysed with Spearman’s Rho. To examine the dependence of one outcome variable on other variables simultaneously, multiple regression was performed. All calculations were done using GraphPad Prism 4.0 or SPSS 11.5. A P-value <0.05 is marked with *, P < 0.01 with ** and P < 0.001 with ***.

Results

Flow cytometry

Freshly isolated PBMC samples from 77 out of 80 ESRF patients and from 17 HC were evaluated for lymphocyte subsets including the CD4+CD25bright+, CD4+CD25+ and CD4+CD25negdim T-cells (Figure 1A). Our flow cytometric results are summarized in Table 2. Absolute numbers of CD19+ B-cells, CD3+CD16+CD56+ NK-cells, CD3+ cells and CD4+ T-cells were significantly lower in ESRF patients than in HC. The absolute number of regulatory T-cells, defined as the CD4+CD25bright+ T-cell population [16,18], was also lower in ESRF patients than in HC (Figure 1B, P < 0.05) as was the case for the CD4+CD25+ population (P = 0.08) and the CD4+CD25neg population (P < 0.01). The percentage of CD4+CD25bright+ cells of CD4+ T-cells was not different from HC (Figure 1C) as was the case for the CD4+CD25+ population and the CD4+CD25neg population. The ratio of CD4+CD25bright+...
Fig. 1. Flow cytometric results from whole blood. (A) Dotplot of lymphocytes stained for CD4 and CD25 with a representative example of the gated CD25* + CD25neg, CD25* + and CD25neg gated areas. (B) Absolute numbers of peripheral CD4+CD25*+ T-cells were significantly lower in all patient groups compared to HC (all groups *P* < 0.05). (C) The percentage of CD4+CD25*+ cells of peripheral CD4+ T-cells was not different in any group of patients when compared to HC. (D) Dotplot of lymphocytes stained for FoxP3 and CD127 with a representative example of the gated FoxP3+CD127neg area from the CD25*+ gate in Figure 1A. (E) Dotplot of the isotype controls IgG2A-APC and IgG1-FITC for FoxP3 and CD127, respectively. (F) Whole blood of an additional cohort of patient with ESRF was stained for FoxP3 and CD127. Here, the expression of FoxP3 by CD4+CD25*+ cells was slightly lower when compared to HC in HD and ND patients (*P* < 0.05 and *P* < 0.01, respectively). Also this expression of FoxP3 was lower in ND patients when compared to PD patients (*P* < 0.05). (G) The expression of CD127 by CD4+CD25*+FoxP3+ cells was not different between the groups of patients. HD = haemodialysis, PD = peritoneal dialysis, ND = no dialysis, HC = healthy controls.

...
The mRNA expression level of FOXP3 in PBMC was not significantly different between patients (N = 14) and HC (N = 17, Figure 3A, P = 0.49), whereas the expression level of FOXP3 mRNA was lower in patients (N = 13) than PBMC of HC (N = 17, Figure 3B, P = 0.0002).

Next, we studied the IL-2 and FOXP3 gene expression level in allo-Ag stimulated PBMC from ESRF patients and HC. The expression level of IL-2 mRNA was not different between patients (N = 14) and HC (N = 17, Figure 3A, P = 0.49), whereas the expression level of FOXP3 mRNA was significantly higher in patients (N = 13) than PBMC of HC (N = 17, Figure 3B, P = 0.0002).

Expression levels of IL-2 and FOXP3 mRNA

To determine whether in the studied cohort the IL-2 pathway is activated, we measured IL-2 mRNA expression levels in PBMC from 16 patients with ESRF (HD N = 7, PD N = 5, ND N = 4) and 17 HC. RT-PCR analysis indeed showed that the expression level of IL-2 mRNA was significantly higher in patients than in HC (Figure 2A, P = 0.0002). The most specific marker for CD4+CD25bright regulatory T-cells is FoxP3 [11]. No difference was found in the expression level of FoxP3 mRNA between patients and HC (Figure 2B, P = 0.32).

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Proliferative responses

Proliferative responses of freshly isolated PBMC were studied in 60 out of 80 patients. ESRF patients and HC showed comparable proliferative responses to mitogen PHA (Figure 4A). In contrast, proliferation of PBMC to

<table>
<thead>
<tr>
<th>Cell subsets</th>
<th>HD (N = 37)</th>
<th>PD (N = 26)</th>
<th>ND (N = 14)</th>
<th>HC* (N = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leuko (×10^6/l)</td>
<td>4.6 (2.5–9)</td>
<td>7.2** (4.8–12.4)</td>
<td>5.4 (3.6–8.6)</td>
<td>5.0 (3.8–7.3)</td>
</tr>
<tr>
<td>B cells (CD19)</td>
<td>44*** (2–318)</td>
<td>77** (18–365)</td>
<td>68* (26–308)</td>
<td>117 (29–469)</td>
</tr>
<tr>
<td>NK cells (CD3+CD16/56+)</td>
<td>131*** (40–703)</td>
<td>153*** (47–389)</td>
<td>181** (38–416)</td>
<td>272 (159–857)</td>
</tr>
<tr>
<td>CD3</td>
<td>809 (161–1745)</td>
<td>693* (322–1879)</td>
<td>721 (383–1588)</td>
<td>1011 (464–3020)</td>
</tr>
<tr>
<td>CD8</td>
<td>302 (55–768)</td>
<td>220 (59–737)</td>
<td>321 (100–539)</td>
<td>269 (113–1330)</td>
</tr>
<tr>
<td>CD4</td>
<td>478* (93–1375)</td>
<td>462* (160–1355)</td>
<td>422** (261–1454)</td>
<td>622 (331–1687)</td>
</tr>
<tr>
<td>CD25neg</td>
<td>172* (75–687)</td>
<td>99*** (46–494)</td>
<td>122** (71–538)</td>
<td>297 (111–625)</td>
</tr>
<tr>
<td>CD25+</td>
<td>274 (62–816)</td>
<td>333 (85–861)</td>
<td>290 (140–916)</td>
<td>368 (200–1147)</td>
</tr>
<tr>
<td>CD25bright+</td>
<td>39* (9–102)</td>
<td>36* (14–115)</td>
<td>34* (12–84)</td>
<td>50 (21–153)</td>
</tr>
</tbody>
</table>

HD = haemodialysis, PD = peritoneal dialysis, ND = no dialysis, HC = healthy controls.

*For one HC the staining of CD25 was insufficient to distinguish CD4+ cells for their expression of CD25.

Absolute numbers in cell/µl, median (range), *P < 0.05 versus HC, **P < 0.01 versus HC, ***P < 0.001 versus HC.

Fig. 2. Basal mRNA expression levels of IL-2 and FOXP3 in PBMC. (A) The peripheral mRNA expression level of IL-2 was higher in patients (N = 16) as in healthy controls (HC, N = 17, P = 0.0002), (B) while the mRNA expression level of FOXP3 in PBMC was not significantly different (P = 0.32). ■ = HD = haemodialysis, ● = PD = peritoneal dialysis ▼ = ND = no dialysis.

Fig. 3. Expression of IL-2 and FOXP3 mRNA in allo-Ag stimulated PBMC. PBMC were cultured with allo-Ag for 7 days. (A) The expression level of IL-2 mRNA after allo-Ag stimulation of PBMC from patients (N = 14) and healthy controls (HC, N = 17) was comparable (P = 0.49). (B) The expression level of FOXP3 mRNA was significantly higher in allo-Ag stimulated PBMC from HC (N = 17) than from patients (N = 13, P = 0.0002). ■ = HD = haemodialysis, ● = PD = peritoneal dialysis ▼ = ND = no dialysis.

Fig. 4. Proliferative responses of PBMC. (A) The proliferative response of freshly isolated PBMC from ESRF patients and HC to PHA was comparable (HD N = 26, PD N = 18, ND N = 13, HC N = 17). (B) Comparing the proliferation of PBMC from patients and HC (N = 17) to allo-Ag demonstrated that proliferation was low by PBMC from HD patients (N = 29 P < 0.0001), PD patients (N = 19 P = 0.009) and ND patients (N = 12 P = 0.04). HD = haemodialysis, PD = peritoneal dialysis, ND = no dialysis, HC = healthy controls.

Proliferative responses

Proliferative responses of freshly isolated PBMC were studied in 60 out of 80 patients. ESRF patients and HC showed comparable proliferative responses to the mitogen PHA (Figure 4A). In contrast, proliferation of PBMC to
Fig. 5. Expression of CD25 by cell fractions isolated by autoMACS. (A) Representative example of the isolated CD25^{bright}+ fraction stained for its expression of CD4 and CD25, with gated areas of the CD25^{bright}+ (I), CD25^{-} (II) and CD25^{-} (III) fraction (B–C). Analysis of the isolated CD25^{bright}+ fractions from patients and healthy controls (HC) demonstrated that most of these cells resided in gate I (D). Isolated CD25^{-/dim} fraction (E–F). Analysis of the isolated CD25^{-/dim} fractions from patients and HC demonstrated that most of these cells resided in gates II and III. Due to the low number of isolated cells this flow cytometric analysis could not be performed for all patients and HC samples.

Fig. 6. Proliferative responses of PBMC to allo-Ag before and after depletion of CD25^{bright}+ cells. (+) PBMC that followed the autoMACS procedure as described in the ‘Materials and methods’ section and (−) CD25^{-/dim} cells. Depletion of CD25^{bright}+ cells from PBMC resulted in a significant increase in the proliferative response in HC and ND patients, but not for PD patients and HD patients. HD = haemodialysis (N = 33), PD = peritoneal dialysis (N = 23), ND = no dialysis (N = 14), HC = healthy controls (N = 17).

allo-Ag was lower in ESRF patients than in HC (Figure 4B, P < 0.0001). No significant difference was found in the proliferative capacity of PBMC between the different patient groups.

**Phenotypical characterization of isolated fractions by autoMACS**

The isolated CD25^{bright}+ and CD25^{-/dim} fractions contained a comparable percentage of CD4^{+}CD25^{bright}+, CD4^{+}CD25^{+} and CD4^{+}CD25^{-} cells for patients and HC (Figure 5A–F). We were able to determine the FoxP3 protein expression by isolated fractions from seven patients (ND N = 2, PD N = 3, HD N = 2). Here, the median expression of FoxP3 by the isolated CD25^{bright}+ fraction was 73% (range 40–85%) and of the CD25^{-/dim} fraction only 3% (1–7%). These percentages are in line with our findings on the expression of FoxP3 in the CD25^{bright}+, CD25^{+} and CD25^{-} fractions of PBMC from our additional patient cohort (72%, 7% and 1%, respectively) as well as from our HC (80%, 7% and 1%, respectively).

**The regulatory function of CD4^{+}CD25^{bright}+ cells**

The effect of CD25^{bright}+ cell depletion from PBMC on direct alloresponses was determined in the MLR. The increase in proliferation reflects the regulatory capacities of the depleted fraction. Indeed, in HC, we measured a vigorous increase of the proliferative response to allo-Ag after depletion of the CD25^{bright}+ fraction (Figure 6,
ESRF and CD4^+CD25^+FoxP3^+ T-cells

Fig. 7. Cocultures of CD25\textsuperscript{bright} cells with CD25\textsuperscript{neg/dim} responder cells. (A) In HC, adding CD25\textsuperscript{bright} cells to allo-Ag stimulated CD25\textsuperscript{neg/dim} responder cells at several ratios showed a dose-dependent inhibition. (B) This dose-dependent effect was also seen in ND patients, (C) less outspoken in HD patients and (D) not seen in PD patients. The isolated CD25\textsuperscript{bright} cells did not proliferate upon stimulation. HD = haemodialysis (N = 33), PD = peritoneal dialysis (N = 23), ND = no dialysis (N = 14), HC = healthy controls (N = 17).

Fig. 8. Suppression of CD25\textsuperscript{neg/dim} cells by CD25\textsuperscript{bright} cells calculated as the percentage of inhibition (%IH). Inhibition of proliferation of CD25\textsuperscript{neg/dim} cells by CD25\textsuperscript{bright} cells calculated as the %IH was dose dependent in HC and all patient groups. Also, the %IH was lower in patients than in HC. This was significant when CD25\textsuperscript{bright} cells were added to CD25\textsuperscript{neg/dim} cells at the ratio of 1:10 (median %IH: 68% HC versus all patients 42%, P < 0.001). The impaired suppressive function of CD25\textsuperscript{bright} cells in ESRF patients was most explicit in patients on dialysis: ND 47%, PD 41% and HD 30%. Importantly, the %IH did not correlate with proliferation of the allo-Ag stimulated CD25\textsuperscript{neg/dim} cells (HC: P = 0.16; patients P = 0.15), indicating that activated cells do respond to the suppressive signals of CD25\textsuperscript{bright} cells.

To determine whether the patients underlying disease, age, gender, HD/PD/ND treatment and time on dialysis influenced the function of CD25\textsuperscript{bright} T-cells, a multiple regression analysis was performed. This analysis showed no association between the number of CD25\textsuperscript{bright} T-cells, their proportion and function with one of these endpoints.
Discussion

To assess whether the characteristic overactivated but compromised immune system of ESRF patients is caused by an impaired function of CD4+CD25bright+FoxP3+ T-cells, we analysed their number and regulatory capacities. First, we demonstrated that the number of peripheral CD4+CD25bright+ T-cells in ESRF patients was low, whereas their proportion of CD4+ T-cells was not different between ESRF patients and HC. Second, while the proliferation of PBMC from ESRF patients upon stimulation with allo-Ag was affected, it appeared that there was also a defect in regulation by CD4+CD25bright+ T-cells.

Several explanations for the immunodeficient state observed in ESRF patients have been described, including a decreased capacity of antigen presentation and a low number of circulating lymphocytes [4–6,27,28,32,33]. The cause of low numbers of peripheral CD4+CD25bright+ T-cells in ESRF patients is at present unclear. An explanation may be that CD4+CD25bright+ T-cells like CD4+ T-cells from ESRF patients show increased susceptibility for apoptosis resulting from their continuous activation by T-cells from ESRF patients and HC. This may explain the significantly low expression of FOXP3 mRNA in ESRF patients and HC [21–24].

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